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Autor:	Aas, Gregor / Maier, Josef / Baltisberger, Matthias
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Morphology, isozyme variation, cytology, and reproduction of hybrids between *Sorbus aria* (L.) Crantz and *S. torminalis* (L.) Crantz

Gregor Aas¹, Josef Maier², Matthias Baltisberger³ and Sabine Metzger¹

¹ Chair of Forest Pathology and Dendrology, ETH Zürich, CH-8092 Zürich, Switzerland

² Udalrichstr. 15, D-80933 Munich, Germany

³ Geobotanical Institute, ETH Zürich, Zollikerstr. 107, 8008 Zürich, Switzerland

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Abstract

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In the Reppischtal valley (near Zürich, Switzerland) *Sorbus aria* and *S. torminalis* grow as well as numerous presumably hybridogeneous forms (*S. latifolia*). This study was based on the hypothesis that these are hybrids and part of an apomictic taxon. In order to examine this hypothesis, the morphological as well as the isozyme and cytological variation of these trees were analysed and investigations on their reproduction were carried out. Furthermore, isozyme analyses were effected in *S. latifolia* growing on another site (Birnberg, to the south of Baden, Germany).

S. latifolia varies morphologically. Apart from the intermediate individuals, there are individuals that are more or less similar to *S. aria*. *S. latifolia* and *S. torminalis* differ clearly from each other. The isozyme analyses revealed that the seven tested (morphologically intermediate) individuals from Reppischtal are in fact six different genotypes. Also the tested individuals from Birnberg showed genotypic variation. *S. latifolia* produces viable seeds sexually. The seedlings are mainly diploid ($2n=34$), one plant proved to be triploid ($3n=51$).

The results obtained suggest the conclusion that *S. latifolia* in the Reppischtal valley is not a genetically uniform, apomictic taxon. It is much more likely that the individuals are a complex of hybrids, at least partially capable of sexual reproduction, and various forms of back-crosses more or less similar to *S. aria*. However, the possibility that apomictic forms occur within this complex cannot be ruled out on the basis of this study. Further investigations will be necessary. The taxonomic consequences of this variation are being discussed.

Key words: *Sorbus aria*, *Sorbus torminalis*, *Sorbus latifolia*, morphology, isozymes, cytology, hybrids, apomixis, taxonomy.

1. Introduction

In Central Europe, the genus *Sorbus* L. contains the following diploid species ($2n=34$) with sexual reproduction: *Sorbus aria* (L.) Crantz (whitebeam), *S. aucuparia* L. (rowan), *S. chamaemespilus* (L.) Crantz (dwarf medlar), *S. torminalis* (L.) Crantz (wild service tree) and *S. domestica* L. (service tree) (Liljefors 1953, 1955, Düll 1959, Maier 1994a). All species of *Sorbus* – except *S. domestica* – can (in certain combinations) produce diploid and fertile hybrids amongst themselves. Furthermore, numerous taxa originated by hybridization are known, often polyploid and endemic (surveys by Düll 1959, Maier 1994a, b). Many of these are described as species (microspecies) and are regarded as apomicts (apomixis understood as asexual development of seeds; Asker and Jerling 1992).

Intermediate and presumed hybridogeneous forms between *S. aria* and *S. torminalis* also belong to these species. Düll (1959) describes them as hybrid wild service tree, *S. latifolia* s.l. According to Seybold (1992), they form a group (aggregate) that includes the hybrids of *S. aria* and *S. torminalis* as well as a number of apomictic microspecies derived from them. Morphologically, they are all similar to *S. latifolia* (Lam.) Pers. (Fontainebleau service tree, Düll 1959) found in the woods of Fontainebleau (to the south of Paris). Di- or polyploid taxa of the *S. latifolia* aggregate have been known in England (Wilmott 1934, Warburg 1957, Clapham et al. 1987, Sell 1989), in the Czech Republic (Challice and Kovanda 1986, Jankun and Kovanda 1987, 1988, Kovanda 1984), in Hungary (Karpati 1960) and in Germany (Düll 1959, 1961, Meierott 1986, Suck and Meyer 1990). Kägi (1924), Kummer (1943) and Moor (1967) describe sites in Switzerland, where *S. latifolia* can be found, as does Seybold (1992) in the adjoining Baden-Württemberg (Southern Germany). In many cases, these forms are described as species (microspecies). It is, however, questionable, whether these species are actually always morphologically distinct units and reproductively isolated from each other. Often there is a lack of relevant investigations concerning morphological variation, cytology, and the reproductive behaviour. New methods to describe the genetic variation allow a better understanding of the differentiation between these taxa. By means of isozyme analysis, it is possible to clarify to what extent such microspecies show clonal structures as a result of asexual reproduction, that is, to what extent morphologically similar individuals represent varied genotypes (Proctor et al. 1989, Proctor and Groenhof 1992).

In the Reppischtal valley (near Zürich) there are sites with *S. aria* and *S. torminalis* as well as numerous individuals of *S. latifolia* (Aas and Storrer 1993, fig. 1). It, therefore, seemed reasonable to analyse these groups of trees thoroughly within a case study, starting out with the hypothesis that *S. latifolia* in this region is an apomictic taxon, originated by hybridization. The subject of the morphological investigation was the analysis of the variation of *S. latifolia* as well as that of *S. aria* and *S. torminalis*. The aim of isozyme analysis was to clarify to what extent morphologically intermediate trees and their descendants are genetically uniform or varied individuals. Furthermore, studies were carried out to clarify whether intermediate forms are able to produce viable seeds sexually, and to find out whether or not di- or polyploid seedlings actually originate from this stage.

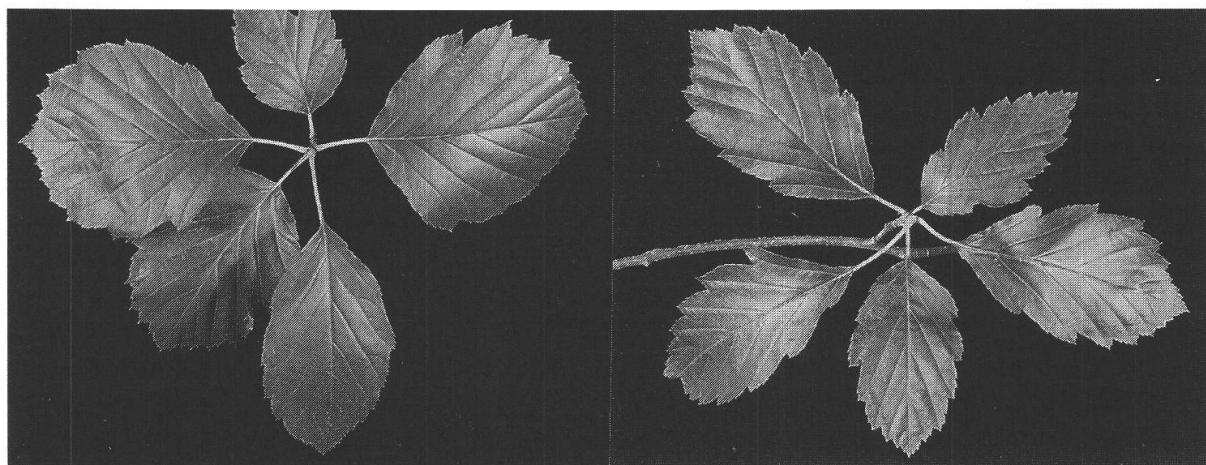


Fig. 1. Leaves of two individuals of *Sorbus latifolia* (both from Reppischtal) illustrating morphological differences within the taxon.

