

DNA fingerprinting and biometry can solve some taxonomic problems in apomictic blackberries (*Rubus* subgen. *Rubus*)

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ABSTRACT

The feasibility of using DNA fingerprinting and biometry in apomictic blackberries (*Rubus* subgen. *Rubus*) was investigated on five sets of material, chosen to represent various taxonomic problems: 1. Four similar but according to biometrical results distinct species were compared: the Swedish *R. pseudopallidus* and *R. hartmanii*, and the German *R. fuscus* and *R. pallidus*. Surprisingly, *R. hartmanii* and *R. fuscus* exhibited identical DNA fingerprints, whereas the other taxa in this group could be clearly distinguished; 2. *R. insularis* and *R. septentrionalis*, both from Sweden, were rather similar but still well separated by each of the two methods; 3. Samples of *R. infestus* from Sweden and Germany were almost identical with DNA fingerprinting and did not differ appreciably with biometry; 4. Samples of *R. polyanthemus* from Sweden and Germany were completely identical with DNA fingerprinting and deviated only slightly with biometry; and 5. *R. scheutzii* from Sweden and *R. muenteri* from Germany could not be separated with either method.

KEYWORDS: Taxonomy, microspecies, apomixis, DNA analysis, M13 probe, (AC)/(TG) probe, Rosaceae.

INTRODUCTION

Taxonomists have for a long time struggled with the notorious blackberries, *Rubus* subgen. *Rubus* (Rosaceae). These perennial, bushy plants are characterized by vigorous vegetative reproduction, either through root suckering or through tip rooting. Thus a genetically homogeneous clone, consisting of several ramets, may spread over a substantial area. The taxonomic problems are, however, caused primarily by the system for seed reproduction. Most species in this group are polyploid and pseudogamous, i.e. the egg cell is not fertilized but pollination is still necessary to trigger embryo development. The few existing diploid species instead appear to be sexual (Gustafsson 1930). Crossing experiments between polyploid species have demonstrated that apomixis is not complete since at least a minor proportion of the resulting offspring carry some paternal traits and thus emanate from sexual seed set (Lidforss 1905; Nybom 1988). Moreover, many of these interspecific hybrids in their turn appear to have reverted to sexuality (Lidforss 1905; Nybom 1995).

In Europe, Sudre (1908–1913) recognized over 100 blackberry species, many of which were split into units of lower rank. The fact that his index contains more than 3000 names is, however, also caused by a vast number of synonyms. At the beginning of this century, a considerable number of botanists – often non-professionals – were occupied with *Rubus* taxonomy but activity eventually dwindled. A new boost was delivered by the work of Weber (1972) on the *Rubus*-flora in northern Germany and the Nordic countries, and a comprehensive treatise of the British brambles published

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by Edees & Newton (1988). In a later study, Weber (1985) arranged the *Rubus* taxa into four different taxonomic categories: 1. widespread species with a distribution of more than 500 km in diameter; 2. regional species with a distribution of 20–250 km in diameter; 3. local species with a distribution of less than 20 km in diameter; and 4. individual species consisting of a few vegetatively derived plants. Only the first two categories were thought to merit taxonomic interest.

Phenotypic variation in *Rubus* is to a large extent associated with environmental factors, thus rendering identification of isolated populations very difficult. At the end of the last century, several Swedish *Rubus* species with a restricted distribution were described as new species. Later, many of these were lumped with previously described species, often of British or German origin. In some cases, this led to a rather intense debate among *Rubus* taxonomists.

Rubus taxonomy has always emphasized morphological characters, and the different species have been defined as morphologically uniform groups of plants. Often a major part of the taxonomic work has been based on subjective evaluation of dried herbarium specimens. In other apomictic genera, morphological data have sometimes been subjected to more sophisticated biometrical treatments, e.g. in *Crataegus* (Dickinson & Phipps 1985; Dickinson 1986). Also allozyme electrophoresis has yielded valuable results in apomicts, e.g. in *Antennaria* (Bayer 1989) and *Taraxacum* (Van Oostrum *et al.* 1985). Recently, the very sensitive method of DNA fingerprinting has provided an efficient means to identify genotypes and to separate clones in, for example, *Taraxacum* (Van Heusden *et al.* 1991) and *Rubus* (Nybom & Schaal 1990; Antonius & Nybom 1994). To what extent DNA fingerprinting could be considered a generally useful tool for taxonomy in apomicts is, however, not yet investigated. In this study we therefore attempt to solve some taxonomic problems in *Rubus* subgen. *Rubus* using two different approaches: DNA fingerprinting and biometry.

1. *R. pseudopallidus* (C. E. Gustafsson) Å. Gustafsson is a species endemic to a restricted area on the Swedish east coast. When first described, suggestions were made that this could be a subspecies of *R. hartmanii* Gandoger (Lund 1877), which also occurs on the Swedish east coast and, very rarely, in Great Britain (Edees & Newton 1988). Areschoug (1886), however, treated it as identical to *R. pallidus* Weihe, whereas several other botanists regarded it as *R. fuscus* Weihe & Nees (Hylander 1941; Oredsson 1970; Weber 1972). Both *R. pallidus* and *R. fuscus* are widespread in N.W. Europe but do not otherwise occur in Sweden (Weber 1972). Thus, does *R. pseudopallidus* merit species rank, or should it be synonymized with any one of *R. fuscus*, *R. pallidus* or *R. hartmanii*?

2. *R. septentrionalis* Watson occurs in a very restricted area on the Swedish west coast, in southern Norway, in northern Denmark and in Great Britain (Weber 1984; Edees & Newton 1988). Sometimes this taxon has been considered a subspecies of *R. insularis* F. Aresch. (Areschoug 1886; Weber 1972). This latter species occurs in the southernmost part of Sweden, in Denmark and in the eastern parts of Germany (Pederson & Schou 1989). Therefore, should *R. septentrionalis* and *R. insularis* be treated as separate species?

3. The endemic species *R. taeniarum* Lindeberg has been described from a restricted area on the Swedish west coast (Lindeberg 1858). However, it was soon afterwards synonymized with *R. infestus* Weihe & Nees (Focke 1877), a species which occurs in Great Britain, Germany and Denmark (Weber 1972; Edees & Newton 1988). Should *R. taeniarum* and *R. infestus* be treated as separate species?

