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

Genetische Vielfalt und Auftreten von systemischen Erkrankungen der Aurebe (*Vitis vinifera ssp. silvestris*) entlang der Donau

Ferdinand Regner[1], Robert Hack[1], Helmut Gangl[2], Gerhard Leitner[2] , Karin Mandl[1] and Wolfgang Tiefenbrunner[2]

1) HBLA u. BA für Wein- und Obstbau, Wiener Str. 74, A-3400 Klosterneuburg, Austria,
2) Bundesamt für Weinbau, A-7000 Eisenstadt, Gölbeszeile 1, Austria

correspondence E-mail: Wolfgang.Tiefenbrunner@bawb.bmlfuw.gv.at
Ferdinand.Regner@hblawo.bmlfuw.gv.at

Summary

In the riparian woods of Danube and March east of Vienna 87 specimens of *Vitis vinifera ssp. silvestris* were geneticly analyzed and compared. The *Silvestris* population can be splitted into six distinct groups, but their distribution cannot be explained by the geographical distance. In the current situation the genetic variability will be reduced significantly by a loss of some of the wild grapevines.

The incidence of diseases as bacterioses, viroses and nematodes transmitting nepoviruses to this vines were registered. None of the analyzed specimens suffered from *Agrobacteruim vitis* induced crown gall. Only some vines are infected by viral pathogens as GLRaV I and SLRV. Thus the wild vine do not constitute a risk for the surrounding grape plantations. On the other hand, an extension of the 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 differ morphometrically significantly from others found on arable soils or isolated on the remaining research area.

Keywords: 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 seemed to have changed during the 20th century – Kirchheimer 1955 recognized only 20 specimens in the Lobau and 25 near Orth and some isolated ones on both banks of the Danube between Vienna and the Austrian-Slovakian border. He was quite sure, that the subspecies was extinct in the floodplains of the March river. During the next fourty years, there was little interest concerning the wild grape in Austria. Since the “Nationalpark Donauauen” and protection areas of the World Wildlife Fund near Regelsbrunn/Danube and Marchegg/March was founded the wild grapevine of Austria returned into the limelight of protectional and scientific research.

In 1996 the WWF started a cultivation/recultivation programme for the wild grape, that should make its extinction in Austria less likely. 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, under the support of C. Fraissl (2003) began to map the wild vine within the national park Donauauen. They found about 180 individual vines.

Our research activity was started in 2001. The first intention was to investigate how many individuals are remaining and if not mapped individuals are still existing. The main interest was focused on the genetic variability of the wild grape and their diseases. Especially, the

incidence of viroses and bacterioses was analysed. Hence it was evaluated if actually transmission from the cultivated grapevine of the nearby vinegrowing regions or vice versa would occur. Thus the presence of viral pathogens and their vectors was proved. Especially nepoviruses easily can transferred by nematodes and therefore constitute a menace for the wild grapevines.

Materials and methods

The position of each analysed wild grapevine was registered using Global Positioning System (GPS). Therefore anybody has the chance to follow 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 the wild grape was gained by genotyping all individuals with 18 SSR markers. As the first step DNA was extracted from young leaves of natural grown shoots by following the protocol described by Thomas *et al*. (1993) modified by Regner *et al*. (1998). PCR conditions were applied as already used for identification of grapevines (Regner et al. 2000). The amplified allelic fragments were separated on a 6% polyacrylamide gel on the 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. The data were analysed by using Microsat program and the multivariate comparison was drawn with the PhyQuest program.

From the roots, tendrils and shoots of the plant, samples for the identification of viral and bacterial (*Agrobacterium vitis*) pathogens were taken. 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 (SLRV), Tomato Ringspot virus (TomRSV „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. Different to the method no additional extension time was applied. The primers acs1, acs2 and vis1, vis2 were purchased from VITOLAB.

For the record of the soilborne virus vectors soil samples mainly from the rhizosphere of the wild vine were taken with a cylindrical 22 mm diameter soil auger. The samples were from a depth of 0 down to 90 cm and had a volume of ca. 340 cm^3. For the extraction an Oostenbrink-Elutriator was used (sampling sieve width: 150 μm). All nematodes were extracted and the one from the family Longidoridae were identified on the species level. For the identification the following polytomous keys were used: genus *Longidorus*: Chen et al. 1997 and Supplement 1 - Loof & Chen 1999 -, *Xiphinema* with exclusion of *X. americanum s. l.*: Loof & Luc 1990 and Supplement 1 - Loof & Luc 1993 - and 2 - Loof et al. 1996.