2. Material and methods

2.1 Sample sites

The *Sorbus*-species studied grow in the Reppischtal valley, situated in the canton of Zürich (Switzerland) between Birmensdorf and Urdorf (Tab. 1, detailed description of the site in Aas and Storrer 1993). By far the largest part of the individuals analysed is on a southern to south-western orientated slope. To draw comparison, a second site with *S. aria*, *S. terminalis* and *S. latifolia* was chosen, to carry out isozyme analysis. This site is on a steep limestone debris slope with a shallow soil layer. The slope is on the mountain Birnberg near Griessen, in the region of Klettgau (Southern Baden, Germany) (Tab. 1).

Tab. 1. Details on the sites of the *Sorbus* species in Reppischtal and on Birnberg (coordinates taken from maps of Switzerland, *Landeskarten der Schweiz* 1:25 000, no. 1091 Reppischtal, no. 1051 Birnberg).

	Reppischtal	Birnberg
Coordinates	ca. 672 500 to 673 500/ 247 625 to 247 850	ca. 673 250/274 150
Altitude above sea-level	450–580 m	480–550 m
Exposition	S to SW	W to SW
Geological subsoil	Calcareous upper freshwater molasse with alternating layers of solid sandstone and soft marlaceous soil	Upper jurassic (Malm)
Forest type	Beech forests of varied forms	Pubescent oak forest

2.2 Morphological investigations

2.2.1 Material

Investigations were carried out on *S. aria*, on *S. torminalis* and their intermediate forms. While sampling, each tree was *a priori* allocated to one of the following groups (morphotypes):

1. *S. torminalis*
2. intermediate (*S. latifolia*)
3. *S. aria*
4. doubtful

The morphological characteristics of the leaves were of prime importance for this allocation. The classification of the types 1 to 3 were carried out according to the instructions to be read in Düll (1959), Hess et al. (1977) and Seybold (1992). The conclusive factor for the distinction between *S. aria* (group 3) and trees of doubtful affiliation (group 4) was the shape of the leaf margin. Those trees with a serrate or biserrate, but not lobed leaf margin were allocated to *S. aria*. Trees with lobes at the leaf margin, which would otherwise have been allocated to *S. aria* with reference to their other characteristics, were classified as doubtful (group 4).

Altogether, studies were carried out on 72 trees of the valley of Reppischtal. Only individuals with a minimum diameter at breast height of 10 cm were selected. According to the *a priori* classification these were:

- 18 *S. torminalis*
- 13 *S. latifolia*
- 17 *S. aria* and
- 24 trees of doubtful affiliation

The trees were selected randomly and the number of individuals per group (1–4) selected did not correspond with the actual numerical ratio within the selected forest stand; (the latter to be read in Aas and Storrer 1993).

Sampling took place in early June 1993. By using a telescopic secateurs, two to three non flowering short shoots of each tree were cut off. The leaves of these shoots were preserved as herbarium specimens. According to studies carried out on *Crataegus* spp. (Rosaceae) (Phipps and Muniyamma 1980) and on *Sorbus* spp. (Sell 1989), leaves of short shoots are more suitable for taxonomic purposes than those of long shoots, as the intra-individual variability of the leaves of short shoots is lower. Furthermore, it is recommendable to use leaves of a comparable position on the shoot itself (Dickinson and Phipps 1984). The topmost as well as the bottom leaf of the shoot (that is the leaf closest to the apex and to the base of the shoot, respectively) have, therefore, not been taken into consideration in this study. Five leaves were randomly selected out of the remaining collective (that is short shoot leaves of a middle position on the shoot) in order to determine the necessary data.

2.2.2 Methods

Determination of data. – The values of the following 13 parameters were ascertained per leaf (fig. 2).

1. Length of petiole
2. Length of lamina
3. Relative length of petiole (=quotient 1. to 2.)
4. Max. width of lamina
5. Shape of lamina (=quotient 2. to 4.)
6. Width of lamina at 1/10 of length
7. Width of lamina at 9/10 of length
8. Shape of leaf base (=quotient 6. to 4.)
9. Shape of leaf apex (=quotient 7. to 4.)
10. Angle of leaf base
11. Depth of lateral incisions

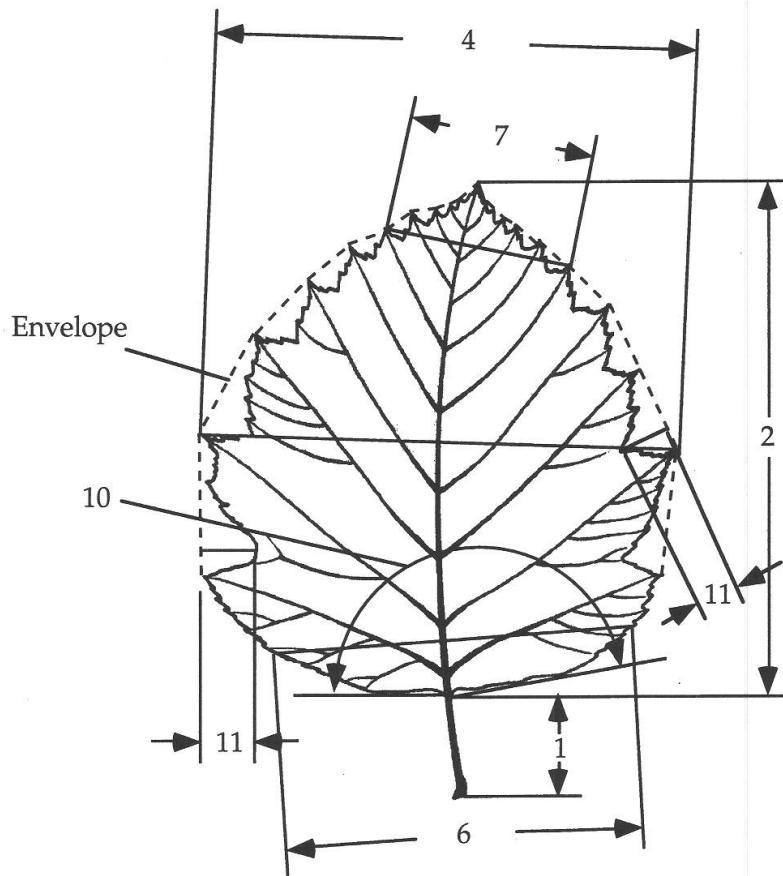


Fig. 2. Ascertained leaf-morphological parameters.

12. Relative depth of lateral incisions (=quotient 2 \times 11. to 4.)
13. Number of pairs of 1st order lateral veins

The parameters 4, 6, 7 were measured vertically to the leaf midrib and refer to the width of the envelope of the lamina, which is the straight connection of the leaves' tips of the teeth and lobes respectively. Parameter 10 is the angle of the tangents between the leaf base and the leaf margin. To determine parameter 11, the depths of the largest lateral incisions were measured vertically to the envelope of the lamina on both longitudinal leaf halves. The two results were then averaged to get one value per leaf. Parameter 13 is the average of the number of pairs of lateral veins of both longitudinal leaf halves. – Data were ascertained by means of measuring the leaves with the image analysing system MacFolia™ (Regent Instruments INC. and Aas 1993), a computerised measuring programme developed especially for the recording of leaf-morphological parameters. The leaves were scanned and thus shown on the screen, whereupon the values could either be determined automatically ("length of petiole", "length of lamina") or interactively (e.g. "depth of lateral incisions").