4. *R. polyanthemus* Lindeberg was first described from Sweden where it occurs in a very restricted area on the southwestern coast (Lindeberg 1883). Since then, it has also been reported from Ireland, Great Britain, Denmark and Germany (Weber 1985; Pedersen & Schou 1989). Can the Swedish and German populations of *R. polyanthemus* be treated as conspecific?

5. It has been suggested that *R. scheutzii* Lindeberg, endemic to the Swedish east coast, is identical to *R. muenteri* Marsson, a species that only occurs in Germany (Areschoug 1886; Weber 1985). Are *R. scheutzii* and *R. muenteri* separate species?

MATERIALS AND METHODS

Nomenclature is according to Weber (1972), except in two cases: the Swedish *Rubus* population which Weber regarded as *R. fuscus* in 1972, is here called *R. pseudopallidus* (as suggested by Weber,

pers. comm., 1992); and *R. insularis* subsp. *confinis* in Weber (1972) is here called *R. septentrionalis* in accordance with Weber (1984).

DNA FINGERPRINTING

Recently hypervariable regions of genomic DNA have been detected, which can be used to yield genotype-specific 'DNA fingerprints' in various organisms (Jeffreys *et al.* 1985; Nybom 1991, 1993). This so called minisatellite-DNA consists of a number of tandem repeats of a core sequence with c. 10–60 base pairs. Restriction enzymes will usually cut the DNA at either end of a series of tandem repeats. The length of the resulting DNA-fragment is therefore proportional to the number of repeats. Hybridization of sample DNA to a minisatellite DNA probe allows us to analyze a considerable number of such DNA fragments simultaneously.

Fresh *Rubus* leaves were collected in Sweden and Germany (Table 1, Fig. 1). Plants were considered from different localities if collected at least 1 km apart. Voucher specimens are deposited at LD. Determinations of the German plants have been verified by Prof. Dr Dr H. E. Weber.

DNA was extracted from fresh or frozen leaf material and subsequently digested with the restriction enzyme *Hae*III. The resulting DNA fragments were size-separated by electrophoresis in an agarose gel, denatured and transferred to a nylon filter by Southern blotting. These nylon filters were hybridized to a ^{32}P -labelled minisatellite DNA probe derived from the M13 bacteriophage (Vassart *et al.* 1987). Autoradiography was carried out for 2–12 days at -80°C with intensifying screens. Residual probe was stripped off and filters rehybridized, this time to a synthetic (AC)/(TG)

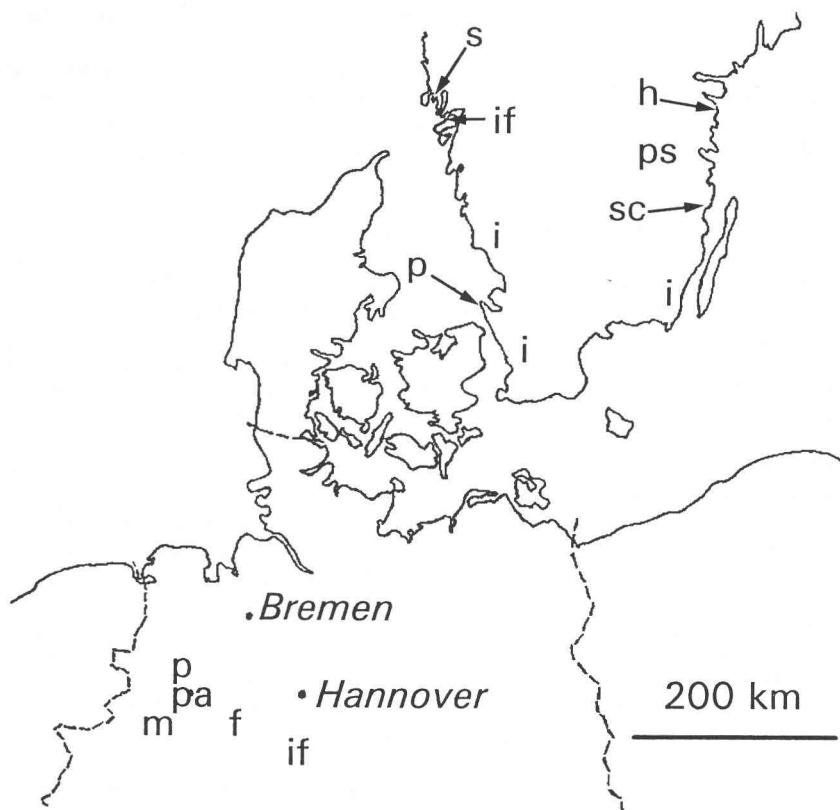


FIGURE 1. Map of southern Sweden, Denmark and northern Germany showing where material of *Rubus* spp. was collected for the DNA fingerprinting. f = *R. fuscus*, h = *R. hartmanii*, i = *R. insularis*, if = *R. infestus*, m = *R. muenteri*, p = *R. polyanthemus*, pa = *R. pallidus*, ps = *R. pseudopallidus*, s = *R. septentrionalis*, sc = *R. schcutzii*. Some letters represent several closely situated localities.

TABLE 1. ORIGINS OF THE *RUBUS* MATERIAL USED FOR DNA FINGERPRINTING AND THE DISTANCE BETWEEN THE TWO MOST DISTANT LOCALITIES

Species	Sweden			Germany		
	Number of localities	Number of shrubs	Maximum distance (km)	Number of localities	Number of shrubs	Maximum distance (km)
<i>R. fuscus</i>	0	0	—	1	1	—
<i>R. hartmanii</i>	7	8	7	0	0	—
<i>R. infestus</i>	4	11	21	1	2	—
<i>R. insularis</i>	5	6	180	0	0	—
<i>R. muensteri</i>	0	0	—	2	3	7
<i>R. pallidus</i>	0	0	—	1	1	—
<i>R. polyanthemus</i>	2	2	2	1	1	—
<i>R. pseudopallidus</i>	4	6	5	0	0	—
<i>R. scheutzii</i>	6	10	20	0	0	—
<i>R. septentrionalis</i>	2	4	7	0	0	—

polydinucleotide (Lönn *et al.* 1992). For a more detailed description of DNA isolation and hybridization with the M13 probe, see Nybom & Schaal (1990). Labelling and hybridization with the (AC)/(TG) probe was performed in the same way as with the M13 probe except that the final washes were in 0.2× SSC instead of 2×.

