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Structure and inheritance of ribosomal DNA variants in cultivated and wild hop, *Humulus lupulus* L.

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Abstract Genetic variation was assessed among cultivated and wild hop, *Humulus lupulus,* by restriction fragment length polymorphisms (RFLPs) of the ribosomal RNA genes (rDNA). Two rDNA length variants of 10.3 and 9.3 kbp represented by three phenotypes designated A, B and C were detected with *Xho*I. Restriction-site mapping showed that hop rDNA is structurally similar to those of most higher plants. A high level of homogeneity existed in rDNA repeat lengths among the diverse hop genotypes. Generally, phenotype A was predominant in wild and cultivated European and Asian genotypes; phenotype B in North American cultivars; while phenotype C was present only in native North American hop, providing a potential molecular marker for the identification of this germ plasm. The rDNA data provided genetic evidence for the separation of native and cultivated American genotypes and supports the hypothesis that North American hop cultivars are of hybrid origin from European and native American genotypes. The segregation of rDNA phenotypes in four F_1 families suggests that a single locus with two co-dominant alleles controls genetic variability for rDNA variants in hop.

Key words Ribosomal DNA · *Humulus lupulus* · Mendelian inheritance

Introduction

Humulus lupulus L. (hop) is a dioecious, climbing perennial plant in the family Cannabaceae. It is cultivated in over 30 countries for its female inflorescences which are spotted with lupulin glands that contain resins and essential oils (Neve 1991). The resins contribute bitterness, while the essential oils provide flavor and aroma to beer and ales (Neve 1991). Hop breeding is complex since male plants are of no breeding value. Generally, female plants are open pollinated and selection for specific agronomic traits are made from within a seedling family, usually the F_1 generation. Hop breeders have generally improved varieties by selection criteria based on the phenotype. Since a plant's phenotype is determined not only by its genetic composition but also by the environment, the phenotype provides an imperfect measure of its genetic potential (Tanksley et al. 1989). DNA polymorphisms are independent of environmental conditions and restriction fragment length polymorphisms (RFLPs) are useful for obtaining genetic information and for generating linkage maps (Beckmann and Soller 1983). Although hop has been in cultivation for over a 1000 years, there is no information on the extent of genetic diversity and variability among wild and cultivated genotypes.

The multi-copy ribosomal RNA gene family is a useful marker for genetic and evolutionary studies in plants (Appels and Dvorak 1982). Ribosomal DNA is a length of DNA encoding the genes for the 18S, 5.8S and 26S ribosomal RNAs (Rogers and Bendich 1987). It is composed of large tandemly repeated arrays of transcribed coding regions separated by intergenic spacer (IGS) regions (Appels and Honeycutt 1986). The coding sequences of the rDNA units are homogeneous within a species whereas the intergenic spacer sequences are generally heterogeneous (Appels and Dvorak 1982). This heterogeneity is due mainly to a variable number of sub-repeated sequences in the IGS which alters the length of the IGS region giving rise to different rDNA length variants within a species (Rogers and Bendich 1987). These length differences can be detected by restriction-endonuclease and Southern-blot analysis. Variation in the rDNA repeats has been useful in addressing questions in population genetics (Schaal et al.

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1987; Capossela et al. 1992) and phylogenetics (Zimmer et al. 1988; Govindaraju et al. 1992), for initiating RFLP maps (Jellen et al. 1994), and for detecting alien chromatin within a species (Appels et al. 1986). Variation in rDNA is also considered a useful genetic marker for breeding applications (May and Appels 1987; Tremousaygue et al. 1988).

The objectives of the present study were to: (1) assess molecular genetic variability in hop using restriction fragment length polymorphisms (RFLPs) of the ribosomal RNA genes in cultivated and wild hop, and (2) examine the genetics and inheritance of hop rDNA variants and establish whether length heterogeneity would be useful as a genetic marker for hop breeding.