Evaluation of data. – For each of the 13 parameters, mean values were calculated per tree. These mean values were used for the cluster analytical grouping of all trees as well as for the discriminant analytical separation of the groups *a priori* classified. The description of the variability within the groups was effected by means of Box-and-Whisker-Plots on the basis of the individual values of all the leaves. Two medians differ from each other, provided that the standard deviations do not overlap (95% interval of confidence, Lozán 1992). – The parameters 3, 9, 10, 12, and 13 were employed to carry out the multivariate statistical analysis (cluster- and discriminant analysis). The remaining parameters were not taken into consideration as they stood in correlation with one of the parameters

employed ($r > 0.8$, Spearman rank correlation). The Euclidean Distance was chosen as measure of distance for the cluster analytical grouping; the linking of the clusters was effected according to the Ward-method. The statistical evaluations were carried out by means of SYSTAT, a Macintosh programme package.

2.3 Isozyme analyses

2.3.1 Material

1. *Adult trees*. – In Reppischtal and in Birnberg, 30 morphologically intermediate trees (*S. latifolia*) were selected randomly as well as 17 *S. aria* and 15 *S. torminalis* (tab. 2). The *S. latifolia* of Reppischtal were trees whose intermediate status was confirmed on the basis of the morphological analysis carried out (see chapter 3.1). – In the autumn of 1993, branches were cut off the selected trees, just before the leaf fall. The buds of these branches were removed in the laboratory. After removing the outermost scales, the green buds were pulverised in liquid nitrogen and stored at -70°C for further use.

Tab. 2. Isozyme analyses: number of tested trees of Reppischtal and Birnberg.

	Reppischtal	Birnberg	Total
<i>Sorbus aria</i>	9	8	17
<i>Sorbus torminalis</i>	10	5	15
<i>Sorbus latifolia</i>	7	23	30
Total	26	36	62

2. *Single tree progenies*. – At the beginning of the second vegetation period, seedlings grown from seeds of three selected intermediate trees (see chap. 2.4) were tested electrophoretically. The number of seedlings per mother tree were 7, 7 and 6, respectively. Young leaves (max. four weeks after unfolding) were harvested, pulverised in liquid nitrogen and stored at -70°C for further use.

2.3.2 Methods

Electrophoresis. – Extraction of the isozymes from the plant material was effected by means of a 0.12 M tris-HCl buffer (pH 7.3), which was mixed with 4 w/v% polyvinylpyrrolidone (PVP), 1 v/v% mercaptoethanol and 3.2 mM EDTA. For better dissolving ca. 30 v/v% undissolving PVP was added. The extract was absorbed by means of filter paper wicks (size: 7 x 4 mm) and these were used as specimen carriers in the gel. Separation of isozymes was effected by means of horizontal starch gel electrophoresis (Müller-Starck and Ziehe 1991, Müller-Starck 1993, Müller-Starck pers. comm.). The enzyme systems mentioned in tab. 3 were analysed with the following buffer-systems:

1. Ashton:

Electrode-buffer: 0.05 M LiOH/0.19 M boric acid, pH 8.1

gel-buffer: 0.05 M tris/8 mM citric acid and 10 v/v% electrode-buffer, pH 8.1.

2. Tris-citro:

Electrode-buffer: 0.14 M tris/0.043 M citric acid, pH 7.5

gel-buffer: electrode-buffer/H₂O (1:2.5).

Tab. 3. Isozyme analysis: analysed enzymes, their abbreviations and codes (E.C.) plus the applied buffer-systems.

Enzyme	Abbreviation	E.C.	Buffer-system
Isocitric dehydrogenase	IDH	1.1.1.42	Tris-Citro
6-Phosphogluconate dehydrogenase	6 PGDH	1.1.1.44	Histidine-HCl
Phosphoglucose isomerase	PGI	5.3.1.9	Ashton
Phosphoglucomutase	PGM	2.7.5.1	Histidine-HCl
Shikimate dehydrogenase	SKDH	1.1.1.25	Tris-Citro

3. Histidine-HCl:

Electrode-buffer: 0.14 M tris/0.043 M citric acid, pH 7.5

gel-buffer: 0.05 M L-histidine-HCl/1.4 mM titriplex II/0.17 M Tris, pH 6.2.

In the adult trees, analysis were carried out on the enzymes 6 PGDH, PGI, PGM and SKDH, in the single tree progenies on the enzymes IDH, PGI, PGM and SKDH.

The gels contained 11 w/v% (Ashton) respectively 12 w/v% (tris-citro, histidine-HCl) starch (hydrolysed, Sigma). The separation was carried out with a constant voltage of 20 V/cm (Ashton) respectively 30 V/cm (tris-citro, histidine-HCl) with a time of migration of about 5 (Ashton), 6 (tris-citro) and 7 (histidine-HCl) hours. Staining of the isozymes was effected as described by Müller-Starck (1993).

Evaluation. – The zymograms were evaluated visually. The individual banding patterns were divided into zymogram-phenotypes. The analyses focus on the determination of the variation of morphologically intermediate individuals (*S. latifolia*). The phenotypes of *S. aria* and *S. torminalis* were only taken into consideration when species-specific variation had been ascertained.

2.4 Viability of seeds

In the autumn of 1992, fruits of four *S. latifolia* were collected in the Reppischtal valley. The seeds were separated from the flesh of the fruit, stratified and sown separately (according to their respective parent tree) at the beginning of April 1993 (treatment of the fruits and stratification of the seeds according to Frehner and Fürst 1992).

2.5 Chromosome number

For the determination of the chromosome number, offsprings of three *S. latifolia* (see section 2.4) were used at seedling stage. The number of analysed individuals per mother-tree were 7, 4 and 2 respectively. Root tips were processed following the method described by Dyer (1963): pretreatment for 1 hour with colchicine (0.05%), fixation in ethanol/acetic acid (3:1), staining and squashing in lactopropionic orcein. The small size of chromosomes (ca. 1–2 µm) made counting rather difficult. For this reason, 5–10 cells in metaphase were chosen in each individual.