The programme PhyQuest was used for a multivariate comparison of biometrical data (the method is described in Tiefenbrunner, 2002). Species and local populations of the nematode family Longidoridae were compared. Within the genus *Longidorus* the characters: body length, body diameter at vulva, tail length, body diameter at anus, oral aperture to vulva, odontostyle, oral aperture to guiding ring, body diameter at guiding ring and body diameter at lip region were used. 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 five 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, in the east of Vienna.

Area I surrounds the riparian woods of the March north of Marchegg and very near to the Slovakian border. 31 samples of wild grapevine individuals were taken. Most sites are very close to river branches. *Vitis vinifera ssp. silvestris* occurs nested. Most individuals have not developed inflorescences and therefore the sex could not de determined. Two were definitively females and six males. 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).

From **area II** some soil samples (merely to close a relatively large geographical gap) were taken but this region lacks wild vines.

Area III, the southern bank of the Danube near Regelsbrunn, hosts only few wild grapevines. They are all males and all growing on *Populus* hybrids close to the river.

Area IV is the floodplain north of the Danube near Orth/Danube. Samples of 23 *Vitis* individuals were taken. 11 were females, 9 males, one hermaphrodite and 2 with unknown sex. They are relatively homogenously distributed. As *Vitis* hosts *Cornus sanguinea* and *Crataegus monogyna* are dominating. A lot of the sampling sites are located far away from the river or its branches, and thus have a drier soil. More details about this area can be derived from Tiefenbrunner et al. (2004 a).

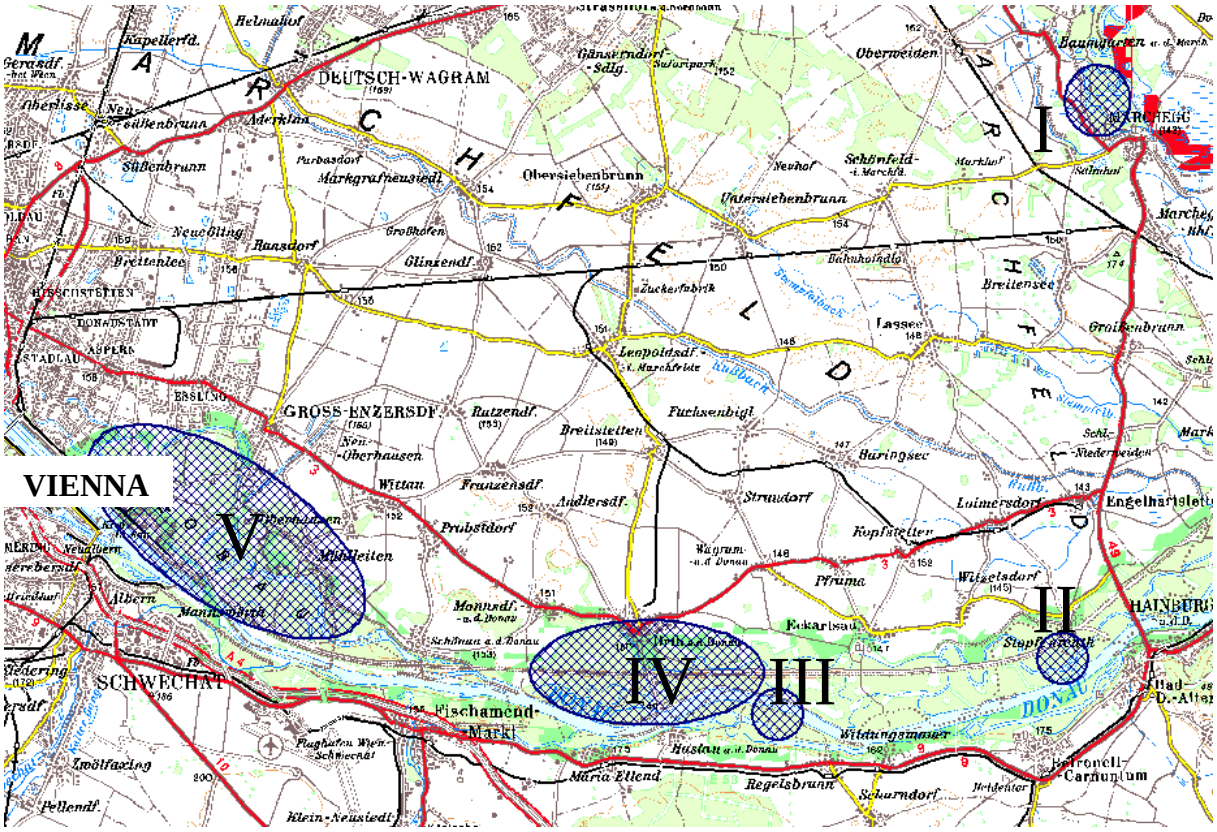
The riparian woods (Lobau) of the Danube near and east of Vienna are **area V**. Here samples of 29 vines were taken. As in area IV, *Cornus sanguinea* and *Crataegus monogyna* are very common hosts, as well as *Populus* species (Tab. 1). Many of the sites are some hundred metres away from the next river branch. Due to late sampling the sex was not determined.

