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Assessment of compatibility among *Armillaria cepistipes, A. sinapina,* and North American biological species X and XI, using culture morphology and molecular biology

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Abstract: Ten single-spore isolates each of *Armillaria sinapina, A. cepistipes,* and North American biological species (NABS)X and XI were paired in all combinations. A second set of ten single-spore isolates of each species was likewise paired. Each pairing was duplicated for a total of 3280 pairs. Using the standard morphological criteria (e.g., fluffy, crustose) to assess the pairings, *A. sinapina* exhibited the following compatibility levels: 68% with itself, 5.5% with *A. cepistipes,* 3.5% with NABS X, and 5% with NABS XI, respectively. *Armillaria cepistipes* was rated 53%, 6.5%, and 57% compatible with itself and NABS X and XI, respectively. NABS X was 70% selfcompatible and 4.0% compatible with NABS XI. NABS XI was 55% selfcompatible. The intergenic spacer region of the rDNA of each isolate was amplified using polymerase chain reaction and digested with *Alu* I. Seven distinct restriction fragment length polymorphic (RFLP) patterns were observed: two in *A. cepistipes,* two in *A. sinapina,* one in NABS X, one in NABS XI, and one that was common to NABS XI and *A. cepistipes.* By using the RFLP patterns as markers to assess the outcome of the pairings, it was determined that, in general, the morphological ratings were an accurate reflection of nuclear combination. Combining the RFLP and pairing results confirms that NABS XI is compatible with *A. cepistipes* and should be considered conspecific. *A. sinapina,* NABS X, and *A. cepistipes* are compatible to a very limited degree in culture, but not enough evidence exists to warrant reducing any to synonomy. Therefore, we believe NABS X should be formally described as a new species of *Armillaria.*

Key Words: intergenic spacer, incompatibility groups, polymerase chain reaction, restriction fragment length polymorphisms

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INTRODUCTION

The relatedness among the ten North American biological species (NABS) of *Armillaria* (Fr.:Fr.) Staude described by Anderson and Ulrich (1979) and their five European counterparts (EBS; Korhonen, 1978) was initially examined by Anderson et al. (1980), who demonstrated compatibility in culture between several NABS and EBS, which indicated conspecificity. With the exception of EBS B, each EBS was found to be compatible with a single NABS; EBS B was found to be compatible with both NABS IV and X.

Anderson (1986) reported that NABS IV and V were compatible and NABS IV was abandoned. NABS V was described as *A. sinapina* by Bérubé and Dessureault (1988). Romagnesi and Marxmüller (1983) equated EBS B as *A. cepistipes* Velenovsky. Bérubé et al. (1996) more thoroughly examined the relationship between *A. sinapina* and *A. cepistipes* and found these species to be 2.1% and 6.7% compatible when ambiguous pairings were included as positives. In their opinion, this amount of compatibility did not indicate conspecifity.

The relationship of NABS X to *A. cepistipes* remained to be examined critically. Although, Anderson et al. (1980) reported that these species are at least 50% compatible, they used NABS X single-spore isolates that originated from a single collection. Morrison et al. (1985) reported complete incompatibility between two collections of NABS X and four single spores of *A. cepistipes.* Also, they reported partial compatibility between *A. cepistipes* and two collections of NABS XI, a group that they had described from British Columbia. Banik et al. (1996) reported at least 60% compatibility among four new collections of NABS XI from the Olympic Peninsula of Washington and five single spores of *A. cepistipes.* In both of these studies, the number of testers used from each species was limited.

The work discussed so far was based exclusively on pairings in culture using the technique originally described by Hintikka (1973). Because of variation in culture morphology and intensity of reactions, such pairings are at times difficult to interpret, especially when dealing with isolates from species that are only partially compatible. Another method used to examine relationships between species involves molec-

ular biological techniques. Anderson et al. (1989) used ribosomal DNA (rDNA) polymorphisms to divide EBS and NABS into six different classes. *Armillaria sinapina, A. nabsnona* Volk & Burdsall, and NABS X were placed in a class that was related to a class containing *A. cepistipes, A. gallica* Marxmüller & Romagnesi, and *A. calvescens* Bérubé & Dessureault. Using sequence data from the rDNA intergenic spacer (IGS) region, Anderson and Stasovski (1992) reevaluated these two classes and concluded they were so closely related that they should be considered a single species cluster that shares a common ancestry. North American biological species XI was not included in either of these studies.