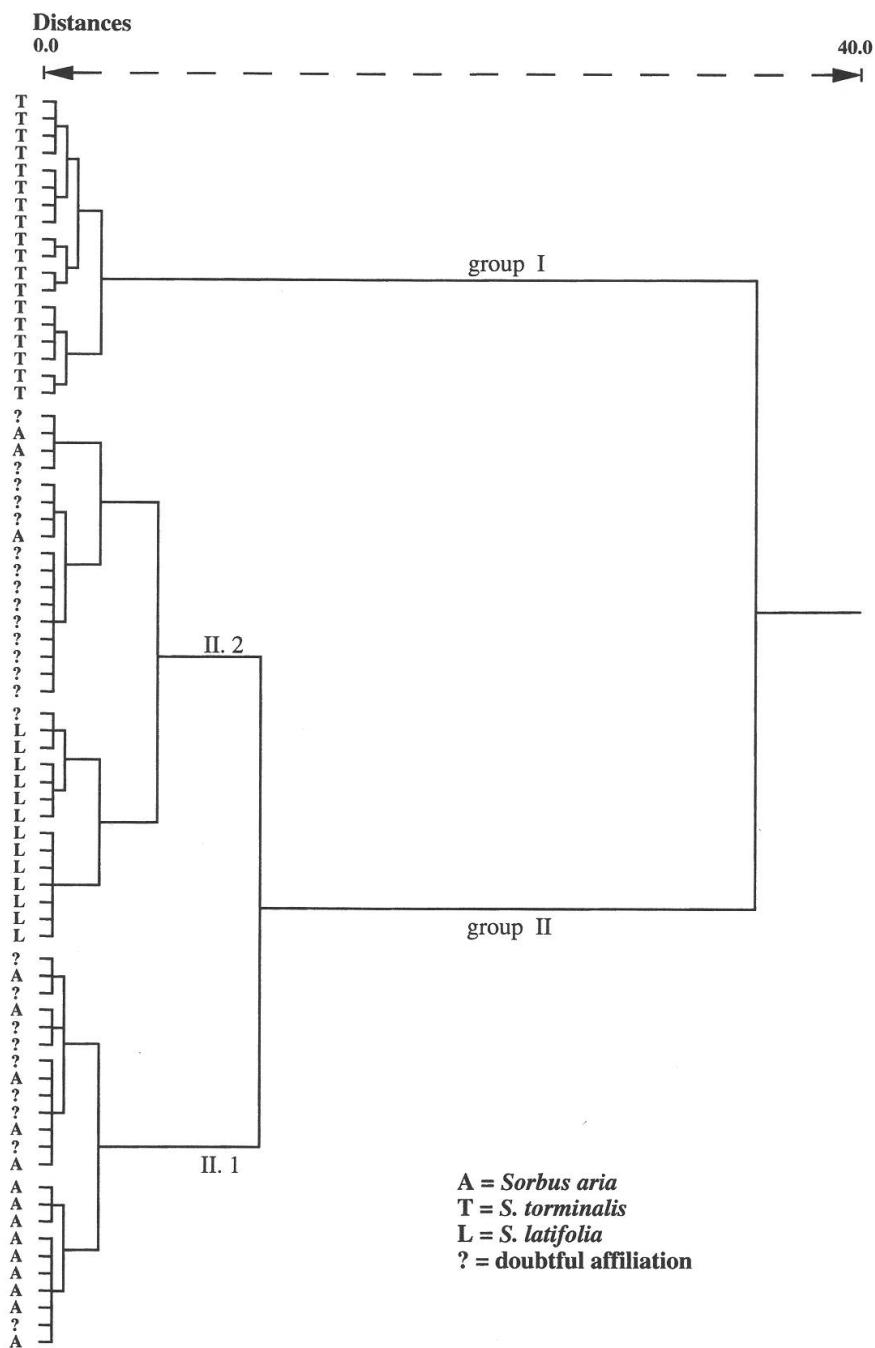


Fig. 3. Morphological investigations: cluster analytical grouping (dendrogram) of the studied trees.

Results

3.1 Morphological investigations

3.1.1 Cluster analytical grouping

Fig. 3 shows the results of the cluster analytical grouping. The 72 trees are divided into two groups (*I*, *II*). Group *I* contains all the 18 trees determined *a priori* as *S. torminalis*, group *II* all those determined either as *S. aria*, as intermediate or as of doubtful

affiliation. Group *II* consists of two subgroups: Subgroup *II.1* contains 14 (of the total of 17) *S. aria* and 9 trees of doubtful affiliation. Subgroup *II.2* further divides into two units: 3 *S. aria* and 14 trees of doubtful affiliation form one group, all the intermediate individuals and one tree determined *a priori* as one of doubtful affiliation form the other one.

3.1.2 Univariate statistics

Fig. 4 shows the frequency distribution of various characteristics in the studied trees. The distributions are usually characterised by a distinct maximum. In some cases ("length of petiole", "depth of lateral incisions", "number of pairs of lateral veins") a second, lower peak apart from the first main peak is marked. Fig. 5 shows the frequency distribution of the values "relative depth of lateral incisions" only of *S. aria* and trees of doubtful affiliation.

Median, standard deviation and range of the parameters are shown in fig. 6. *S. torminalis* and *S. aria* differ significantly from each other in all characteristics. The distributions of the values of "relative depth of lateral incisions" and "relative length of petiole" do not overlap. The medians of *S. latifolia* are in between those of *S. aria* and *S. torminalis*, except for the characteristics "max. width of lamina" and "shape of leaf apex". In most of the cases, the medians of *S. latifolia* are closer to those of *S. aria*.

Medians and variability of the individuals of doubtful affiliation differ in most of the parameters only very slightly from those of *S. aria*. There is, however, a significant difference in "relative depth of lateral incisions" and "relative length of petiole" as well as in "shape of leaf apex". Individuals of doubtful affiliation are more similar to *S. torminalis* only with regard to the characteristic "shape of leaf apex".

3.1.3 Separation of the *a priori* groups by discriminant analysis

Fig. 7 shows the distribution of the trees in the discriminant plane formed by discriminant function 1 and 2. Function 1 clearly separates *S. torminalis* from *S. aria* and trees of doubtful affiliation. The trees determined as *S. latifolia* are all situated between *S. aria* and *S. torminalis*. They are, however, all in all, closer to the scatter plot formed by *S. aria* (and by the individuals of doubtful affiliation) than to the one formed by *S. torminalis*. Function 1 does not separate *S. aria* and the trees of doubtful affiliation. In contrast to function 1, function 2 allows a partial differentiation between the two groups (not void of overlapping).

Within the discriminant function 1 (canonical correlation $r=0.98$) the variables "relative length of petiole" and "relative depth of lateral incisions" are the most important separating factors, followed by "number of pairs of lateral veins", "shape of leaf apex" and "angle of leaf base". Within the function 2 ($r=0.58$) the characteristics "shape of leaf apex" is by far the most important separating factor.

Tab. 4 contains the results of the classification of the trees on the basis of their discriminant values. All *S. torminalis* and all *S. latifolia* are allocated in the same way as *a priori*. One tree allocated to *S. aria* at sampling is assigned to the group of the trees of doubtful affiliation, five individuals of this group are allocated to *S. aria*.