Materials and methods

Plant material

The plant material consisted of 118 cultivated and native European, Chinese and North American hop genotypes. A list of the plants used in this study is presented in Table 1. Some of these plants are

Table 1 List of hop genotypes/cultivars used to examine rDNA variation and inheritance in hop. M and F represent male and female genotypes. The locations by state and population number of North American native genotypes are indicated

Genotype	Gender (M/F)	Description cultivar /native	Origin
21015	F	Tettnanger	European
21051	F	Apollon	European/North American [a]
21087	M	*	Native European
21090	M	*	Native European
21110	M	*	European/North American
21116	F	Brewers Gold	European/North American [a]
21117	M	Native	American (WI)
21132	M	*	European/North American
21184	M	*	North American[b]
21234	M	*	European[d]
21235	M	*	European[d]
21237	M	*	European[d]
21549	M	Native	American (NE 1)
21552	F	Native	American (MO 4)
21558	M	Native	American (MO 4)
21559	F	Native	American (MO 5)
21560	M	Native	American (MO 6)
21561	M	Native	American (IA 1)
21565	F	Native	American (IA 5)
21567	F	Native	American (IA 7)
21568	F	Native	American (ND 1)
21572	M	Native	American (ND 5)
21574	M	Native	American (ND 7)
21575	M	Native	American (ND 7)
21576	F	Native	American (MT 7)
21583	F	Native	American (MT 10)
21587	M	Native	American (MT 13)
21596	F	Native	American (UT 11)
21601	M	Native	American (MT 23)
21605	F	Native	American (WI)
21608	F	Native	American (ND 9)
58016	F	*	Unknown (UT 526-5)
60013	M	Native	American (AZ 1-2)
60015	F	Native	American (AZ 1-4)
60020	F	Native	American (NM 2-4)
60024	F	Native	American (CO 1-2)
60028	M	*	North American (CO)
60028	M	Native	American (CO 2-3)
60035	F	Native	American (CO 7-2)
60038	F	Native	American(WY 3-1)
62013	F	Comet	European/North American [a]
65102	F	L1 Cluster	European/North American [a]
66055	F	First Choice	North American [b]
8254-146	F	*	European[c]
8254-181	F	*	European[c]
8658-039	M	*	European/North American [a]
8659-023	M	*	European
8659-045	M	*	European
8685-014	M	*	European/North American[a]
8693-043	M	*	European/North American [a]
8901-02	F	Native	American (Souris, Canada)

Table 1 (Continued)

Genotype	Gender (M/F)	Description cultivar /native	Origin
8906-01	F	Native	American (Minot, ND)
8906-02	F	Native	American (Minot, ND)
8917-13	M	Native	American (Burlington, ND)
8917-05	F	Native	American (Burlington, ND)
8921-01	M	Native	American (Burlington, ND)
8921-02	F	Native	American (Burlington, ND)
8923-05	M	Native	American (Mohall, ND)
8923-09	F	Native	American (Mohall, ND)
8931-01	F	Native	American (Northgate, ND)
8936-17	M	Native	American (Northgate, ND)
8936-22	F	Native	American (Northgate, ND)
8939-02	M	Native	American (Glen Ewing, Canada)
8940-01	F	Native	American (Oxbox, Canada)
8940-07	M	Native	American (Oxbox, Canada)
8943-08	F	Native	American (Oxbox, Canada)
8944-01	M	Native	American (Oxbox, Canada)
8944-02	F	Native	American (Oxbox, Canada)
8946-01	M	Native	American (Midale, Canada)
8946-04	F	Native	American (Midale, Canada)
8949-02	F	Native	American (Indian Head, Canada)
8949-05	M	Native	American (Indian Head, Canada)
8950-01	F	Native	American (Indian Head, Canada)
8951-04	M	Native	American (Indian Head, Canada)
8951-05	F	Native	American (Indian Head, Canada)
Chinese samples[e]			

[a] Genotypes of mixed origin (European and North American)
[b] Genotypes of North American and Unknown origin
[c] Genotypes of European plus unknown origin
[d] Genotypes of possible European origin
[e] These include 38 native hop from western China. The plants were too young to determine the gender
* Breeding stocks maintained at the Irrigated Agricultural Research and Extension Center, Prosser, Washington, USA

maintained at the Irrigated Agriculture Research and Extension Center (IAREC), Prosser, Washington, USA. The native North-American hop samples were obtained from Dr. A. Haunold, Oregon State Uni- versity, Corvallis, Oregon. The wild Chinese hop samples were grown from seed collected from western China (Xinjiang) by Dr. R. Klein (IAREC). Leaf samples from each plant surveyed were harvested, frozen in liquid nitrogen and stored in a −70 °C freezer until used.