Utilizing Anderson and Stasovski's (1992) sequence data, Harrington and Wingfield (1995) devised a scheme for separating the species of *Armillaria* via restriction fragment length polymorphisms (RFLP) of the polymerase chain reaction (PCR) amplified IGS region. They were able to distinguish 11 species of *Armillaria* from Europe and North America using a combination of five restriction enzymes, most prominently *Alu* I. Isolates of *A. sinapina,* NABS X, and a group of *A. cepistipes* each possessed unique RFLP when digested with this enzyme. The remaining *A. cepistipes* isolates had an *Alu* I RFLP pattern that was shared with *A. ostoyae* (Romagnesi) Herink, *A. gemina* Bérubé & Dessureault, and a group of *A. borealis* Marxmüller & Korhonen isolates. However, this group of *A. cepistipes* could be separated from the other three species by digestion with *Hinc* II. North American biological species XI was not included in this work.

Banik et al. (1996) used the technique devised by Harrington and Wingfield (1995) to aid in the identification of collections from the Olympic Peninsula. The RFLP pattern of the IGS region of four Olympic Peninsula collections of NABS XI was the same as the unique pattern reported for *A. cepistipes* by Harrington and Wingfield (1995). Banik et al. (1996) did not compare any NABS X isolates. The restriction pattern they reported for *A. sinapina* was different from that reported by Harrington and Wingfield (1995), and unlike that of NABS XI or *A. cepistipes.*

Volk et al. (1996) used the PCR technique of Harrington and Wingfield (1995) to verify their identification of NABS IX isolates prior to describing NABS IX as the morphological species *A. nabsnona.* They used two distinct RFLP haplotypes in this species as genetic markers in pairing analysis. When a combination of the two haplotypes was observed as a result of a pairing, the pairing was considered to be positive. The advantage of this technique is that it avoids reliance on morphological indicators, such as culture morphology or clamp connections, when assessing compatibility. These indicators are often affected by factors not related to mating compatibilty, such as growth medium, which can lead to spurious results.

The objective of the study reported here was to define the incompatibility groups that exist in the closely related species *A. cepistipes, A. sinapina, NABS* X, and NABS XI. The use of larger numbers of isolates than used in previous studies was intended to remove ambiguities surrounding their relationships. Additionally, genetic markers of the type used by Volk et al. (1996) were used to assess the reliability of the pairing results. Combining pairing data with RFLP analysis of the IGS region provides a clearer indication of the taxonomic status of the taxa in this group.

MATERIALS AND METHODS

Test isolate selection.—Single-spore tester isolates were identified from 12 collections of *A. sinapina,* 5 collections of NABS XI, and 8 collections of NABS X, via pairings of sibling single-spore isolates in all combinations. Species identifications were based on pairings with known haploid tester isolates, and all pairings were conducted using previously established protocol (Banik et al., 1995). The NABS X isolates were collected from conifer in Idaho (courtesy of G. McDonald). Four of the NABS XI collections were from the Olympic Peninsula of Washington State and the other was from British Columbia (courtesy of D. Morrison). All NABS XI isolates were collected from hardwoods. Three of the *A. sinapina* collections were from Wisconsin, two from Michigan, two from British Columbia, and five from the Olympic Peninsula. The *A. sinapina* collections were from conifer, except two from Wisconsin and one from British Columbia that were on hardwood. Specific collection information for the isolates used is available from the authors upon request.

One single-spore isolate representing each of the mating types identified from each collection was selected and subjected to RFLP analysis of its PCR-amplified IGS region of rDNA with the restriction endonuclease *Alu* I using previously described methods (Harrington and Wingfield, 1995; Volk et al., 1996). Thirty-four isolates of *A. sinapina,* 20 of NABS XI, and 29 of NABS X were analyzed. In addition, 30 single-spore isolates from 17 collections of *A. cepistipes* identified and provided by J. Guillaumin were also RFLP-haplotyped.