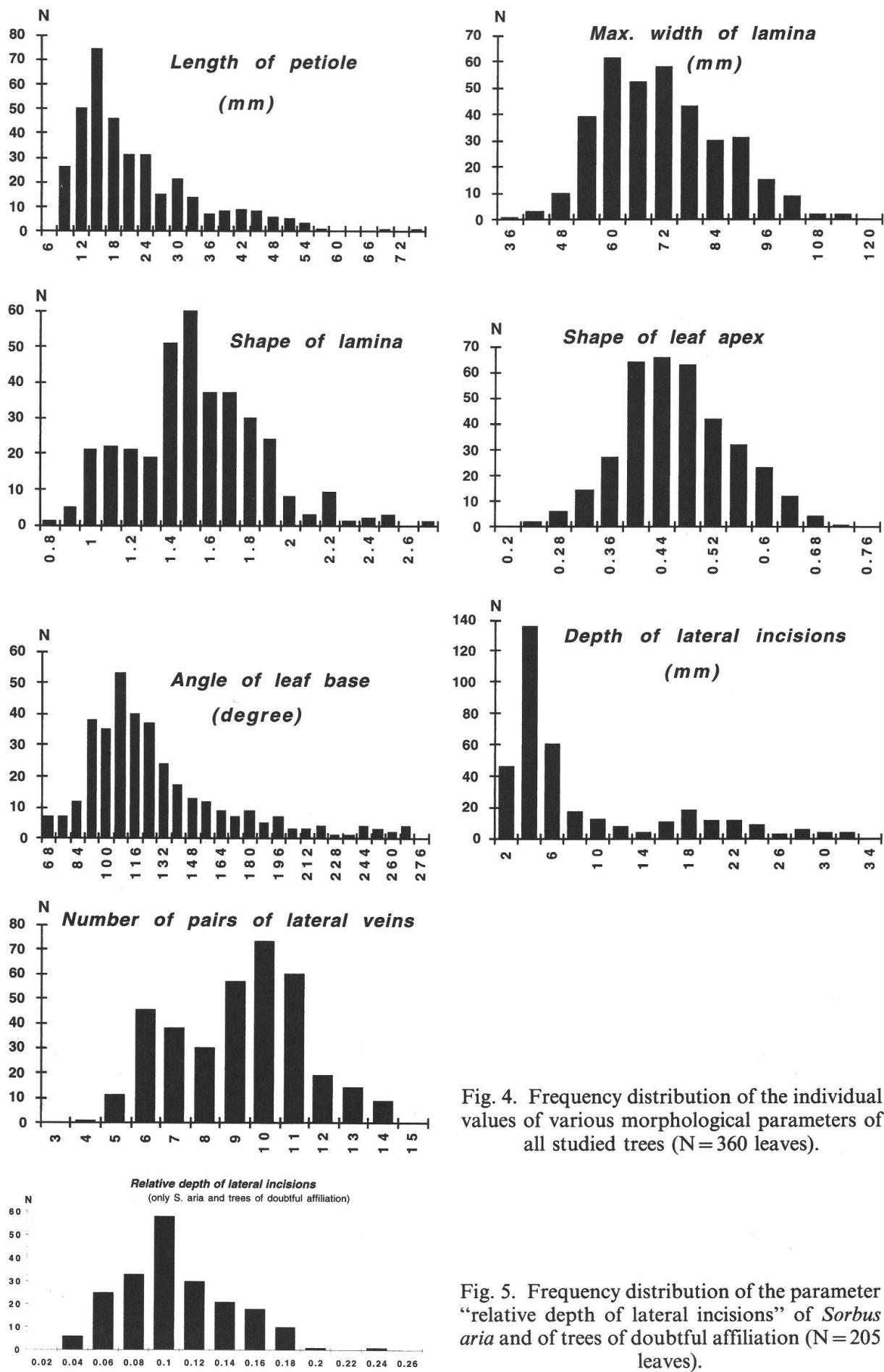


Fig. 4. Frequency distribution of the individual values of various morphological parameters of all studied trees ($N = 360$ leaves).

Fig. 5. Frequency distribution of the parameter “relative depth of lateral incisions” of *Sorbus aria* and of trees of doubtful affiliation ($N = 205$ leaves).

Tab. 4. Morphological investigations: results of the classification of all trees on the basis of discriminant analytical separation of the four *a priori* chosen groups.

	A priori	Discriminant analytical classification				Classification like a priori
		<i>S. torminalis</i>	<i>S. aria</i>	<i>S. latifolia</i>	Doubtful	
<i>Sorbus torminalis</i>	18	18	0	0	0	18 (100%)
<i>Sorbus aria</i>	17	0	16	0	1	16 (94%)
<i>Sorbus latifolia</i>	13	0	0	13	0	13 (100%)
Doubtful	24	0	5	0	19	19 (79%)
Total	72	18	21	13	20	66 (92%)

3.2 Isozyme analysis

3.2.1 Variation of adult trees

Occuring phenotypes:

1. SKDH (fig. 8). *S. aria*, *S. torminalis* and *S. latifolia* only showed one specific banding pattern (phenotype) each. It is remarkable that the zymogram of *S. latifolia* was a combination of the patterns of *S. aria* and *S. torminalis*.
2. PGM (figs. 9a, b). The seven *S. latifolia*-individuals of Reppischtal (fig. 9a) showed five different zymogram phenotypes. The 23 samples from Birnberg (fig. 9b) showed six different patterns.
3. PGI (fig. 10). In PGI on both sites, a total of six different phenotypes could be identified. Pattern II could only be made out in one plant (individual R4, Reppischtal).
4. 6 PGDH (fig. 11). In 6 PGDH, three different phenotypes occurred. Pattern I was proved on both sites, pattern II only in Reppischtal and pattern III only in Birnberg.

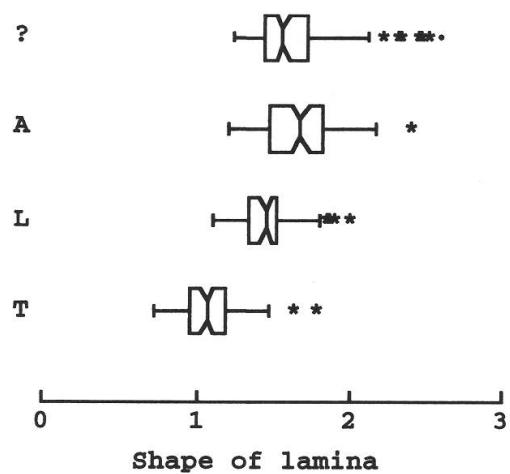
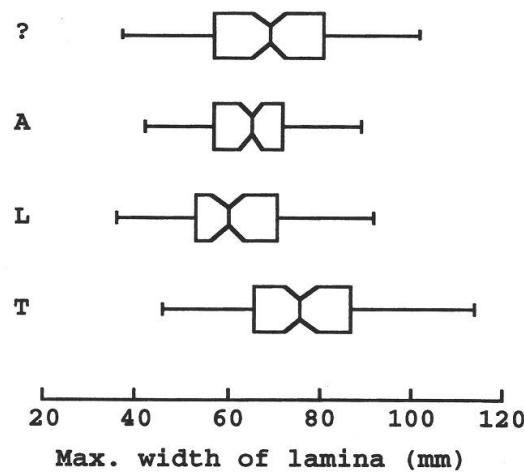
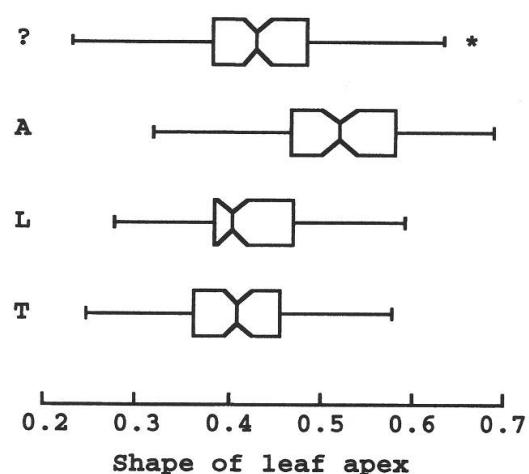
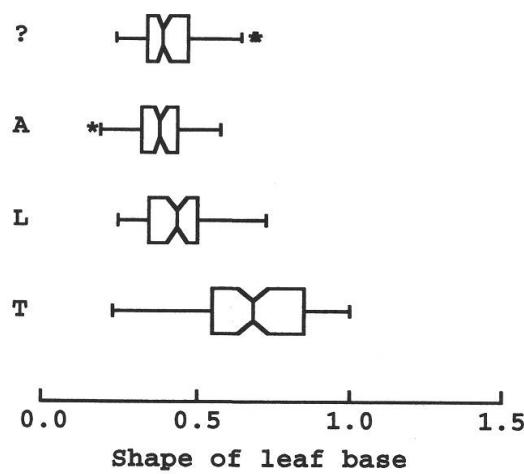
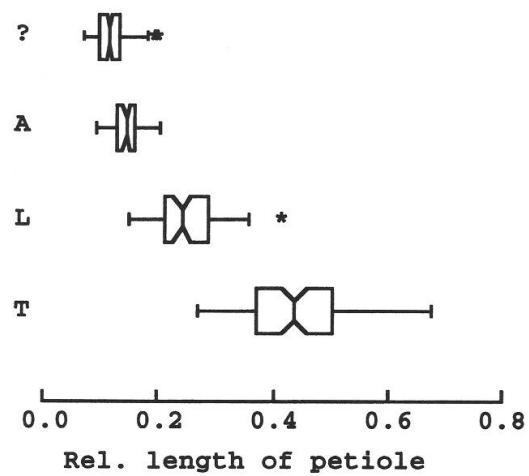
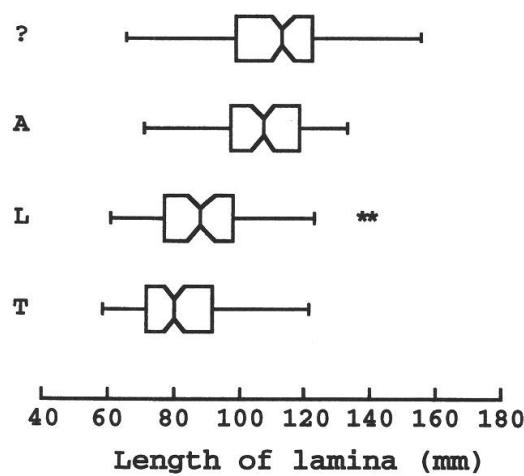
Number of *S. latifolia*-individuals with different isozyme-phenotypes:

1. Reppischtal: Combining the phenotypes of the enzymes PGM (fig. 9a) and PGI (fig. 10) six of the seven *S. latifolia*-individuals turned out to be phenotypically different. Individuals 5 and 6 showed the same patterns in all the tested enzymes.
2. Birnberg: Combining the phenotypes of the enzymes PGM (fig. 9b) and PGI (fig. 10) the 23 *S. latifolia*-individuals represented a total of ten different phenotypes. 13 out of the 23 individuals represented two phenotype-combinations (combination PGM I–PGI I: individuals 4–10, 13; combination PGM I–PGI IV: individuals 1–3, 21, 23).