In the whole, 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's riparian woods can be added to the Freiding/Gußmark map. In area V this map was helpful but also other grapes could be discovered. Finally the total number of definitely known specimens raises to at least 220.

Table 1: *Vitis* hosts

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

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



Genetic analysis

One of the main tasks was, whether the populations of area I, III, IV and V are homozygous. Using eighteen different SSR (simple sequence repeats) loci a general genetic profil determined them as *V. silvestris* genotypes (Tab. 2, Appendix). No individual shows close relationship to our cultivated grapevines. Therefore these individuals represent no source for the *V. vinifera* used nowadays. For comparing their genetic relationship we conducted a multivariate comparison of the distances of all specimens from one area. According to this, area I and III are homogenous, whereas IV and V respectively, are clearly splitted into two genetic distinct groups. In both cases most individuals belong to one group, whereas only a small number (4 or 2) belong to the other. Nei's genetic distance for populations 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 places with the largest geographical distance. The larger group of area IV is genetic less similar to the second group of this area than to the vines of area I or to the larger group of the 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. This two groups are also morphologically very different, concerning the shape of the leaves, the vitality of growth and the ripening time of the grapes.

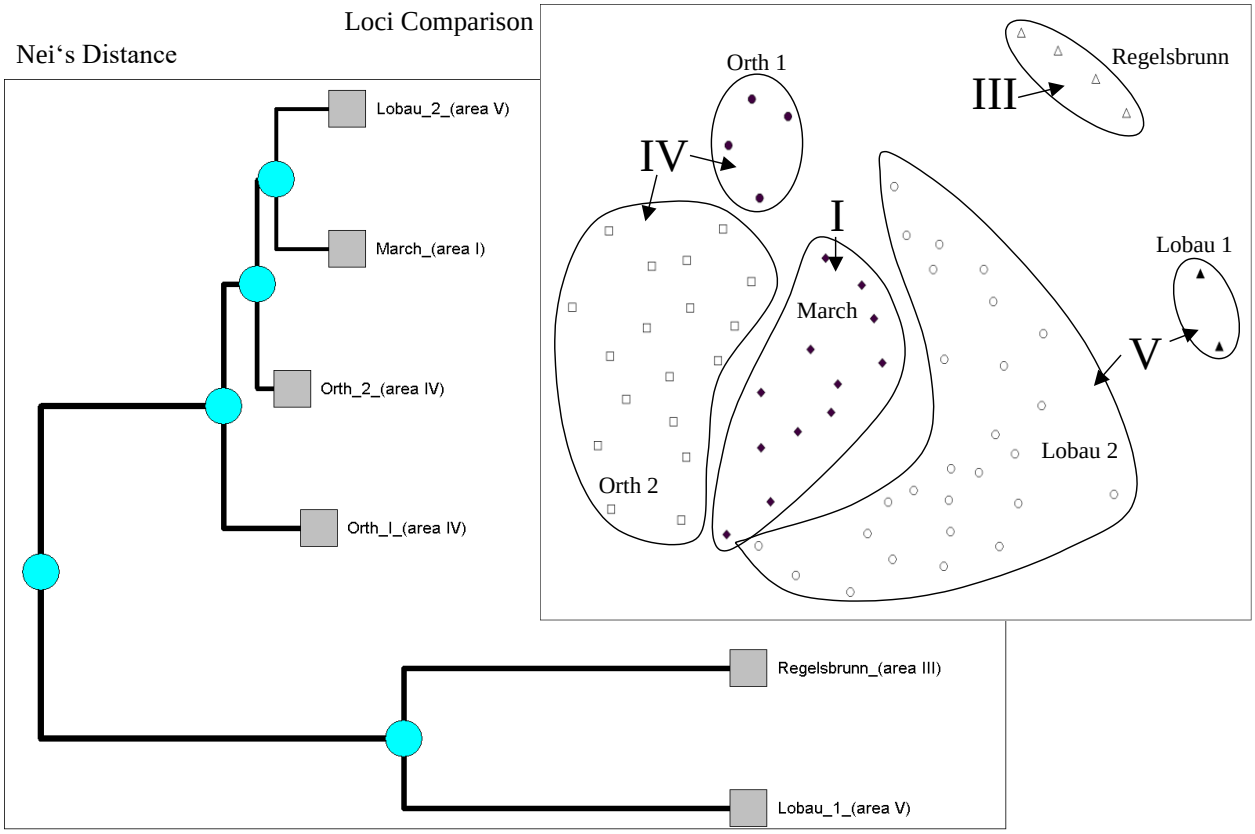
The unexpected pattern of genetic and geographical divergence is not in accordance to the spread of natural populations. The smaller and genetic very divergent groups were maybe carried as seeds in the gut of birds from distant places, or more likely are survivors of an otherwise extinct population. Due to the historical records of the abundance of the wild vines we can suppose that only a few of the autochthonous vines survived. If today wild vines are only a relict of large populations in 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 from native wild vines wine of acceptable quality was produced until 1911. Nowadays only the two vines of this group produce grapes, that could be used for wineproduction.