DNA fragment patterns were scored manually for presence or absence of individual bands. A bandsharing value was calculated for each pairwise comparison between two different plants: $D = 2 \times \text{number of shared fragments} / (\text{number of fragments in plant A} + \text{number of fragments in plant B})$. For a more detailed description of DNA fingerprint evaluation, see Nybom & Rogstad (1990).

BIOMETRY

We have chosen morphological characters for the biometrical investigation according to two criteria: they should be easy to measure on dried material as well as considered generally useful for species identification within *Rubus*. Distribution of prickles on the stem and in the inflorescence is an important character. Some species have prickles of more or less even size, whereas others have a characteristic mixture of both large and small prickles. Thus we noted the frequency of prickles occurring in three different length-classes. Lengths of the petiolules and the shape of the terminal leaflet of the three-, five-, or seven-foliated leaves are often employed to separate species. Number of glands and hairs on the stem and in the inflorescence are also commonly used characters. For the present investigation we ultimately decided on the following measurements: length/width ratio of the terminal leaflet (A/B, Fig. 2); lengths of leaf petiolules (C,D,E, Fig. 2); distance from petiolule base of the middle leaflets (leaf centre) to the point of attachment for the lower leaflet petiolule (F, Fig. 2); length of petiole (G, Fig. 2); number and size distribution of prickles on 5 cm of the stem; number of hairs on 1 cm of the stem; distance from inflorescence apex down to the base of the uppermost inflorescence leaf; number of prickles and glands on the pedicel; number of hairs on the pistils; and number of hairs on the anthers.

Measurements were taken on herbarium specimens in **LD** (Table 2, Fig. 3). The herbarium sheets have been marked with 'TK' and a number from 1 to 212. For each species–country combination we selected 20 specimens that were as complete as possible. We also used plants from our own collections as well as some herbarium specimens in **C** of *R. muensteri* and *R. polyanthemus* from Germany. Still, only five specimens of each of these two species–country combinations were available.

All comparisons were performed between plants of similar stem diameter to correct for variation caused by differences in plant vigour. F-tests were calculated, for each character separately, to determine the level of variation between taxa as compared to within. Multivariate F-tests, including all of the above-mentioned characters, were also performed utilizing the Hotelling-Lawley trace statistic (Morrison 1976). All calculations were performed with SYSTAT for Windows (1992). When necessary, logarithmic transformations were used to adjust for inequality of variances.

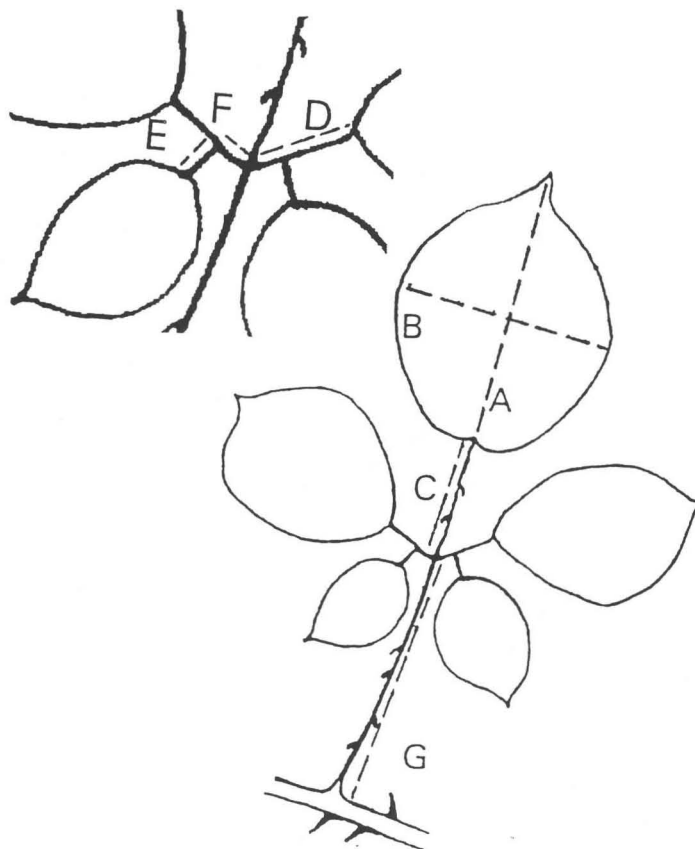


FIGURE 2. Leaf of blackberry (*Rubus* sp.) and enlarged portion of the centre of the leaf with some of the characters used for the biometrical analysis: length/width-ratio of the terminal leaflet (A/B); length of the petiolule of the terminal leaflet (C), middle leaflet (D) and lower leaflet (E); distance from petiolule base of the middle leaflets (leaf centre) to the point of attachment for the lower leaflet petiolules (F), and length of the petiole (G).

TABLE 2. ORIGINS OF THE *RUBUS* MATERIAL USED FOR BIOMETRY AND THE DISTANCE BETWEEN THE TWO MOST DISTANT LOCALITIES

Species	Sweden			Germany		
	Number of localities	Number of shrubs	Maximum distance (km)	Number of localities	Number of shrubs	Maximum distance (km)
<i>R. fuscus</i>	0	0	—	5	17	190
<i>R. hartmanii</i>	10	20	20	0	0	—
<i>R. infestus</i>	13	30	30	5	20	160
<i>R. insularis</i>	12	21	180	0	0	—
<i>R. muenterii</i>	0	0	—	4	5	380
<i>R. pallidus</i>	0	0	—	12	15	150
<i>R. polyanthemus</i>	2	20	5	3	5	300
<i>R. pseudopallidus</i>	5	19	10	0	0	—
<i>R. scheutzii</i>	6	20	25	0	0	—
<i>R. septentrionalis</i>	6	20	5	0	0	—