DNA isolation, restriction enzyme digestion and hybridization

Total genomic DNA was isolated from 3–5 g of frozen leaf tissue using the CTAB procedure of Saghai-Maroof et al. (1984) with minor modifications. The DNA preparations were treated with RNAse A, followed by a single phenol-chloroform extraction and final ethanol-precipitation. Initially, 2–5 µg of DNA from each genotype was completely digested with the restriction endonucleases *Bam*HI, *Ban*I, *Bst*EII, *Dra*I, *Eco*RI, *Hin*dIII, *Hin*fI *Nde*I, *Nsi*I, *Sac*I, *Xba*I, and *Xho*I according to the suppliers instructions (New England Biolabs, New Beverly, Mass., USA). Agarose-gel electrophoresis, Southern transfer to nylon memberanes, molecular hybridization and autoradiographic procedures were carried out as described previously (Pillay and Kenny 1994). Two cloned DNA fragments (pRY12 and pRY18) that make up the entire rDNA repeat from rice were used as hybridization probes (Sano 1989). In addition, rDNA clones from soybean (E. A. Zimmer, Smithsonian Institution) were also used as probes. The number and relative position of the cleavage sites for the enzymes *Eco*RI, *Dra*I, *Sac*I, *Xba*I and *Xho*I were determined by a combination of single and double digestions of the various enzymes.

The sizes of hybridization bands were estimated using a computer program (J. L. Johnson, Virginia Polytechnic Institute and State University, Blacksburg, Va.) based on comparisons to molecular-weight standards including phage lambda DNA digested with *Hin*dIII and a 1-kb ladder marker (Life Technologies, Gaithersburg, Md., USA).

Inheritance of rDNA repeats

The enzyme *Xho*I was selected for examining the inheritance of rDNA variants in hop because it produced differences in banding patterns among some of the parents. Our preliminary study of rDNA variation in hop indicated that there were two rDNA length variants represented by three phenotypes (see Fig. 1a, b). These phenotypes were designated A (lane 1), B (lane 2) and C (lane 3). Prior to this investigation, crosses were made between selected cultivated genotypes in a program breeding for aphid resistance and other agronomic properties in hop. At this stage, no crosses were available between native North American hop (type C) and plants representing phenotypes A and B. For Mendelian analysis of inheritance patterns of rDNA variants, crosses representing the following phenotypic combinations of rDNA repeats were chosen: (1) A × A, (2) A × B, (3) B × A, and (4) B × B. The DNA profiles from F_1 progeny of the four crosses were scored for rDNA phenotypes following digestion with *Xho*I. Table 2 contains information on the parents used in the crosses, their rDNA phenotypes, the number of progeny examined in each cross, and the number and types of rDNA phenotypes detected among the progeny.

Results

Restriction digests

When hop rDNA was digested with *Xba*I, a single band was observed in all the plants, suggesting that there was a single cleavage site for *Xba*I. The *Xba*I digests were, therefore, useful in determining the repeat size of the rDNA units. Cleavage with *Eco*RI produced a constant 3.9-kbp band and either one or two other bands that

differed in length by approximately 1 kbp. Digestion with *Dra*I produced three bands of 2.1, 2.7 and 5.5 kbp. The 2.1-kbp fragment hybridized only to the pRY18 clone. Similarly, the *Sac*I digestion yielded three fragments of 1.6, 2.2 and 5.6 kbp, with the 2.2-kbp band hybridizing only to pRY18. Fragments that hybridized only to pRY18 indicated that restriction sites producing these fragments were located within the coding region. After digestion with *Xho*I and hybridization to both probes, three types of hybridization patterns were detected (Fig. 1). The first pattern (A) consisted of fragments of 10.3, 7.6 and 2.7 kbp. The second pattern (B) was composed of five fragments of 10.3, 9.3, 7.6, 6.6 and 2.7 kbp. The third type (C) included fragments of 9.3, 6.6 and 2.7 kbp. Patterns A and B were present in cultivated and native hop from Europe, China and North America, while pattern C was detected only in native North American hop. The positions of the cleavage sites for *Xba*I, *Eco*RI, *Dra*I, *Sac*I and *Xho*I in hop rDNA are shown in Fig 2. The enzymes *Hin*dIII and *Bst*EII did not cleave hop rDNA. The restriction patterns obtained with *Bam*HI, *Ban*I, and *Hin*fI were complex and require further work before being mapped. Similarly, further research is necessary to locate the sites for *Nde*I and *Nsi*I since these enzymes produced a single fragment of approximately 6.6 kbp in all the plants. A smaller fragment was not observed in any of the plants even after repeated digestions and hybridizations to the rice and soybean rDNA probes.