The larger groups are closer related. This could be an indication that the genetic background of these vines favour their occurrence under the specific climate and growing conditions along the Danube. On the other side the smaller groups could have been reduced due to their individual not adapted genome.

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 relict 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 millenia.

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



Diseases (viruses and bacterioses)

Agrobacterium vitis could not be recorded in any of the analyzed areas. A bit more successful was the proof of viruses. In area IV, six specimens were GLRaV I positive, and one SLRV positive (tab. 3). No evidence for virus induced pathogenes was found in the other areas. We took samples of eight different parts of the plants (roots, woods, tendrils, their peaks, sprouts, inflorescences, leaves and stalks). In the case of GLRaV I, the molecular biological proof was only possible from the inflorescences, in the case of SLRV from the roots. Furthermore it seemed, that only parts of the large vines suffered from a disease and not the whole plant.

Table 3: Virus induced diseases of the grapes of the riparian woods of the Danube and March

VIRUSES	Area I	Area III	Area IV	Area V
	Marchegg	Orth a. d. Donau	Regelsbrunn	Lobau
GLRaV 1	0	6	0	0
SLRV	0	1	0	0

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, no viruses and *Agrobacterium vitis* was detected in wild vines. However, no risk for the economic grown grapevines will derive from the vines of the floodplains. On the other hand, for the future this subspecies will only not be endangered if the plantings are free of viruses and bacteria. In long term the existence of wild vines is dependent of the usage of certified grapevine material.

Nematode vectors of viruses and other Longidorids:

Four nepovirus vector species (Brown & Trudgill, 1997) were registered in the riparian woods of Danube and March. *Longidorus attenuatus*, the vector of 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 probes of meadows and forest soils. The only species strictly associated with the wild grape seems to be *L. elongatus.*

Table 4: Nematodes of the soil samples from the floodplains of Danube and March.

NEMATODS 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	

Area I is dominated by *L. intermedius.* This species is known to be ecologically connected with oak trees. Oak is 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 - ten 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 of 29 samples. Even more abundant per sample is *X. vuittenezi,* but it is not so present. We recognized it in 10 out of 29 samples. *X. vuittenezi* was detected only in area IV and V.

By means of morphometric analysis Longidorids can be determined correctly. The analysis resulted that *L. attenuatus* and *X. vuittenezi* of the riparian woods morphometrically differ from individuals of the same species isolated from vineyard locations. The differences are good enough to misinterpret the identity.