3.2.2 Variation of the single tree progenies

The combined results of the isozyme analysis of the single tree progenies are presented in tabular form (tab. 5–7). The progenies are assigned as offsprings no. 1–3. The numbering of the phenotypes does not correspond with that of the adult trees.

Offspring no. 1 (tab. 5).— In each of PGM and SKDH, three different phenotypes were found, in each of PGI and IDH two. By combining all four systems, it showed that six of the seven tested individuals were phenotypically different. For the individual 5, no difference to the individuals 1, 2 and 3 could be proved.



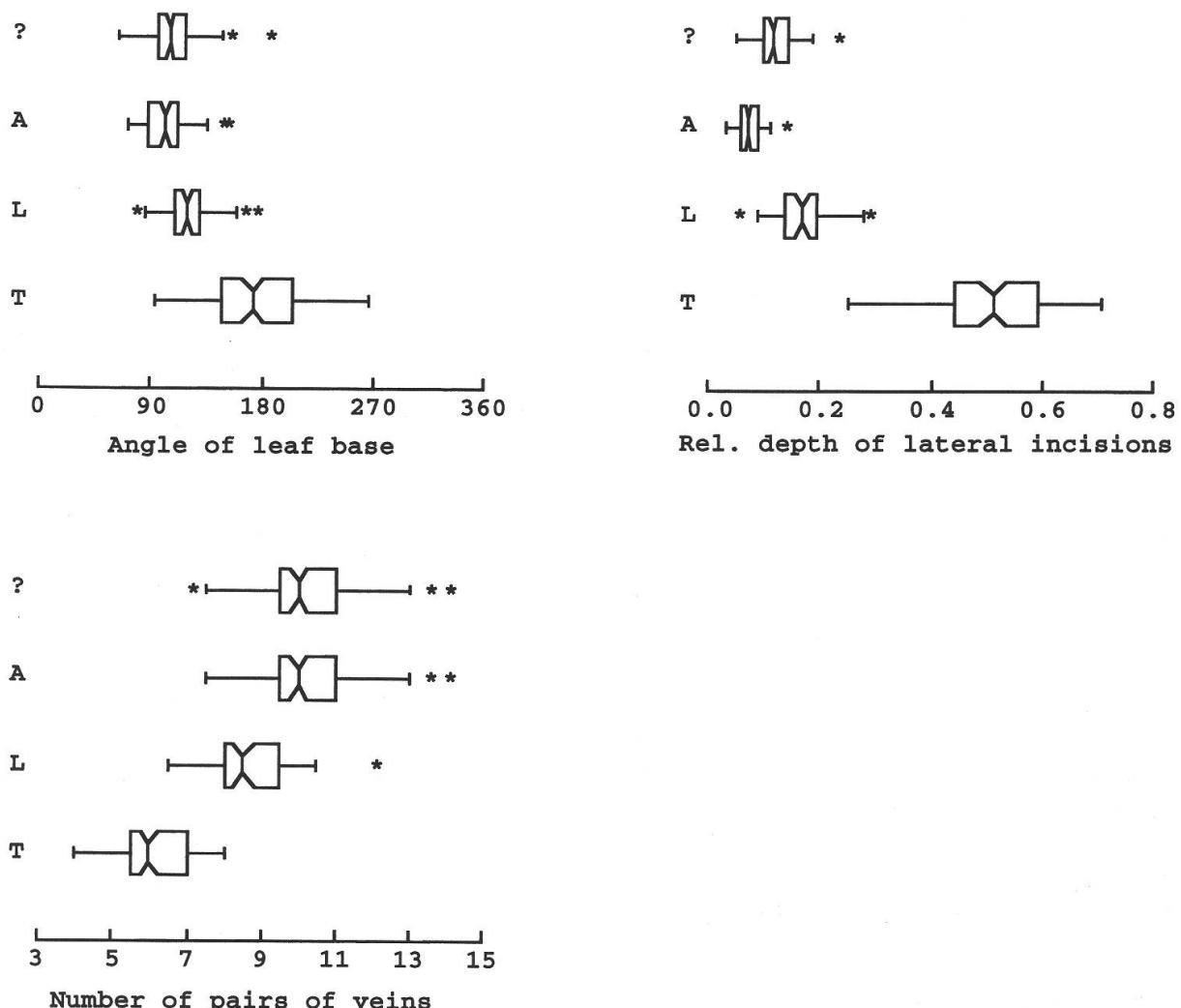


Fig. 6. Box-and-Whisker-Plots of morphological parameters of the four *a priori* chosen groups (A = *Sorbus aria*, T = *S. torminalis*, L = *S. latifolia*, ? = doubtful; N = 360 leaves). The box is the interquartile range (Hspread), the indentation of the box the standard deviation, and the median the centre vertical line in the box. The lines emanating from each box extend to the smallest and largest observation that are less than 1.5 Hspread from the end of the box. Observations outside this range are marked with asterisks.

Tab. 5. Isozyme-phenotypes of the single tree progenies no. 1. For each individual (no. 1–7) and enzyme the respective isozyme-phenotype is indicated by a number ("?" = phenotype not identified).

Individual	no. 1	no. 2	no. 3	no. 4	no. 5	no. 6	no. 7
PGM	1	2	3	3	?	3	3
PGI	1	1	?	2	1	1	1
SKDH	1	1	1	2	1	3	1
IDH	?	1	1	1	1	1	2
Combination	1/1/1/?	2/1/1/1	3/?/1/1	3/2/2/1	?/1/1/1	3/1/3/1	3/1/1/2

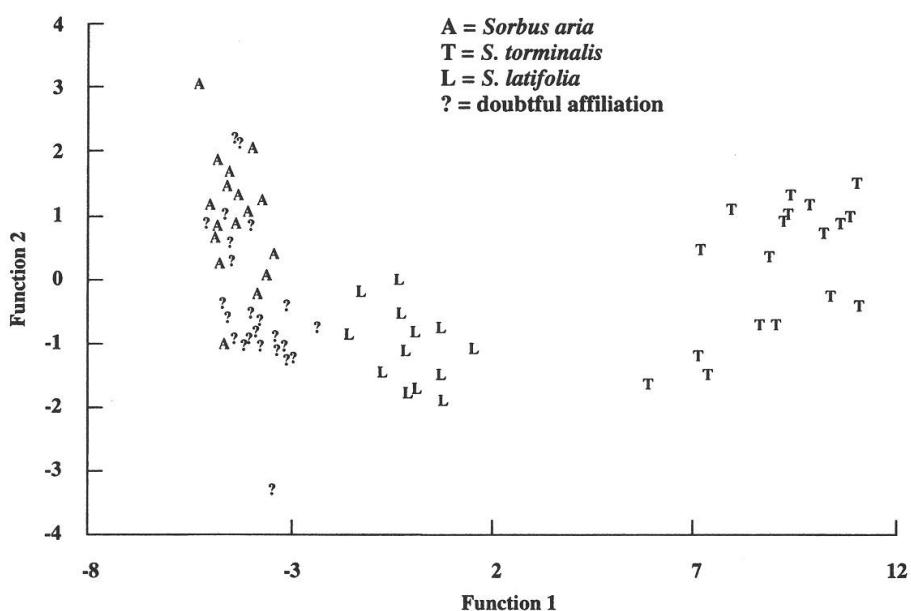


Fig. 7. Discriminant analytical separation of the four *a priori* chosen groups.