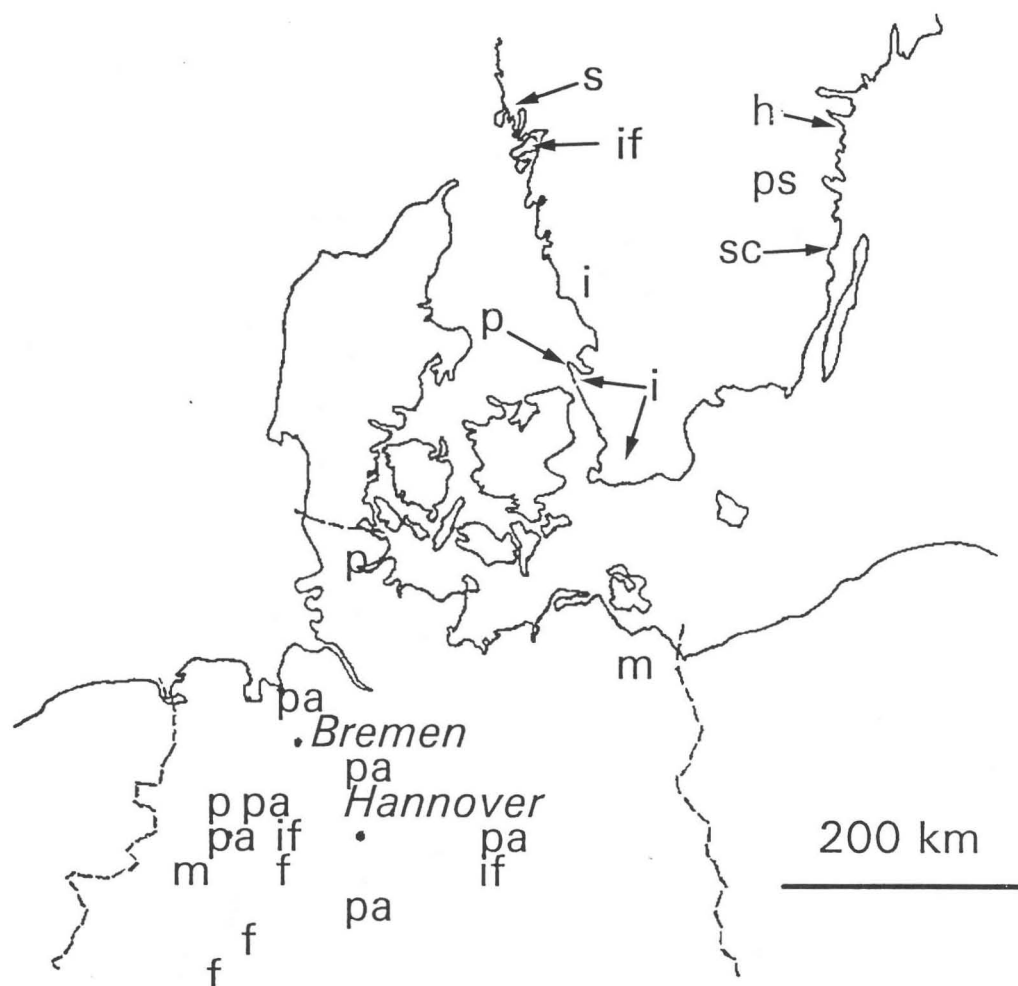


FIGURE 3. Map of southern Sweden, Denmark and northern Germany showing where material of *Rubus* spp. was collected for the biometrical analysis, f = *R. fuscus*, h = *R. hartmanii*, i = *R. insularis*, if = *R. infestus*, m = *R. muenteri*, p = *R. polyanthemus*, pa = *R. pallidus*, ps = *R. pseudopallidus*, s = *R. septentrionalis*, sc = *R. scheutzii*. Some letters represent several closely situated localities.

RESULTS

R. PSEUDOPALLIDUS

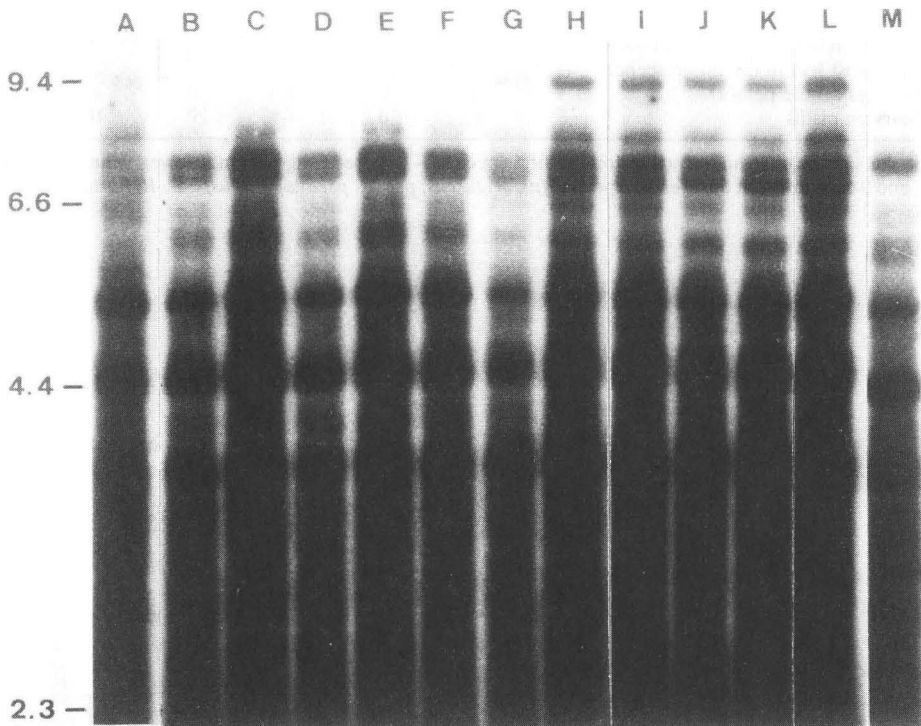
Does *R. pseudopallidus* merit species rank, or should it be synonymized with any one of *R. fuscus*, *R. pallidus* or *R. hartmanii*? Quite surprisingly, the German *R. fuscus* and the Swedish *R. hartmanii* exhibited identical DNA fingerprints with both DNA probes (Table 3, Fig. 4). Barring minor mutations, which do not manifest themselves in DNA fingerprinting, these two species appear to have essentially the same genotype. By contrast, the DNA fingerprint of *R. pallidus* differed considerably from those of *R. pseudopallidus* and *R. fuscus*/*R. hartmanii*. *R. pseudopallidus* was more similar to *R. fuscus*/*R. hartmanii* than to *R. pallidus* (Table 3). Nine fragments could be scored with the M13 probe in *R. fuscus*/*R. hartmanii*, eight in *R. pseudopallidus* and seven in *R. pallidus*. With the (AC)/(TG)-probe 13 fragments could be scored in *R. fuscus*/*R. hartmanii*, 15 in *R. pseudopallidus* and eleven in *R. pallidus*. Intraspecific variation was not encountered.