Fig. 3 shows the result of a multivariate analysis of body proportions. Compared are *Xiphinama vuittenezi* 1) from vineyards and arable land, 2) from area IV (Orth/Danube), 3) from area V (Lobau), and 4) *X. index* and 5) *X. diversicaudatum* as morphometrical similar, but nevertheless clearly distinguishable 'outgroups'. Fig. 2 can be seen as the surface of a hemisphere. *X. index* and *X. diversicaudatum* are laying at the periphery of that hemisphere and are well separable from the other groups. The specimens from area IV are also on the periphery, on both sides between the outgroups. The individuals of area IV are separable from the one from vineyards and arable land (in the centre of Fig. 2), thus indicating, that

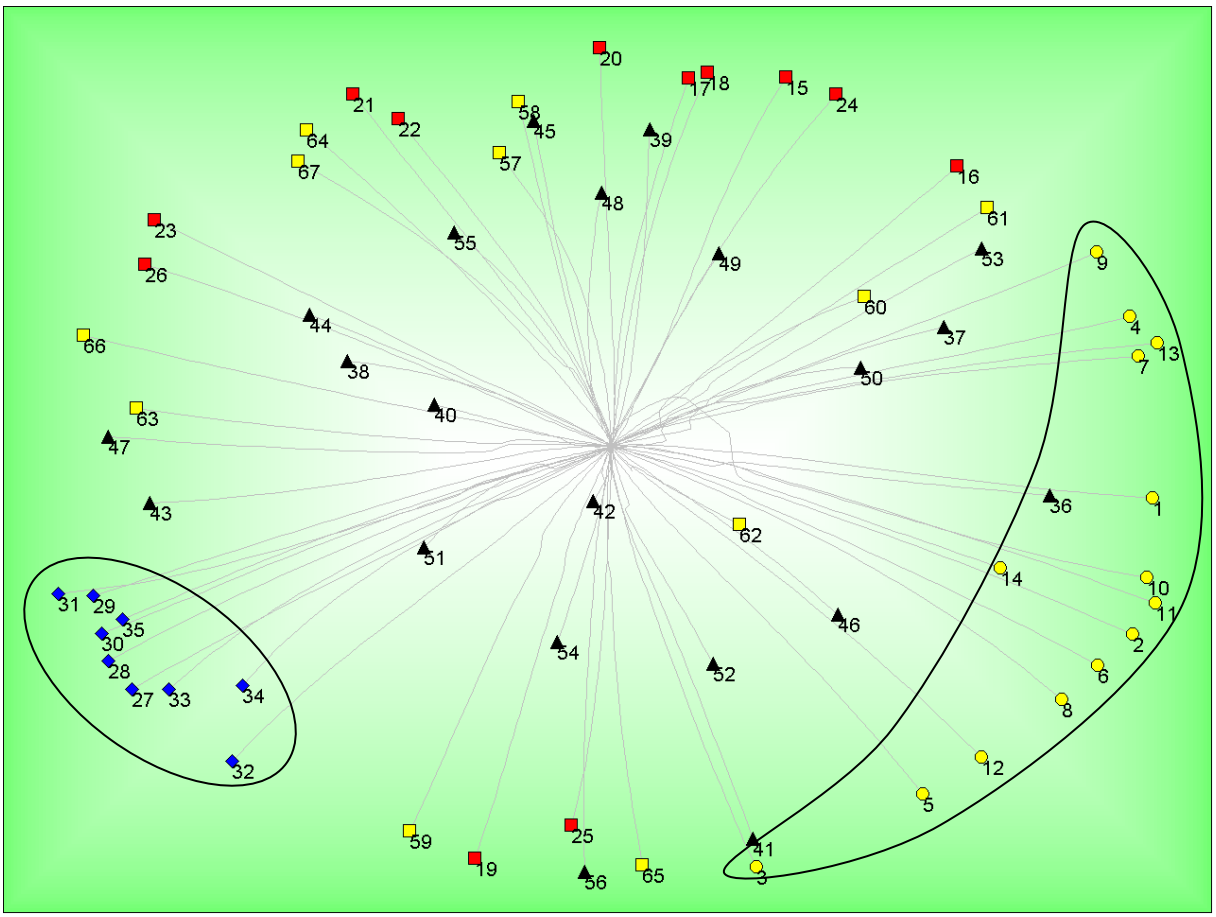
they could belong to different ‘species’. Indeed this was the original interpretation (Tiefenbrunner et al. 2004 a and b) before the animals of area V were known. This one can neither be separated from *X. vuittenzi* from cultivated land nor from the individuals from Orth.

In the last decades within Longidorid systematics, splitting was always (and exhaustive) prefered to lumping. Here we have an example that this may not always be the best strategy, especially if agamotaxons are concerned. It is rather questionable whether the present customs of species description are valid as far as morphometrical characters are concerned. Usually mean and standard deviation of these characters in the analyzed ‘populations’ are given, but it is not considered, that they are – partly highly – correlated. This is the reason, why we made a proportion analysis. To ignore this correlation might bias comparisons.