Offspring no. 2 (tab. 6). – In each of PGI and SKDH, three different phenotypes were found, and four in PGM. By combining the three systems, it showed that all the six individuals were phenotypically different.

Offspring no. 3 (tab. 7). – In PGM six, in PGI four, and in SKDH three different phenotypes were found. By combining these three systems, it turned out that all the seven individuals were phenotypically different.

Tab. 6. Isozyme-phenotypes of the single tree progenies no. 2. For each individual (no. 1–6) and enzyme the respective isozyme-phenotype is indicated by a number ("?" = phenotype not identified).

Individual	no. 1	no. 2	no. 3	no. 4	no. 5	no. 6
PGM	1	2	3	?	?	4
PGI	1	2	2	3	2	2
SKDH	1	1	2	?	3	2
IDH	1	2	1	?	?	1
Combination	1/1/1/1	2/2/1/2	3/2/2/1	?/3/?/?	?/2/3/?	4/2/2/1

Tab. 7. Isozyme-phenotypes of the single tree progenies no. 3. For each individual (no. 1–7) and enzyme the respective isozyme-phenotype is indicated by a number.

Individual	no. 1	no. 2	no. 3	no. 4	no. 5	no. 6	no. 7
PGM	1	2	3	4	5	6	6
PGI	1	1	1	2	3	4	1
SKDH	1	1	2	2	3	2	2
Combination	1/1/1	2/1/1	3/1/2	4/2/2	5/3/3	6/4/2	6/1/2

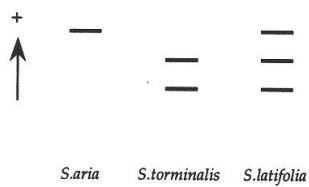


Fig. 8. SKDH: isozyme-phenotypes of *Sorbus aria* (N = 17), *S. torminalis* (N = 15) and *S. latifolia* (N = 30) from Reppischtal and Birnberg (schematic representation).

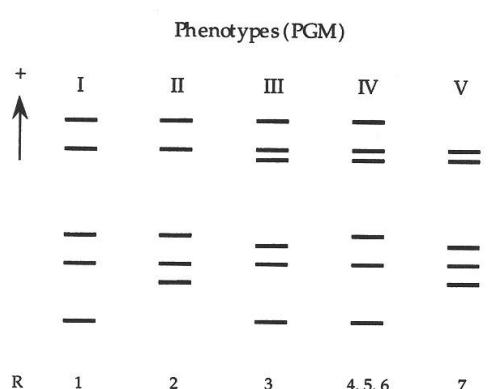


Fig. 9a. PGM: isozyme-phenotypes ($I-V$, schematic) of *Sorbus latifolia* from Reppischtal (individuals R 1-7).

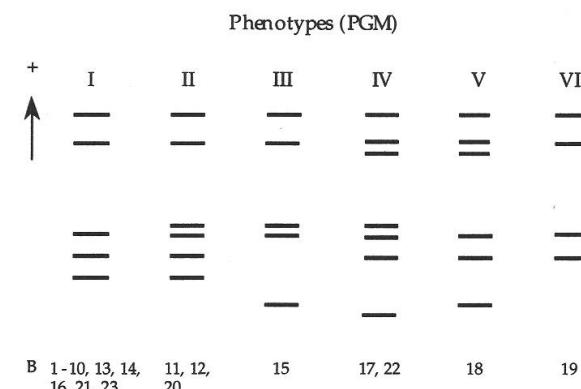


Fig. 9b. PGM: isozyme-phenotypes (I–VI, schematic) of *Sorbus latifolia* from Birnberg (individuals B 1–23).

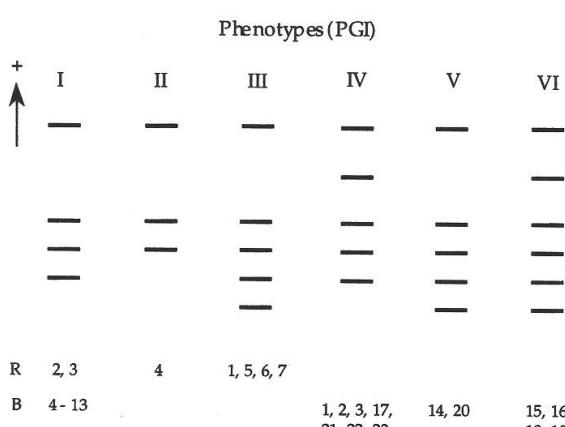


Fig. 10. PGI: isozyme-phenotypes (I–VI, schematic) of *Sorbus latifolia* from Reppischtal (individuals R 1–7) and Birnberg (individuals B 1–23).

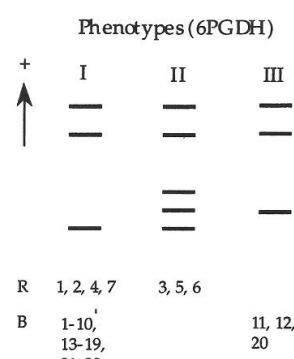


Fig. 11. 6 PGDH: isozyme-phenotypes (I-III, schematic) of *Sorbus latifolia* from Reppischtal (individuals R1-7) and Birnberg (individuals B1-23)

3.3 Viability of seeds

On the whole, about 40% of the seeds germinated. However, the results of the four different parent trees varied significantly (tab. 8).

Tab. 8. Germination results of seeds of morphologically intermediate trees (*Sorbus latifolia*).

Tree	Seeds	Germinated	% Germinated
1	237	172	73
2	200	111	55
3	130	16	12
4	248	25	10
Total	815	324	40

3.4 Chromosome numbers

Out of the 13 seedlings investigated, 12 had a chromosome number of $2n = 2x = 34$, and one had a chromosome number of $2n = 3x = 51$ (fig. 12).

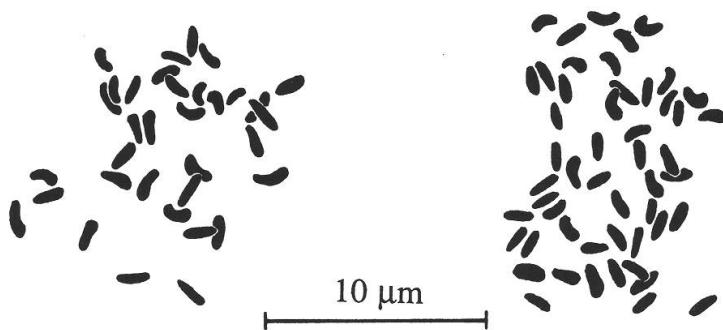


Fig. 12. *Sorbus latifolia*: Somatic metaphase showing different chromosome numbers. Left: seedling with $2n = 2x = 34$; right: seedling with $2n = 3x = 51$.