A multivariate F-test on the biometrical data showed a significant difference between *R.*

TABLE 3. AMOUNT OF BANDSHARING (D-VALUES) AS OBTAINED IN DNA FINGERPRINTING, FOR PAIRWISE COMPARISONS AMONG *RUBUS* SPP.

Results from hybridization with the (AC)/(TG) probe in the upper right half, and from hybridization with the M13 probe in the lower left half.

Species	<i>R. pseudopallidus</i>	<i>R. fuscus</i>	<i>R. hartmanii</i>	<i>R. pallidus</i>
<i>R. pseudopallidus</i>	—	0.79	0.79	0.54
<i>R. fuscus</i>	0.82	—	1.00	0.67
<i>R. hartmanii</i>	0.82	1.00	—	0.67
<i>R. pallidus</i>	0.53	0.62	0.62	—

FIGURE 4. DNA samples from leaves of *Rubus fuscus* (A), *R. pseudopallidus* (B–F), *R. hartmanii* (G–L) and *R. pallidus* (M) digested with *Hae*III and hybridized with the M13 probe. Size markers (kb) were obtained by digestion of λ DNA with *Hind*III.

pseudopallidus and *R. fuscus* ($F=3.25$, $p=0.007$), between *R. pseudopallidus* and *R. hartmanii* ($F=6.98$, $p<0.001$), and between *R. pseudopallidus* and *R. pallidus* ($F=9.46$, $p<0.001$). Single-variate F-tests for the same interspecific comparisons showed significant variation ($p<0.05$) for five, six and seven characters, respectively (Table 4). There was a significant difference with the multivariate F-test also between *R. fuscus* and *R. hartmanii* ($F=7.28$, $p<0.001$), whereas six characters differed significantly with the single-variate F-tests. *R. fuscus* was also well separated from *R. pallidus* with the multivariate F-test ($F=6.93$, $p<0.001$), and yielded significant differences in six characters with the single-variate F-tests. The last pair of species to compare in this group, *R. hartmanii* and *R. pallidus*, differed considerably both with the multivariate F-test ($F=25.77$, $p<0.001$) and with the single-variate F-tests, yielding seven significantly different characters.

TABLE 4. BIOMETRICAL CHARACTERS OF *RUBUS* WHICH SHOWED A SIGNIFICANT VARIATION ($P < 0.05$) WHEN SPECIES WERE COMPARED TO EACH OTHER

Variable	Species					
	<i>fus-pse</i>	<i>har-pse</i>	<i>pal-pse</i>	<i>har-fus</i>	<i>pal-fus</i>	<i>har-pal</i>
Length of the terminal petiolule	-	-	-	+	0	+
Length of the middle petiolule	0	0	0	+	0	0
Length of the lower petiolule	0	0	0	+	-	0
Length of the petiole	-	0	-	+	0	+
Length/width ratio of the terminal leaflet	0	-	+	0	+	-
Placement of the lower leaflets on the petiolules of the middle leaflets	0	+	0	+	0	+
No. of prickles <1.5 mm	+	+	+	0	+	-
No. of prickles 1.5-3 mm	+	+	+	0	+	0
No. of prickles >3 mm	-	0	0	+	-	+
Distance from inflorescence apex to base of the uppermost leaf in the inflorescence	0	+	+	0	0	-
No. of prickles on the pedicel	0	0	0	0	+	0
No. of hairs on pistils	0	0	-	0	0	0

+ denotes a significantly higher value for the first species, - denotes a significantly lower value for the first species, and 0 no significant difference. Species: *fus*, *R. fuscus*; *pse*, *R. pseudopallidus*; *har*, *R. hartmanii*; *pal*, *R. pallidus*.

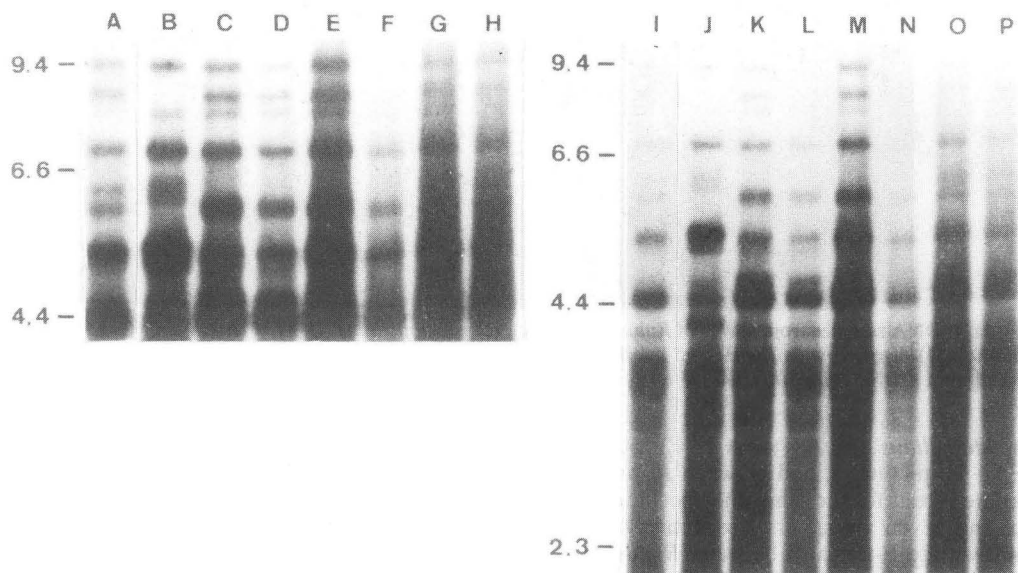


FIGURE 5. DNA samples from leaves of *Rubus insularis* (A, B, G-J, O, P) and *R. septentrionalis* (C-F, K-N) digested with *Hae*III and hybridized with the M13 probe. I-P are identical to A-H except for a different exposure time, A, G-I, O and P represent a genotype, '*R. insularis* II', which differs somewhat from the standard genotype, '*R. insularis* I'. Size markers (kb) were obtained by digestion of λ DNA with *Hind*III.