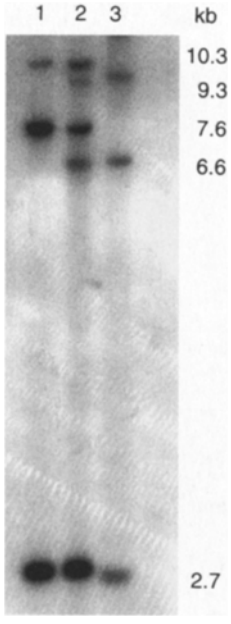

Fig. 1 Southern blot of *Xho*I-digested hop genomic DNA hybridized to pRY12 and pRH18 showing the three rDNA phenotypes: (*lane 1*-phenotype A, *lane 2*-phentoype B, and *lane 3*-phenotype C). The sizes of the rDNA fragments are shown in kbp

Segregation patterns of rDNA length variants

The 26 progeny examined in the cross 8254-181 × 8658-039M (A × A) had only parental (type A) rDNA repeat (Table 2). The rDNA bands in the progeny resulting from the crosses 58016 × 21234M (A × B) and 66055 × 21087M (B × A) segregated in a 1:1 ratio for both A- and B-type repeats (Table 2). In the cross 65102 × 21235M (B × B) both the parents possessed type-B repeats (Fig. 3). Of the 62 progenies examined, 16

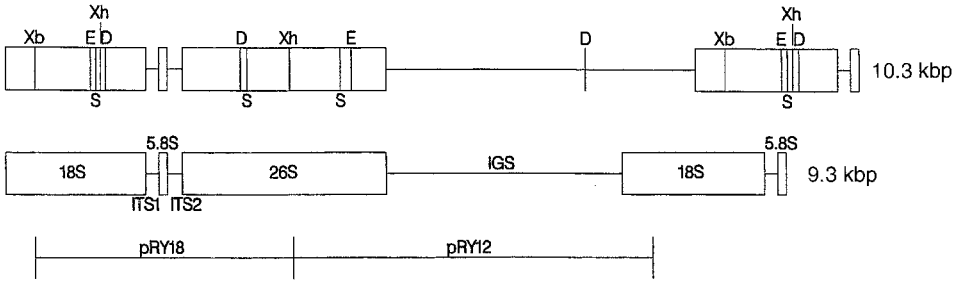

Fig. 2 Ribosomal DNA restriction-site maps of hop showing the location of *Xb* (*Xba*I), *E* (*Eco*RI), *Xh* (*Xho*I), *S* (*Sac*I) and *D* (*Dra*I) sites. The two maps differ only in the length of the IGS. The location of the two rDNA probes pRY12 and pRY18 is also shown

Table 2 Results of segregation analysis of rDNA phenotypes in hop showing identity of parents and their rDNA phenotypes, the number of F_1 progeny examined, the rDNA phenotypes detected among the progeny, expected segregation ratios and χ^2values. F and M denotes female and male. A, B, and C represent the three rDNA phenotypes in hop

Cross (rDNA phenotypes)		Number of F_1 progeny evaluated	rDNA phentypes of individual within each progeny			Expected segregation ratio	χ^2 value
F	M		A	B	C	A : B : C	
8954-181 × 8658-039 (A × A)		26	26	0	0	1 : 0	
58016 × 21234 (A × B)		26	12	14	0	1 : 1	0.15
66055 × 21087 (B × A)		26	12	14	0	1 : 1	0.15
65102 × 21235 (B × B)		62	16	38	8	1 : 2 : 1	5.22