Although the specimens from the Lobau can be seen as a transition, in mean they differ very much from that of Orth/Danube, giving the impression of a long lasting isolated evolution of both groups. Of course, the reasons for the metrical differences may not be geneticly at all, but may be adaptions to ecological factors.

Fig. 3: morphometrical comparison of three local ‘populations’ of *X. vuittenezi*. *X. diversicaudatum* and *X. index* are used as outgroups.

▲: *Xiphinema vuittenezi* from arable land and vine yards
■: *Xiphinema vuittenzi* from the Lobau (area V)
■: *Xiphinema vuittenezi* from Orth/Danube (area IV)
◆: *Xiphinema index*
● :*Xiphinema diversicaudatum*



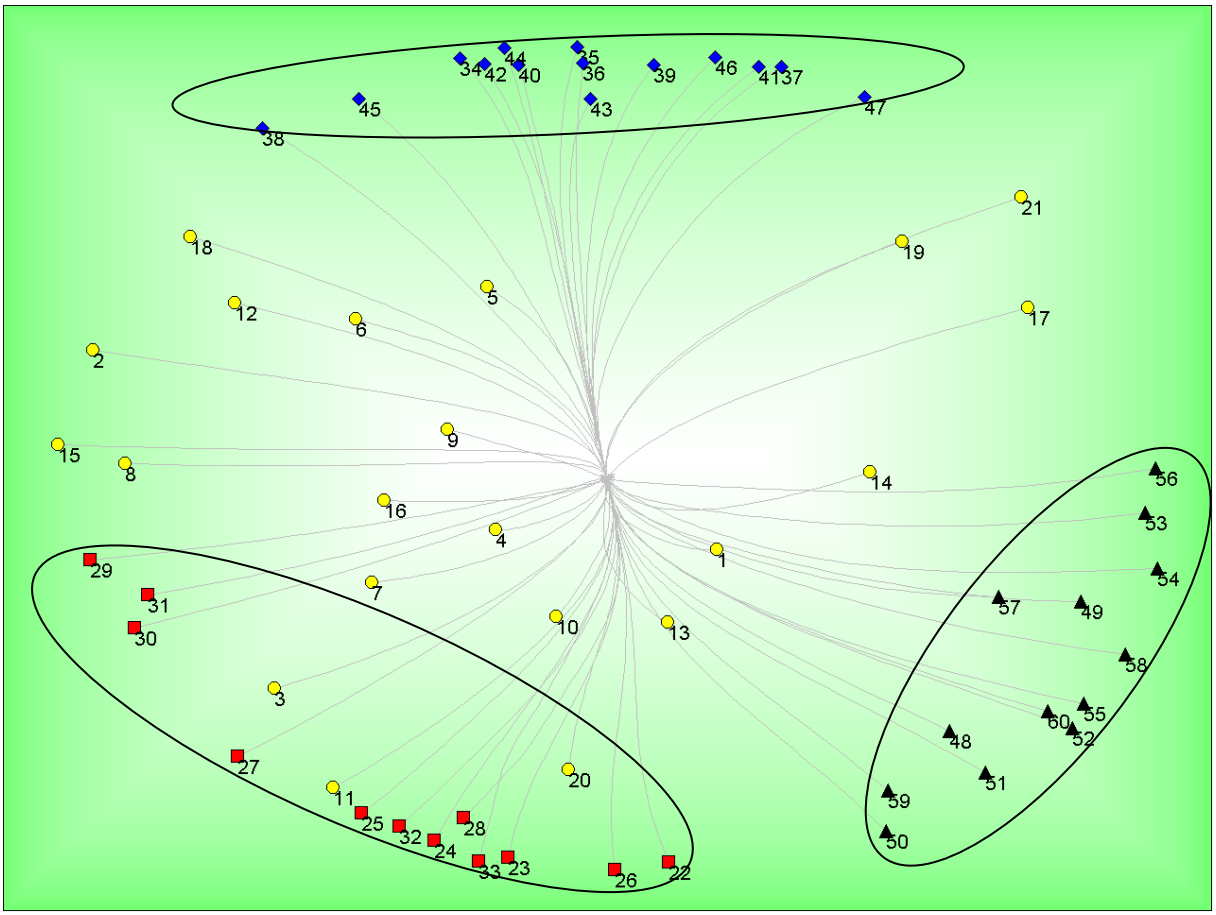
For *L. attenuatus*, the situation is even more complex, because the individuals of the Lobau (area V) are quite different from the one of area IV (Orth/Danube). They can be nearly perfectly separated from one another. There is no great distance between these areas, neither in a geographical, nor in 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, respectively) and the area V 'population'.