R. SEPTENTRIONALIS

Should *R. septentrionalis* and *R. insularis* be treated as separate species? Hybridization with the M13 probe demonstrated the existence of two similar but not identical genotypes of *R. insularis* in our material, '*R. insularis* I' and '*R. insularis* II', differing in two bands (Fig. 5). A comparison of these two genotypes resulted in a D-value of 0.89. The (AC)/(TG) probe, on the other hand, yielded no intraspecific variation. '*R. insularis* I' comprises all plants from Scania in southernmost Sweden and from the Swedish east coast, whereas '*R. insularis* II' comprises material from two close localities on the Swedish west coast. Intraspecific DNA variation was not encountered in *R. septentrionalis*. This species was, however, quite different from *R. insularis*, with a D-value of 0.56 with the M13 probe for comparisons to both '*R. insularis* I' and '*R. insularis* II', and 0.73 with the (AC)/(TG) probe. All genotypes investigated had nine fragments that could be scored with the M13 probe. With the (AC)/(TG) probe 17 fragments could be scored in *R. insularis* and 16 in *R. septentrionalis*.

The multivariate F-test showed a significant difference between *R. insularis* and *R. septentrionalis* ($F=9.30$, $p<0.001$). According to the single-variate F-tests, three characters showed significant ($p<0.05$) variation between taxa: *R. insularis* had fewer prickles in two of the length classes, namely <4 mm and 4–6 mm and the lower leaflets were attached further up on the petiolules of the middle leaflets.

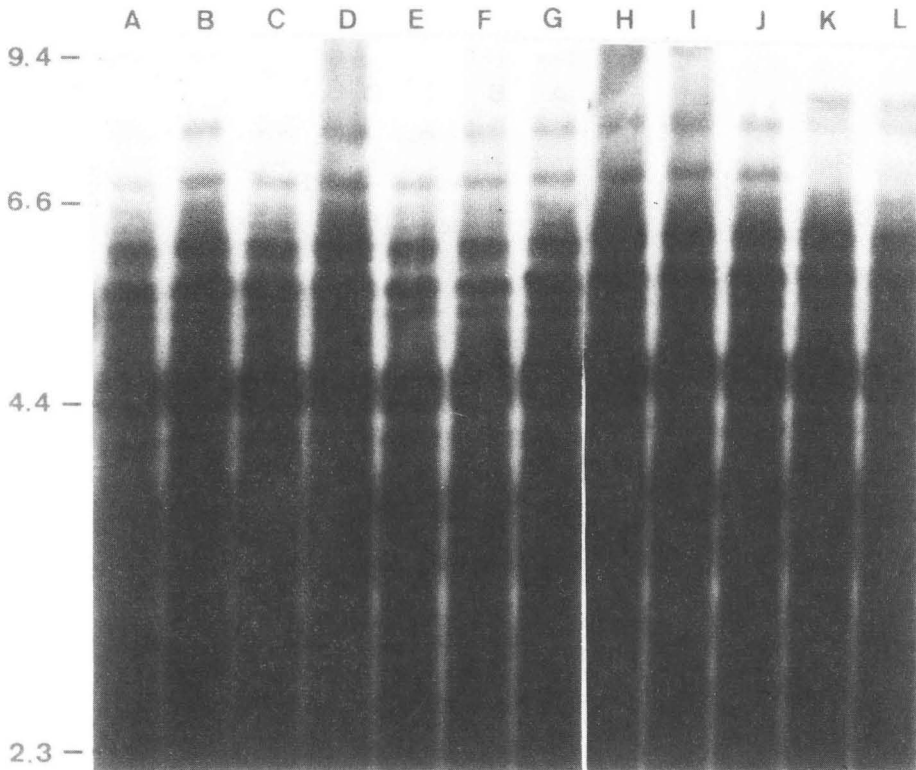


FIGURE 6. DNA samples from leaves of *Rubus infestus* from Sweden (A–J) and Germany (K–L) digested with *Hae*III and hybridized with the M13 probe. Size markers (kb) were obtained by digestion of λ DNA with *Hind*III.

R. INFESTUS

Should *R. taeniarum* and *R. infestus* be treated as separate species? Both DNA fingerprinting probes showed the Swedish collections of *R. taeniarum* to be homogeneous as were also the German collections of *R. infestus* (Fig. 6). Moreover, comparison of (AC)/(TG)-hybridized samples from the two countries yielded identical DNA fingerprints. Somewhat different fragment profiles were, however, obtained after M13 hybridization resulting in a D-value of 0.90, which indicates that genotypes differ somewhat between the two countries. Ten fragments could be scored in both sets of material with the M13 probe and 16 with the (AC)/(TG) probe.

The multivariate F-test showed a barely significant difference between the Swedish and the German material ($F=2.71$, $p=0.038$). Only one character showed significant ($p<0.05$) variation with the single variate F-test: the petiolules of the middle leaflets were longer on the German material.

R. POLYANTHEMUS

Should the Swedish and the German populations of *R. polyanthemus* be treated as conspecific? German and Swedish populations of *R. polyanthemus* had identical DNA-fingerprints with both probes (Fig. 7). Nine fragments could be scored with the M13 probe and 18 with the (AC)/(TG) probe.

By contrast, the biometrical data showed a significant difference between the two sets of material with the multivariate F-test ($F=5.29$, $p=0.005$). According to the single-variate F-tests, three characters showed significant ($p<0.05$) variation between Swedish and German plants: the petiolules of the lower and middle leaflets were significantly longer on the German plants and the lower leaflets were attached further up on the petiolules of the middle leaflets.

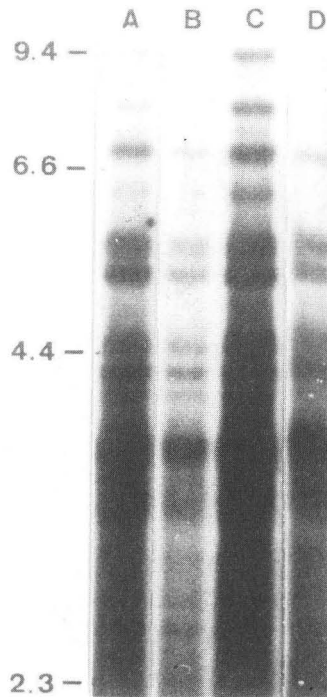


FIGURE 7. DNA samples from leaves of *Rubus polyanthemus* from Germany (A, D) and Sweden (B, C) digested with *Hae*III and hybridized with the M13 probe. Size markers (kb) were obtained by digestion of λ DNA with *Hind*III.