4. Discussion

Apomixis has been proved in the genus *Sorbus* (Liljefors 1953, 1955) including *S. latifolia* (Jankun and Kovanda 1987, 1988). For many of the hybrid taxa between *S. aria* and *S. torminalis* this form of reproduction is described to be either obligatory or facultative (e.g. Düll 1961, Sell 1989). The initial position of this study was, therefore, the hypothesis that in the Reppischtal valley not only *S. aria* and *S. torminalis* grow, but also hybridogenous forms (*S. latifolia*) and that these are part of an apomictic taxon. To verify this hypothesis, the morphological variation was analysed. The trees studied were divided *a priori* into four groups (morphotypes) and by means of variation statistical methods attempts were made to clarify whether or not it was possible to justify this allocation by the actual variation.

In Reppischtal, *S. aria* and *S. torminalis* are morphologically well differentiated taxa. Significant differences – partially even differences void of overlapping – could be made

out in the length of petiole, the angle of leaf base, the number of pairs of lateral veins and the depth of lateral incisions. On the basis of this discontinuous variation, it is relatively simple to determine intermediate phenotypes. It thus proved to be true univariately as well as multivariately (discriminant analysis) that the trees *a priori* – that is more or less intuitively – allocated to the intermediate group are in fact morphologically between *S. aria* and *S. torminalis*. In all probability, these trees are hybrids, or else their offsprings – a statement that is proved by the isozyme variation of SKDH. *S. aria* and *S. torminalis* showed species-specific zymograms in this enzyme. The seven morphologically intermediate individuals tested, however, showed a combination of these banding patterns.

To further interpret the position of these individuals in comparison with the parental species, it is necessary to determine the affiliation of those individuals allocated *a priori* as “doubtful”. Decisive factor to distinguish these trees from *S. aria* was the shape of the leaf margin, that is the depth of the lateral incisions. As could be expected, the two groups have different mean values in this characteristic, however, they also have continuously varying individual values. Furthermore, the other characteristics, except for “the relative length of petiole” and “the shape of leaf apex” indicate a more or less distinct correspondence between the individuals with doubtful affiliation and *S. aria*. This was also shown in the multivariate analyses in which a separation between *S. aria* and the trees of doubtful affiliation was not – or only partly – possible.

A subdivision into two taxa does not seem to be justified in this case. Obviously, both forms are *S. aria* (in the following called *S. aria* s.l.) which shows a wide and continuous variation in the shape of the leaf margin. This is in accordance with Kovanda (1961), who observed individuals with more or less lobed leaves (f. *incisa* (Reichenbach) Javorka) within *S. aria*. In contrast to this, a great deal of literature treats trees that differ from “typical” *S. aria* only by having leaves with deep incisions or lobes respectively, as being a species in their own right (Düll 1959, Maier 1994b). Hatt (1993) succeeded in obtaining the proof for a discontinuity of the characteristic “leaf margin” in a variation statistical analysis of a *S. aria*-population in the Swiss Jura.

An important result is the observation that intermediate trees vary morphologically. Apart from individuals that are between *S. aria* and *S. torminalis* there are those that are more or less similar to *S. aria* s.l. Possibly, these trees are back-crosses – it is, however, interesting that this obviously occurs only with *S. aria*. *S. torminalis* and intermediate trees are significantly separated. The almost continuous variation between intermediate individuals and *S. aria* s.l. makes it impossible to clearly allocate single individuals. In his investigation on *S. latifolia* in Switzerland, Moor (1967) found a wide morphological variation, however, he observed continuous transitions to both parent species.

As one is not able to clearly identify all hybridogeneous individuals, it is not possible to quantify their exact proportion, respectively to quantify the extent of gene introgression on the basis of the studies carried out here. This is even more so, if one considers the statement that ‘intermediate individuals are hybrids’ is not synonymous to the reversed conclusion, that ‘among the morphologically determined *S. aria* s.l. (resp. *S. torminalis*) there are no hybrids’. Rieseberg and Ellstrand (1993) come to the conclusion that hybrids do not absolutely have to be intermediate morphologically, but can, to a large extent, be like their parents. There are, therefore, limits to morphological studies aiming to prove hybridization.

In spite of this limitation, the morphological variation observed only makes sense, if the intermediate trees are genetically different. The isozyme analyses have proved this: six of the seven morphologically intermediate adult trees tested from Reppischtal repre-

sented different zymogram-phenotypes and, therefore, are different genotypes. Furthermore, the isozyme variation of the three offsprings of hybrid parent trees shows that almost all the seedlings are genetically different. This again is only possible if they originated from sexual reproduction. The observation that the seedlings also vary morphologically is well worth mentioning. Within the individual offsprings, there are plants that are difficult to distinguish from *S. aria*, apart from those easily identifiable as being intermediate. The isozyme analysis further shows that on the site of Birnberg *S. latifolia* is not a group of genetically uniform plants (clone) either, although on this site (compared to Reppischtal) relatively fewer isozyme-phenotypes could be ascertained.

In contrast, Proctor et al. (1989) found that various microspecies of the *S. latifolia* aggregate found in the Southwest of England are to a large extent morphologically and isozymically uniform. They have, therefore, come to the conclusion that these groups reproduce apomictically. The morphological variation observed in Reppischtal as well as the fact that intermediate trees mainly produce diploid offsprings sexually provide reason to believe that there is a complex consisting of hybrids with at least partially sexual reproduction and various forms of back-crosses. The continuous variation of *S. aria* and intermediate forms indicates that back-crossing takes place preferentially towards *S. aria*. The thus generated gene introgression between *S. torminalis* and *S. aria* could be an essential reason for the wide variability of *S. aria*, as shown exemplarily with regard to the shape of the leaf margin. In many cases, it is therefore impossible to clearly distinguish morphologically between *S. aria* s.l. and individuals of hybridogeneous origin. A survey of *S. latifolia* in northern Switzerland (Aas and Rudow, in prep.) confirms this statement. According to this investigation, *S. latifolia* occurs more often than presumed. To allocate individuals to *S. torminalis* is in fact quite easy, however, often the question remains whether certain individuals are to be considered as *S. aria* or as hybridogeneous.

Conclusions

In Reppischtal, *S. latifolia* is a morphologically and genetically varying complex of at least partially fertile and mainly diploid hybrids and back-crosses. This stand in Reppischtal differs from other stands described in many articles which present the hybrids between *S. aria* and *S. torminalis* as being morphologically uniform, apomictic taxa easy to distinguish from each other. Düll (1961) describes seven apogamous "fixed" hybrid species of this aggregate in Bavaria and Thüringen (Germany). Sell (1989) describes several such species occurring in the British Isles.

The proof of morphological and genetic variation as well as of the sexual reproduction of *S. latifolia*, however, does not mean that apomixis does not occur. It is definitely possible that on both investigated sites plants of asexual origin exist, that is plants which reproduce either facultatively or obligatorily asexually and thus form small, apomictic groups within the whole complex. It has also to be taken into consideration that apomictic taxa can show certain genetic and morphological variation (e.g. occasional sexual reproduction: facultative apomixis, Asker and Jerling 1992) which makes proof of apomixis difficult. The occurrence of one polyploid individual among the offsprings of intermediate trees from Reppischtal as well as the relatively low isozyme variation of *S. latifolia* on the site of Birnberg could indicate the existence of such apomictic groups (microspecies). If this is assumed, the descriptions of Kummer (1943) and Seybold (1992) that *S. latifolia* contains many fixed microspecies apart from various hybrids are accurate. To gain further insight into this issue, more research is necessary on *S. latifolia*, its

morphological, genetic and cytological variations with emphasis on its reproduction (embryology).

The question of the taxonomic treatment of the observed intermediate forms remains unanswered. As they do not build a distinct, clearly distinguishable group, they can neither be allocated to one of the many described microspecies of the *S. latifolia* aggregate nor does it seem appropriate to describe them as a species in their own right. In the authors' opinion, the most sensible solution is to describe the recognized individuals as hybridogeneous *S. latifolia* sensu lato.

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