After haplotyping, 20 single-spore isolates from each species were chosen to be used in further pairing tests. These were selected from 5 collections of NABS XI, 8 collections of NABS X, 8 collections of *A. sinapina,* and 10 collections of *A. cepistipes.* Selected isolates represented the different haplotypes

identified from each species, and compatible tester isolates from a collection were chosen when available.

Pairing protocol.—The 20 single-spore isolates of each species were divided into 2 sets of 10 (A and B) based on the same protocol by which they were originally selected. Set A isolates from all four species were coded and randomly given a number between 1 and 40. Similarly, Set B isolates were assigned numbers from 41 to 80. All isolates within each set were paired in all possible combinations using previously described protocol. Each pair was assigned a unique number between 1 and 820 for Set A and between 821 and 1640 for set B. Each pairing was replicated for a total of 3280 pairs. Isolates from Set A were not paired with isolates from Set B.

After incubating 3–4 wk at 24 C, the pairings were assigned primary readings of positive, negative, or questionable based on their colony morphology. Positive pairings exhibited a uniform crustose appearance or were brown and appressed without distinction between the two haploids. Negative pairings remained fluffy throughout or exhibited a dark line separating the two haploids. Pairings were considered questionable when (i) both haploid cultures were considered brown and appressed, and were obviously separate but lacked the black line between them, (ii) one haploid culture was crustose or brown and appressed but the other remained fluffy, or (iii) both cultures were predominantly fluffy but with crustose patches.

After scoring, one replicate of each pairing was subcultured by excising a 4 by 40 mm strip from each pairing with a sterile razor blade. The strips were cut perpendicularly to the confrontation zone and adjacent to the seed plugs and placed on 1.5% malt extract, 2.0% agar (MEA) plates and incubated at 25 C. After 4–6 wk, the subcultures were rated as described above and the number of sectors visible from each subculture was recorded. A small piece of each sector was excised and placed in a 10 by 75 mm tube containing 2 mL MEA for storage. The tubes were incubated for at least 4 mo at 25 C and then refrigerated. In subcultures possessing no visible sectors, two pieces of mycelium were excised from the edge of the culture from opposite ends of the strip. The subculture ratings were used to assign a final rating to pairings in which the outcomes from the original scoring did not agree between replicates.

RFLP analysis of pairings.—To assess the reliability of the morphological scoring of the original pairings or subcultures as a measure of nuclear migration, some of the sector cultures were analyzed using the RFLP analysis described above. The detection of a combined RFLP haplotype in a sector was considered indication of nuclear combination and thus a compatible pairing. Only sectors from pairings in which the two haploid tester isolates possessed different haplotypes were analyzed.

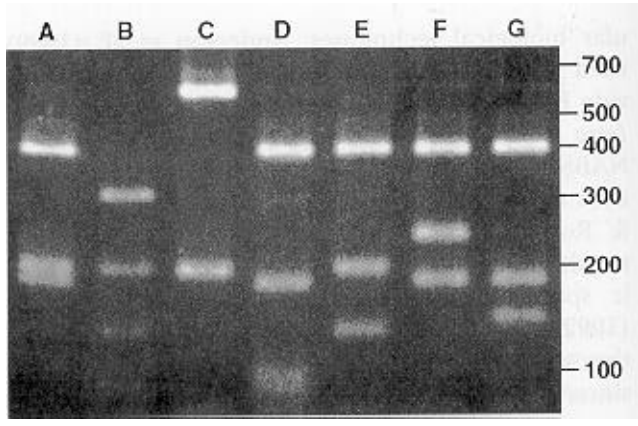

FIG. 1. Representative patterns of *Alu* I digestion products of IGS region of haploid isolates of *A. cepistipes* (patterns A, B, and C), NABS XI (patterns A and D), *A. sinapina* (patterns E and F), and NABS X (pattern G). Size markers in base pairs are shown on far right.