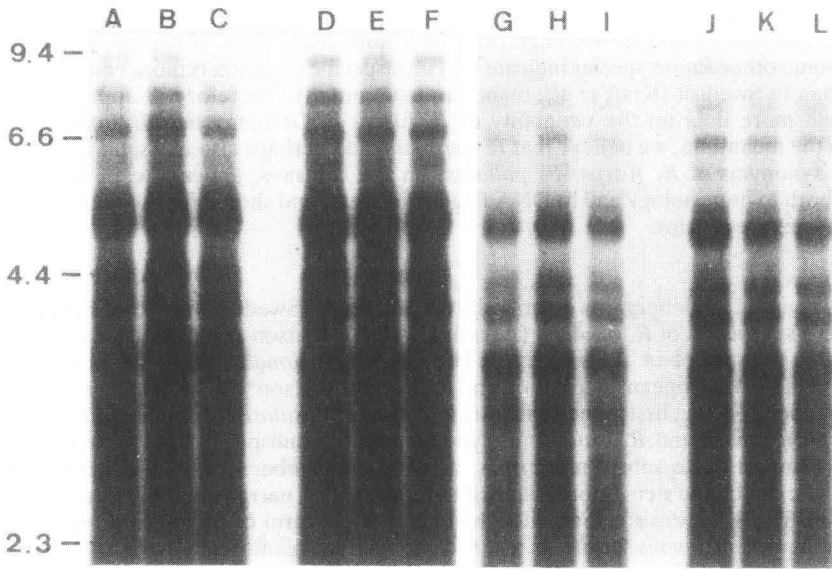


FIGURE 8. DNA samples from leaves of *Rubus scheutzii* (A–C, G–I) and *R. muenterii* (D–F, J–L) digested with *Hae*III and hybridized with the M13 probe. G–L are identical to A–F except for a different exposure time. Size markers (kb) were obtained by digestion of λ DNA with *Hind*III.

R. SCHEUTZII

Should *R. scheutzii* and *R. muenterii* be treated as separate species? *R. muenterii* and *R. scheutzii* had identical DNA fingerprints with both probes (Fig. 8). Nine fragments could be scored with the M13 probe and 15 with the (AC)/(TG) probe.

The biometrical data did not show any significant difference between *R. muenterii* and *R. scheutzii*, neither with multivariate ($F=1.019$, $p=0.481$) nor with single-variate F-tests.

DISCUSSION

R. PSEUDOPALLIDUS

In 1877 Lund described a new blackberry species from the Swedish east coast, *R. mitigatus*. However, he suggested even then that it could perhaps more appropriately be treated as a subspecies of *R. hartmanii*, which occurs about 50 km from the new taxon. According to Lund, *R. mitigatus* deviates from *R. hartmanii* by its thinner leaves that are always green underneath, differently shaped terminal leaflets and inflorescences, and fewer and smaller prickles on the stem. Areschoug (1886) considered the new taxon to be identical to *R. pallidus*, Hylander (1941) regarded it as *R. fuscus*, and in Gustafsson's opinion (1935) it is instead most similar to *R. menkei* Weihe & Nees. *R. pallidus*, *R. fuscus* and *R. menkei* all occur in Germany and/or Denmark. Since the name *R. mitigatus* had already been used for another species, Gustafsson renamed it as *R. menkei* var. *pseudopallidus*. At first Weber (1972) synonymized this taxon with *R. fuscus*, but later changed his mind and now regards it as an endemic species, *R. pseudopallidus* (Weber, pers. comm., 1992).

Surprisingly, *R. hartmanii* has DNA fingerprints identical to *R. fuscus* in spite of a significant biometrical variation between these two species. This discrepancy is probably due to environmentally induced morphological variation, and perhaps also to the occurrence of genetic variation which affects parts of the genome not screened by the DNA fingerprint probes. The inability of DNA fingerprinting to detect somatic mutations has been reported previously in cultivated apple (Nyblom 1990). By contrast, genetic recombination between cross-pollinating genotypes results in easily detected fingerprint variation (Nyblom 1991). Thus *R. hartmanii* and *R. fuscus* probably deviate from each other in somatic mutations only.

Our study shows that *R. pseudopallidus* can be distinguished from *R. fuscus*/*R. hartmanii* as well as from *R. pallidus* by both DNA fingerprint and biometrical data. However, DNA fingerprint studies in some other *Rubus* species indicate that intraspecific variation is more common in German material than in Swedish (Kraft *et al.*, manuscript submitted). Therefore, it appears necessary to obtain much more data on the variability of *R. fuscus* in Germany before finally defining this species. In the meantime, we believe that *R. hartmanii* and perhaps also *R. pseudopallidus* are best treated as synonyms of *R. fuscus*. *R. pallidus*, on the contrary, appears to be more dissimilar according both to morphology and to DNA fingerprint data, and should for the time being retain its status as a separate species.

R. SEPTENTRIONALIS

In 1883, *R. confinis* Lindeberg was described from Bro on the Swedish west coast but in 1886 it was demoted to a subspecies of *R. insularis* (Areschoug 1886). Watson (1946, 1958) claimed that there are two different species in the vicinity of Bro; *R. septentrionalis*, regarded as identical to *R. confinis*, with a west European distribution and *R. broensis* Watson which also occurs sporadically in England. Weber (1972) at first agreed with Areschoug on *R. insularis* subsp. *confinis* and mentioned both *R. septentrionalis* and *R. broensis* as synonyms of this subspecies. Characteristic for subsp. *confinis* in comparison to subsp. *insularis* is, according to Weber, the smaller stature, increased number of prickles on the stem, more rounded terminal leaflet, narrower inflorescence, white petals and green pistils. *R. broensis* is thought to be only a shade form of subsp. *confinis*. Later Weber (1984) gave *R. septentrionalis* species status, and included the plants from Bro in this species, which also occurs in southern Norway, northern Denmark and Great Britain.

Our study clearly suggests that *R. insularis* and *R. septentrionalis* should be treated as two separate species since they are well distinguished both biometrically and with DNA fingerprinting. Interestingly, *R. insularis* is the only taxon to show intraspecific variation within Sweden with DNA fingerprinting. These differences are, however, quite limited and appeared only after hybridization with the M13 probe. The genotype 'R. *insularis* II' was collected from two localities on the Swedish west coast, 13 km apart. These localities are isolated by 160–180 km from the remainder of the Swedish distribution of *R. insularis*.