Using the key of Chen et al. 1997, for the individuals from the 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 unusual short (the F-code concerns the body length). The animals of area IV have the typical code for *L. attenuatus*.

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

■: *Longidorus attenuatus,* mainly from area IV, but also from area II and I
●: *Longidorus attenuatus* from area V
▲: *Longidorus leptocephalus*
◆:*Longidorus intermedius*



Acknowledgement

We are grateful to the WWF, the Nationalpark Donau-Auen GmbH and the Österreichische Bundesforste for their support, especially (in alphabetical order) Johann Bammer, Christian Fraissl, C. Freiding, C. Gußmark, Franz Klein, Anton Kotacka, Franz Kovacs, Günther Lutschinger and Josef Wedenig.

Literature

Arnold, C., Gillet, F., Gobat, J.M., 1998, Situation de la vigne sauvage Vitis vinifera spp. silvestris en Europe, Vitis 37,4,159-170.

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.

Bowers, J.E., Dangl, G.S. and Meredith, C.P. 1999: Development and characterization of additional microsatellite DNA markers for grape. Amer. J. Enol. Vitic. 50(3): 243-246

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(1), 15-28.

Gangl H., Leitner G., Tiefenbrunner, W. , 2001: Rebschädigende Viren, Bakterien und bodenbürtige Vektoren im österreichischen Weinbaugebiet Carnuntum, Mitteilungen 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, Zeitschrift für Botanik, Bd. 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). Supplement 1, Nematology, Vol. 1(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, Systematic Parasitology, 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: supplement 1, Systematic Parasitology, 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: supplement 2, Systematic Parasitology, 33, 23-29.

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.; 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.; Kaserer, H.; Eisenheld, C.; 2001: Weitere Sortenanalysen bei Rebe mittels genetischer Marker. Mitteilungen 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., and 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(7),845-852.

Tiefenbrunner, W., Regner, F., Mandl, K., Leitner, G., Gangl, H., 2004: 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 soilborne vectors, Plant Genetic Resources Newsletter, in press.

Tiefenbrunner, A., Tiefenbrunner, W., 2004: 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, in press.

Table 2, Appendix: Alleles of analysed wild vines – examples of each group.