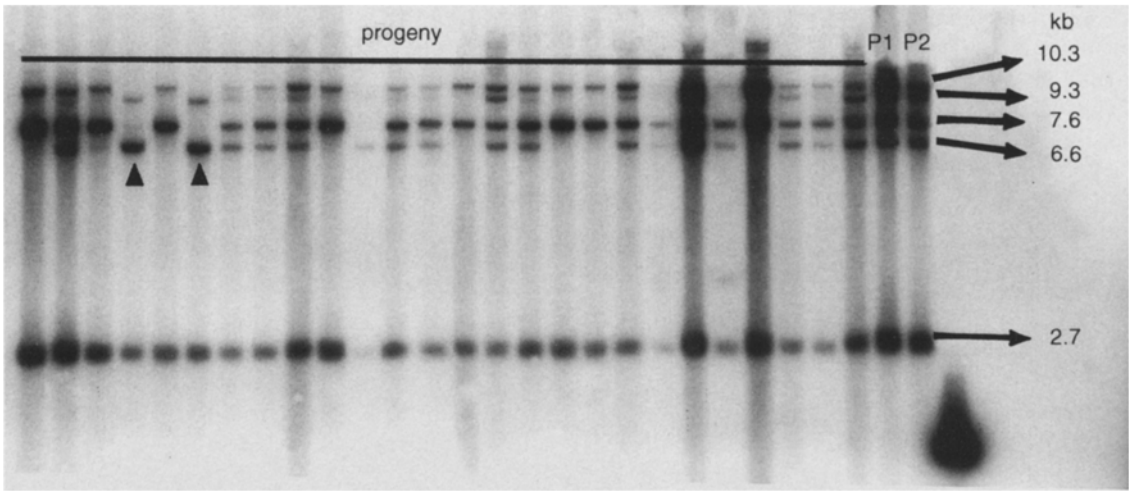

Fig. 3 Autoradiograph of *Xho*I patterns of rDNA in a type B × B cross. Segregation of rDNA repeat sizes is visible. The *arrowheads* shows type-C repeats. *P1* and *P2* represent the rDNA patterns of the parents

had the A phenotype, 38 had a B phenotype, and 8 progeny displayed a C phenotype (Table 2).

Discussion

Ribosomal DNA Structure in hop

Physical mapping showed that there are two rDNA repeat length variants in hop (Fig. 2). These variants, of 10.3 and 9.3 kbp, are within the size range of those observed for most flowering plants as described by Rogers and Bendich (1987). Since the length of the coding region is highly conserved in most plants, variation in the length of the rDNA repeat was attributable to variability in the IGS. It has been observed in both plants and animals that length differences in rDNA repeats is often due to the repetition of subrepeated elements of 100 to 350 bp in the IGS (Apples and Honeycutt 1986). This may be true also in hop, although its subrepeat structure has not been determined. Except for length differences, no restriction-site polymorphisms were detected in the rDNA of the hop genotypes. The restriction-enzyme map of hop rDNA (Fig. 2) is similar to those described for many other plant species. For example, the *Xba*I site at the 5′ end of the 18S gene, and the *Eco*RI, *Xho*I and *Dra*I sites in the 26S region have also been observed in most plant species investigated. The additional *Eco*RI site in the coding region of hop has been shown to be present in species of *Solanum* (Borisjuk et al. 1994), in *Theobroma cacao* (Laurent et al. 1993), in *Hevea brasiliensis* (Besse et al. 1993), and species of *Brassica* (Delseny et al. 1990). The locations of the three *Sac*I sites in the coding region of hop are similar to those observed in species of *Lisianthius* (Sytsma and Schaal 1990), *Brassica* (Delseny et al. 1990), *Helianthus* (Rieseberg 1991), and *Kriga* (Kim and Mabry 1990). These results are congruent with other studies in indicating the conservative nature of the rDNA gene family.