Sectors resulting from 39 pairings from Set A and Set B in which both replicates had a positive primary reading were analyzed for RFLP, as were sectors from 74 pairings with negative and 5 pairings with questionable primary readings. Pairs to be analyzed were chosen from all possible species combinations. In choosing test pairs, an attempt was made to include as many different isolates of each species as possible.

Sectors from 27 positive, 79 negative, and 19 questionable subcultures of pairs whose primary readings did not agree between replicates from sets A and B were subjected to RFLP analysis. Pairs to be analyzed were chosen from all possible species combinations.

Sector isolates to be tested were transferred from the storage tubes to Petri plates containing 4.0% water agar. Following 1–3 wk incubation at 25 C, three hyphal tips were excised with the aid of a dissecting microscope and placed in Petri plates containing MEA. After incubating for 3 wk at 25 C, at least one of the three hyphal tip cultures was used in RFLP analysis using the method previously described. If morphological variability was observed among the hyphal tip cultures, RFLP analysis was done on each morphological type.

RESULTS

Test isolate RFLP haplotypes.—The undigested amplification product of the IGS region was about 920 bp for all isolates. The 80 isolates chosen for use in pairings yielded 7 (A-G) different haplotype RFLP patterns when digested with *Alu* I (FIG. 1, TABLE I). Pat-

TABLE I. *Alu* I restriction fragment sizes of IGS region of seven haplotypes identified from 80 single-spore isolates

Haplotype designation	Species	Fragment size (bp)[a]
A	*A. cepistipes*, NABS XI	398 (390–407), 200 (197–203), 184 (180–188)
B	*A. cepistipes*	311 (303–319), 200 (195–205), 142 (137–147)
C	*A. cepistipes*	589 (576–602), 204 (200–208)
D	NABS XI	402 (395–409), 184 (178–190), 99 (96–102)
E	*A. sinapina*	426 (418–434), 210 (204–216), 142 (138–146)
F	*A. sinapina*	416 (407–425), 253 (248–258), 192 (187–197)
G	NABS X	406 (399–413), 190 (186–194), 153 (149–157)

[a] Fragment sizes were interpolated from DNA size standards using a semilogarithmic scale. Values were obtained by averaging the determinations obtained from 20 measurements of each haplotype from at least 10 different gels. 95% confidence intervals are shown in parentheses.

terns A, B, and C were found in *A. cepistipes* and patterns A and D were present in NABS XI. *A. sinapina* exhibited patterns E and F, and only pattern G was found in NABS X.

Pairings.-For each replicate in each set, there were 55 intraspecies pairings per species and 100 interspecies pairings for each of 6 possible combinations (TABLE II). Intraspecies compatibility in Set A ranged from 25-35 positive of 55 (46–64%), while those in Set B exhibited 15-39 positive pairings (27–71%). Interspecies pairings among Set A isolates were all between 0–2% compatible except for the *A. cepistipes*-NABS XI pairings, which exhibited 68% and 56% compatibility in the two replicates (TABLE II). Interspecies pairings in Set B isolates were 1–9% compatible except for the *A. cepistipes-NABS* XI combination, which exhibited 46% and 39% compatibility in each replicate (TABLE II). Information on specific pairing results is available from the authors upon request.

Of the 820 pairings of Set A isolates, 102 (12%) had primary readings that did not agree between replicates. Based on subculture morphology 66 of these pairings were designated as positive, 23 negative, and 17 questionable. Primary readings of 175 (21%) Set B pairings did not agree between replicates, of which 76 were designated as positive, 82 negative, and 16 questionable based on subculture morphology.