probes	VVS2	MD5	MD7	MD27	VRZag62	VRZag79	MD 6	MD 8	MD 24	MD 25	MD 28	MD 36	VMC 62	VRZag7	VRZag 12	VRZag 15	VRZag 21	VRZag 67
L 5	132/151	231	240	188	195	252	199/206	139	212	257/268	237	254/295	224	157	155	181	192	153
L 6	132	231	240/264	189	198	239/252	199	139	212/216	257/268	238/266	295	228	157	155	177/201	192/196	153
L 7	132/159	233/265	240/254	189/217	195	246/258	205/210	141/175	206/216	241/268	220/238	234/41/48/54	213/224	157/192	142	166	202/206	139/157
L 8	132/159	234/266	240/254	189/217	188/195	146/258	204/209	142/175	206/226	241/268	220/238	240/254	213/224	157/192	141	166	201/205	139/157
L 9	131/151	231	240/264	189	195	239/252	199	139	212	268	238/266	254/295	224/228	109/157	155	179/181	192/196	153
L 10	136/143	231	240/264	189	195	252	199	139	212	268	238/266	254/295	224/228	109/157	155	181	192/195	153
L 11	131	231	240/264	189	195	252	199/206	139	212/216	256/268	238/266	295	224/228	157	155	181/189	192/196	132
L 12	151	231	242/264	189	195	252	200/207	139	212/216	251/257	238	295	209/224	157	155	177/181	192/196	153
L 13	151	231	264	189	195	252	199	139	212/216	257	238	295	209/224	157	155	181/201	192	132/153
L 14	151	227/231	240	189	185/195	252/256	199/206	139	212	268	237	295	224/228	157	155	185/201	192	153
L 21	132	227	240/264	189	195	248/256	199/206	139	212/216	257	237/265	254/294	223/228	109/156	155	181/188	186/192/197	153
L 22	132/151	232	239/263	188	195	246/252	199	139	212	269	237/265	294	223	156	155	199/201	191/195	153
L 23	132	232	239	188	195/197	252/256	199	139	212	256/270	(254)/265	295	223/228	156	155	182/201	191/195	153
L 24	151	228/232	241/249	188	195/197	246/252	199	139	212/216	251/257	237/265	254/295	223/228	156	155	189/201	191/195	153
L 25	132	232	239/249	188	195/197	246/252	199	139	212/216	257/268	263/265	295	228	156	155	181/201	191/196/201	132/153
R 1	137/141	252/264	253	197/215	189/191	256/262	204/214	175/177	206	239/241	235	240	213	178/187	142/171	164/166	199/206	157/167
R 2	137/141	252/264	253	197/215	189/191	256/262	204/214	175/177	206	239/241	235	240	213	178/187	142/171	164/166	199/206	157/164
R 3	137/141	252/264	253	197/215	189/191	256/262	199/204/14	175/177	206	239/241	235	240	213	178/187	142/171	164/166	199/206	157/164
Orth 1	128/133	226/236	239/263	189	195	252	200/207	139	195/213	256	236	254/295	224	109/156	155	201	192	152
Orth 2/0	128/133	228/232	241	189	195	252	207	139	217	256/268	238/266	255/295	224	156	155	181/201	196	153
Orth 3/0	133/152	228/232	241/265	192	195	252	184/200	139/141	213/217	247/257	238/266	295	224/232	156	155	181/203	192/204	153
Orth 4	146/152	232	241	190/1	195	252/256	200/207	139/141	213/217	256	254/266	254/295	224/241	156	155	181	196	153
Orth 5	128/133	232	239/263	189	195	252	207	140	217	256/268	237/265	295	224	156	155	177/181	192	151/153
Orth 6	133/152	232	243/265	189	195	252	207	139	195/213	256	238	254/295	210/228	156	155	181/201	192	152/155
Orth 8	128/133	232/240	241	192	195/197	252	200/207	142	213/217	256	238/242	295	224/228	109	155	181	196	151/153
Orth 9	133/146	232/266	239/253	189/211	193/195	256/260	214	139	207/217	240/256	218/266	241/295	210/214	156	141/157	177/185	196	153
Orth 11	133/152	232	240/264	191	195	252	200	139	213/217	250/268	266	295	224/228	156	155	177	192/196	153
Orth 14	142/153	232	263	189	195	252	207	140	195/213	250/256	238/266	295	225/229	156	155	189/201	192/196	153/155
Orth 15	133/142	232	259/264	189	195	246/252	200/207	139/40	213/219	251/257	238/266	254/295	224/228	156	155	181/201	192/196	153/155
Orth 16	128/152	232	241	192	195	252	200/207	139	195/213	250/256		295	255/259	156	155	177/181	192/196	153

Orth 17	133/152	232	239/40	189/191	195/197	252/256	184/200	139	198/217	250/256	235/238	245/295	210/224	156	155	181/201	192/196	153
Orth 18	133/152	232/238	241	189/191	195	252	199/206	139	213/217	256/268	238/166	245/295	224	156	155	199	194	153
M 1	132/152	228	240/258	189	194/198		199	139	213/217	257/269	265	295	220/224	156	155	181/201	196	132/153
M 6	152	228/232	240/258	181/189	196	244/252	199/208	139	213	268	237/265	254/295	220/224	109/156	155	177/203	192	153
M 10	132/152		240	189/191	193/196	252/256	199	139	213/217	257/269	265		220/224	156	155	178	192/196	130/153
M 12	139	228/232	240/264	189	195	252	206	139	213/217	251/269	237/265	254	220/224	156	155	177/181	192/196	153
M 13	132	228	240	189	195	252	199/206	139	213	257	237/265	295	220/224	156	156	177	192/196	153
M 17	132	228	250/264	189	193/195	252	199	139	213	251/269	265	295	220/224	109/156	155	178	194/196	153
M 20	152	228/232	240/251	189	195	252	199/207	139	213/217	257	265	270/295	220/224	109/156	155	178	192/196	153
M 24	132	232	256/264	189	193/195	252	199/206	139	213/217	257/268	237/265	295	220/224	156	155	177	192/196	132/153
M 25	132	228/240	264	191	193/195	252/256	199/206	139	213	268	237	295	224/228	156	155	181/203	192/196	132/153
M 27	132	228/232	240/242	189	196	252	199	139	213/217	251/257	265	254	220/224	156	155	177/189	192/196	153
M 28	132	228/232?	240/258	125	193/196	252	199/206	139	213	257		254	224/228	109/156	155	177/189	192/196	153