The results of the present study showed a high level of homogeneity among the ribosomal RNA genes within the diverse hop genotypes. The degree of intraspecific rDNA variation in plants has been found to be stochastic, with some species showing high levels of polymorphism and others with little or no variation. For example, no detectable rDNA variation was evident in some species of *Vicia*(Lamppa et al. 1984) and *Glycine* (Doyle and Beachy 1985), and in *Lupinus luteus* (Rafalski et al. 1983). By contrast, exensive rDNA length polymorphism has been reported at the intraspecific level for wheat (Appels and Dvorak 1982; May and Appels 1987), barley (Allard et al. 1990), species of *Aegilops* (Kim et al. 1992), *Vicia faba* (Yakura et al. 1984), *Brassica* (Delseny et al. 1990), and *Theobroma cacao* (Laurent et al. 1993). In the genus *Avena*, geographically diverse *A. sativa* cultivars displayed little or no rDNA variation while extensive polymorphisms were demonstrated in *A. byzantina* and the wild species *A. sterilis* and *A. fatua* (Jellen et al. 1994). The reason(s) for the extensive polymorphisms in rDNA in some species and its absence in others is unknown. A number of hypotheses have been suggested: (1) concerted evolution which results in the homogenization of rDNA repeats being gradual in some species and rapid in others (Sytsma and Schaal 1990); (2) natural selection and /or artificial selection, which is considered to have shaped rDNA length variants in cereal species and their wild ancestors (Saghai-Maroof et al. 1984; Cluster et al. 1984; Flavelll et al. 1986; Allard et al. 1990; Rocheford et al. 1990; Rocheford 1994); and (3) random events, such as genetic drift, small population size and geographical isolation, which have also been implicated in determining rDNA variation (Sytsma and Schaal 1990; Jellen et al. 1994).

In *A. sativa* it was proposed that the rDNA locus is closely linked to another gene that controls an important adaptive or agronomic character and which has been under selection (Jellen et al. 1994). This was

believed to explain the lack of variability in the rDNA patterns of *A. sativa*. A similar reason may account for the homogeneity of rDNA patterns in hop. We have described elsewhere (Pillay and Kenny 1996) that, since hop is clonally propagated, a superior cultivar from one country has often assumed a different name when introduced to other countries. This implies that there is a limited number of traditional cultivars in hop, although many cultivar names are in existence. Our unpublished data from a more widespread RFLP study in hop using genomic DNA clones also showed a high degree of similarity in DNA banding patterns in a diverse array of cultivars suggesting that the hop genome is, in general, highly homogeneous.

One of the interesting observations from the present study was the presence of phenotype C in all the native North American genotypes examined so providing a molecular marker for this germ plasm. Since it is not possible to definitely distinguish native and cultivated hop on the basis of external morphology, native North American hop could now be identified on the basis of its rDNA pattern at a very early growth stage. This information would be a value to breeders, growers and researchers interested in pursuing the evolution of hop. Although hop is capable of producing fertile seed the plant is, in general, clonally progagated. Breeders interested in introgressing genes from native hop into cultivated forms will now be able to genetically characterize their source material, especially if one receives clonal material for propagation. Wild hop has already proved to be a source of valuable characters such as high alpha-acid content, resistance to *Verticillium albo-atrum* and downy mildew (Neve 1991).

Small (1978, 1980) divided wild North American hops into three varieties primarily on the basis of leaf characteristics. Two of these varieties, *H. lupulus* var. *neomexicanus* and *H. lupulus* var. *lupuloides*, were shown to be more closely related to North American cultivars than the third, *H. lupulus* var. *pubescens*, which appeared very distant. Although the rDNA data do not support splitting of wild North American hop into varieties, they do provide a genetic basis for the separation of wild and cultivated hop.

With a few exceptions, rDNA patterns were useful in distinguishing hops of Eurasian and North American origin. The Chinese hop genotypes, native European genotypes (21087, 21090), genotypes of definite European origin (21015, 21987, 21090M, 8659-023M, 8659-045M), and others that could only be of European origin (8254-146, 8254-181), were all characterized by phenotype A. In contrast, North American genotypes had both the type-A and the type-B repeat. Similarly, genotypes of likely mixed North American and European origin had both type-A and -B repeats. Hop is considered to have originated in central Asia and migrated east to America and west to Europe (Neve 1991). The presence of phenotype A in all the native hop samples from China provides information on the evolution and geographic radiation of the species. The preponderance of phenotype A in the European genotypes suggests that these plants have retained the ancestral rDNA pattern. It is believed that present-day North American hop cultivars developed as a result of hybridization between introduced European cultivars and wild North American plants (Neve 1991). The presence of both type-A and -B repeats in the North American cultivars is in agreement with this proposal. Cultivated hop was introduced into North America from Europe in 1629 (Neve 1991). If native North American hops were direct descendants from Chinese ancestors, then rDNA data suggest that the migration has resulted in two distinct hop populations. This conforms with the observation that the Y chromosome in European hops is different from that in native America plants (Neve 1991).