RFLP analysis of pairings.—RFLP pattern types were determined for 238 hyphal tip isolations from sector cultures representing 119 pairings in which the primary readings of both replicates agreed (TABLE III). Twenty-four of 39 (62%) pairings that were positive based on the primary readings exhibited a combined

TABLE II. Number of pairings rated positive, negative, or questionable based on colony morphology for pairings between haploid isolates of *A. sinapina,* NABS X, NABS XI, and *A. cepistipes*[a]

	Set A isolates			Set B isolates		
Species pair	Positive	Negative	Questionable	Positive	Negative	Questionable
sinapina-sinapina	33/32 (59)	17/22 (36)	5/1 (6)	35/39 (67)	15/14 (15)	5/2 (4)
sinapina-NABS X	0/0 (0)	99/100 (100)	1/0 (0.5)	1/3 (2)	97/93 (95)	2/4 (3)
sinapina-NABS XI	0/2 (1)	100/98 (99)	0/0 (0)	5/9 (7)	90/90 (90)	5/1 (3)
sinapina-cepistipes	0/1 (0.5)	93/98 (96)	7/1 (4)	3/2 (2.5)	95/92 (94)	2/6 (4)
NABS X-NABS X	35/34 (63)	15/17 (24)	5/4 (8)	31/26 (29)	17/21 (35)	7/8 (14)
NABS X-NABS XI	1/0 (0.5)	95/97 (96)	4/3 (3.5)	1/4 (2.5)	92/94 (93)	7/2 (4.5)
NABS X-*cepistipes*	2/1 (1.5)	93/93 (93)	5/6 (5.5)	4/8 (6)	86/86 (86)	10/6 (8)
NABS XI-NABS XI	31/35 (60)	11/13 (22)	13/7 (18)	21/15 (33)	28/35 (57)	6/5 (10)
NABS XI-*cepistipes*	68/56 (64)	22/33 (28)	10/11 (11)	46/39 (43)	40/51 (46)	14/10 (12)
cepistipes-cepistipes	30/25 (50)	20/25 (41)	5/5 (9)	20/16 (33)	28/34 (56)	7/5 (11)

"Values are number of pairings rated as belonging to each class based on 100 pairings between species and 55 pairings within species. Each set consists of 10 haploid isolates of each species, The value for the first replicate appears to the left of the slash and that for the second replicate to the right. Values in parentheses indicate average percentage of pairings from the two replicates in each category.

TABLE III. Number of pairings between *A. cepistipes, A. sinapina,* NABS X, and NABS Xl with primary ratings that agreed among replicates that yielded subcultures exhibiting a combined RFLP pattern

	Primary culture rating					
	Set A isolates			Set B isolates		
Species pair	Positive	Negative	Questionable	Positive	Negative	Questionable
sinapina-sinapina	4/5	0/3	—	4/5	—	—
sinapina-NABS X	—	1/5	—	—	0/5	—
sinapina-NABS XI	—	0/5	—	0/3	0/5	—
sinapina-cepistipes	—	0/7	—	—	0/5	—
NABS X-NABS XI	—	0/5	—	0/1	0/5	—
NABS X-*cepistipes*	—	0/6	0/2	0/1	0/5	1/1
NABS XI-NABS XI	2/5	—	—	0/1	2/5	—
NABS XI-*cepistipes*	2/5	0/4	0/1	5/5	2/4	0/1
cepistipes-cepistipes	3/4	1/1	—	4/4	2/5	—

[a]Values express number of pairings yielding subcultures that expressed combined RFLP patterns per total number of pairings sampled of each rating class for that species pair combination. Subcultures analyzed were selected from one replicate of each of two sets of pairings. For each pairing sampled, at least two sectors of resultant subcultures were RFLP typed. No data are presented for NABS X self-pairings because only one RFLP type was present in this species, and thus a combined pattern type did not exist.

pattern in at least one sector of the resulting subcultures as did 8 of 75 (11%) pairings that were rated as negative and 1 of 5 rated as questionable (TABLE III).

The RFLP patterns were determined for 305 hyphal tip isolations from sectors of subcultures from 119 pairings in which the primary readings of the two replicates did not agree. Subcultures from 13 of 28 (46%) pairings rated as positive, based on subculture morphology, exhibited combined RFLP patterns, as did 36 of 74 (49%) pairings rated as negative. In addition 4 of 17 (24%) subcultures rated as questionable possessed combined RFLP patterns (TABLE: IV).