R. INFESTUS

Lindeberg (1858) described *R. taeniarum* as a new species from central Bohuslän on the Swedish west coast, where it is fairly common in a small area (Oredsson 1974). Both Focke (1877) and Areschoug (1886) instead treated these populations as identical to *R. infestus*, although Lindeberg (1887) never agreed. Thirty years later, Neuman (1915) came to the conclusion that *R. taeniarum* is well separated from *R. infestus*. Gustafsson (1938) also argued that *R. taeniarum* should be regarded as a species. Weber (1972), on the other hand, treated *R. taeniarum* as a synonym of *R. infestus*. Apart from a larger stem diameter in German plants, these were almost identical to the Swedish material in our study, with both biometrical and DNA fingerprint data. It seems likely that the few recorded differences are due only to environmental influences and/or somatic mutations, and therefore we conclude that the Swedish populations should be treated as *R. infestus*.

R. POLYANTHEMUS

R. polyanthemus was described by Lindeberg (1883) from southern Sweden. This species has also been called *R. pulcherrimus* Neuman and *R. neumanii* Focke but these synonyms were due to differing opinions about nomenclature rather than about the taxonomy. The DNA fingerprints were identical for the Swedish and German plants investigated, whereas biometrical data showed some significant differences. We believe that this, still rather restricted, morphological variation can be explained by differences in growing conditions and/or somatic mutations. In either case, the two populations must be very closely related and should be treated as a single species.

R. SCHEUTZII

Lindeberg described *R. scheutzii* as a new species in his exsiccata *Herbarium Ruborum Scandinaviae* in 1885. This species is fairly common in a small area on the Swedish east coast (Oredsson 1974). Areschoug (1886) regarded it as identical to the Central European *R. muensteri*, but Gustafsson (1924) claimed that they are well separated with *R. muensteri* being overall more delicate.

Since we obtained no variation between the two species with biometrical or DNA fingerprint data, there appears to be no reason to separate *R. muenteri* and *R. scheutzii*. This is in agreement with the most recent taxonomic treatments, where the Swedish populations are included under *R. muenteri* (Weber 1985; Pedersen & Schou 1989).

CONCLUSIONS

Blackberry taxonomy is usually based almost exclusively on the subjective evaluation of various morphological characters. Published descriptions have mentioned how species differ or are identical for certain characters without any explanation of the basis for these statements. No serious attempts have been made to investigate the extent to which these characters might be prone to environmentally induced variation. Recently, more objective interpretations of morphological data have proven useful for the separation of species in critical groups like the apomictic *Crataegus* (Dickinson & Phipps 1985). Multivariate statistical methods applied to flowers, fruits and leaves of *Crataegus* sect. *Crus-galli* L. yielded results in close accord with previous classifications. However, comparison of widespread plants, as in the present study, may be greatly confounded by environmental influences on the phenotype. Nevertheless, we chose to work exclusively with dried herbarium specimens in order to determine how valid are these types of data. For future taxonomic work, we strongly recommend that measurements are taken instead on plants grown in a randomized design in an experimental garden to circumvent the problem of phenotypic plasticity. Unfortunately this approach is very time-consuming and expensive.

The recent development of molecular methodology has greatly improved the tools at our disposal. Estimates of genetic variability can nowadays be obtained that are completely independent of where the plants were grown. So far, the most sensitive of these methods in distinguishing different plant genotypes is DNA fingerprinting by hybridization of DNA samples to minisatellite DNA probes (reviews in Nybom 1991, 1993). DNA fragment profiles are individual-specific in cross-pollinated plants, as demonstrated for example in the dioecious North American box elder, *Acer negundo* L. (Nybom & Rogstad 1990). Clones may also be identified and delimited as in North American quaking aspen, *Populus tremuloides* Michx. (Rogstad *et al.* 1991). In population studies of *Rubus*, varying levels of genetic variation have been encountered, which are closely associated with the reproductive system in these species (Nybom & Schaal 1990; Antonius & Nybom 1994).

DNA fingerprinting has also yielded results that appear promising for taxonomic applications in apomictic plant groups. Thus Van Heusden *et al.* (1991) collected plants of several apomictic species of *Taraxacum* from the Netherlands, France and Czechoslovakia. In one species they found the same DNA fingerprint in material from France and Czechoslovakia and only a slight deviation in material from the Netherlands. Another DNA fingerprint was found to be identical in material from three, supposedly different species in the Netherlands. These taxa could be separated on minor morphological characters, possibly due to mutations within a clonal line or by recombination between very closely related genotypes.

In our study, intraspecific DNA variation was extremely low. Complete DNA fingerprint homogeneity in material collected from large parts of the distributional area in Sweden has been encountered also in other blackberry species like *R. nessensis* W. Hall, *R. grabowskii* Weihe ex Günther *et al.* and *R. pedemontanus* Pinkwart (Kraft *et al.* 1995; Kraft *et al.*, manuscript submitted). Apparently, the Swedish populations of each of these species are derived from a single recombinational event. The resulting genotypes have subsequently spread over large areas, presumably by a combination of vegetative reproduction and dispersal of apomictic seed. In some cases, like *R. hartmanii*/*R. fuscus*, morphological variation occurs in spite of identical DNA fingerprints. Most likely, this variation is caused by environmental influences and/or somatic mutations. A series of studies on some closely occurring stands of *R. nessensis* similarly yielded identical DNA fingerprints in spite of substantial differences in plant demography and flowering phenology (Kraft *et al.*, manuscript submitted). We believe that variability in morphological characters caused by somatic mutations is, in most cases, not prominent and consistent enough to merit species rank for the resulting variants. Plant populations that have identical DNA fingerprints should, therefore, in general be regarded as conspecific. On the other hand, different DNA

fingerprints may be encountered within what is commonly accepted as a species. As long as this species is morphologically well defined, we see no practical reason to split it into several taxa.

Biometrical analysis and DNA fingerprinting yielded consistent results in three of our five case studies. In the remaining two cases, taxa with identical DNA fingerprints differed in morphological characters. The additional heterogeneity recorded with biometrical data is probably to a large extent due to environmental influences. Distinct genetic variation is instead more easily detected by DNA fingerprinting, as demonstrated by the two deviating plants of *R. insularis* from the Swedish west coast. However, DNA fingerprinting is relatively expensive and time-consuming and cannot, therefore, be used for large series of material. Ideally, a combination of DNA fingerprinting and biometry should be applied in studies of taxonomic problems in apomictic genera. Such studies would also increase our understanding of the genetic structure and evolution of these intriguing plant groups.

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