Inheritance of rDNA variants in hop

A test of "goodness of fit" of observed to expected numbers in the second and third crosses (Table 2) gave a $\chi^2_{1df} = 0.15$, assuming segregation in a 1:1 ratio. In the B × B cross, three phenotypic classes segregated in a 1:2:1 ratio with a chi-square $\chi^2_{2df} = 5.22$. These results suggest that the segregation ratios did not deviate from the expected values. It was interesting to note that in the cross 65102 × 21235M, representing two cultivars, a native North American rDNA type segregated out (Fig. 3). The appearance of phenotype C in the F_1 progeny of this cross can be explained by considering the pedigree of the female parent (65102). This genotype (cv *Cluster*) is presumed to be a hybrid between the native North American and the European (possibly English) hop established in the 1700s or 1800s (Neve 1991). Consequ ently, cv *Cluster* still harbors the 9.3-kbp rDNA repeat.

The segregation patterns of rDNA phenotypes suggest that a single locus with two co-dominant alleles controls genetic variability for rDNA in hop. A model (Fig. 4) is proposed to explain the genotypes of the rDNA genes in hop. Phenotype A is assigned the aa genotype and phenotype C is given the a'a'genotype. The model predicts that phenotype B represents a combination of the A and C patterns.

Fig. 4 Diagrammatic representation of ribosomal DNA phenotypes and assigned genotypes in hop

genotypes	aa	aa'	a'a'
10.3	-	-	
9.3		-	-
7.6	-	-	
6.6		-	-
2.7	-	-	-
phenotypes	A	B	C

Although many studies have addressed the structure and variation of rDNA in plants, relatively few authors have examined the inheritance of rDNA repeats in plants. For example, allotetraploid *Brassica napus* combined the rDNA profiles of its diploid parents (Palmer et al. 1983). Two loci each with two codominant alleles controlled genetic variability for rDNA in barley and segregation of rDNA repeats followed Mendelian principles (Saghai-Maroof et al. 1984). The rDNA repeat lengths in *Tolmies menziesii* and *Tellima grandiflora* were combined in naturally occurring intergeneric hybrids (Doyle et al. 1985). In teosinte × maize hybrids, both parents contributed equally to the rDNA profiles of their progeny. However, rDNA repeats were not inherited in a strict Mendelian fashion in a proportion of these hybrids (Zimmer et al. 1988). By contrast, rDNA inheritance occurred in a typical Mendelian manner in *Pisum sativum* (Ellis et al. 1984; Polans et al. 1986), *Triticum* (Snape et al. 1985), and *Pinus sylvestris* (Karvonen and Savolainen 1993).

The Mendelian inheritance of rDNA genes may be of practical importance for hop breeding, especially if genes of agronomic significance are associated with this locus. For example, the maize dwarf mosaic virus gene, *Mdm1*, co-segregates with the nucleolar organizer region in maize (Simcox et al. 1995). Mapping of the rDNA genes to a specific chromosome (s) would provide an important starting point for the establishment of an RFLP map. By determining linkage relationships among rDNA genes, RFLPs and traits of agronomic interest, plant breeders will be able to identify plants carrying useful genes in segregating progenies.

The present study showed a low level of genetic variability in the nuclear rDNA of cultivated and wild hop. Whether hop is a survivor of a single bottleneck event is a matter of conjecture. The rDNA data provide a molecular marker for native American hop and genetic evidence for the separation of native and cultivated American genotypes. The data also confirm that American cultivars developed as a result of hybridization between European cultivars and native North American hop. Ribosomal DNA length phenotypes showed simple Mendelian inheritance in hop.

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