Overall, subcultures from 86 of 238 unique pairings analyzed for RFLP exhibited combined patterns. Seventeen (20%) of these exhibited combined patterns in all sectors tested, 39 (45%) exhibited such patterns in half the sectors tested, and 5 (6%), 10 (12%), and 13 (15%) exhibited combined patterns in one-fourth, one-third, and two-thirds of the sectors, respectively. Morphological variability among hyphal tip isolates from a single sector was observed for 46 sectors from 39 different subcultures. In 11 of these sectors, both haploid and combined RFLP patterns were detected from different hyphal tip isolations taken from the same subculture sector.

TABLE IV. Number of pairings between *A. cepistipes, A. sinapina,* NABS X, and NABS Xl with primary ratings that did not agree among replicates that yielded subcultures exhibiting a combined RFLP pattern[a]

	Subculture rating					
	Set A isolates			Set B isolates		
Species pair	Positive	Negative	Questionable	Positive	Negative	Questionable
sinapina-sinapina	—	0/2	—	3/4	2/2	—
sinapina-NABS X	—	0/1	—	0/4	0/2	0/1
sinapina-NABS XI	0/1	—	—	0/1	2/8	0/1
sinapina-cepistipes	—	1/5	—	2/3	0/2	1/1
NABS X-NABS Xl	—	2/4	—	0/3	2/8	—
NABS X-*cepistipes*	—	2/5	1/3	1/3	2/6	0/3
NABS XI-NABS Xl	2/4	—	—	—	3/3	—
NABS XI-*cepistipes*	—	4/5	0/3	—	12/16	0/2
cepistipes-cepistipes	4/4	—	2/2	1/1	4/5	0/1

[a]Values designate the number of subcultures that expressed combined RFLP patterns per total number of subcultures sampled of each rating class for that species pair combination. Subcultures analyzed were selected from one replicate of each of two sets of pairings. For each pairing sampled, at least two sectors were RFLP typed. No data are presented for NABS X self-pairings because only one RFLP type was present in this species, and thus a combined pattern type did not exist.

TABLE V. Number of pairs rated positive after reconciliation of data from two replicates, for two different sets of pairings of *A. sinapina, A. cepistipes,* NABS X, and NABS XI in all combinations[a]

Species pair	Set	Positive rating: Both replicates	Subculture	RFLP	Total
sinapina-sinapina	A	30	5	0	35
	B	30	9	2	41
sinapina-NABS X	A	0	0	1	1
	B	0	6	0	6
sinapina-NABS XI	A	0	1	0	1
	B	3	5	2	10
sinapina-cepistipes	A	0	4	1	5
	B	0	7	1	8
NABS X-NABS X	A	32	11	0	43
	B	20	15	0	35
NABS X-NABS XI	A	0	2	2	4
	B	1	4	2	7
NABS X-*cepistipes*	A	0	2	3	5
	B	1	9	3	13
NABS XI-NABS XI	A	26	13	0	39
	B	11	5	5	21
NABS XI-*cepistipes*	A	53	15	4	72
	B	33	9	14	56
cepistipes-cepistipes	A	22	13	3	38
	B	11	9	6	26

[a] Positive ratings were assigned based on occurrence of positive primary rating for both replicates of a given pair. If replicates did not agree, ratings were assigned based on subculture readings. RFLP data indicative of nuclear combination were used when primary or subculture readings were negative or questionable. Values are based on 55 intraspecies and 100 interspecies pairs.

Final assessment of compatibility.-The final assessment of the compatibility of the isolates in Sets A and B is shown in TABLE V. The assessments allocate the primary readings of all agreeing pairs or subculture readings by nonagreeing pairs as the definitive reading on each pairing. These readings are modified where RFLP data indicate that nuclear combination has occurred, indicating compatibility that was not identified by the morphological methods. This occurred in 8 pairs where both replicates had negative primary readings and in 1 pair where both replicates were questionable. Also 36 nonagreeing pairs with negative subculture readings and 4 with questionable subculture readings were designated compatible based on the presence of combined RFLP patterns. The final percentage of compatibility among all the species, averaged over both sets of isolates, is shown in TABLE VI.

TABLE VI. Combined percentage of pairings rated as positive overall, from two sets of isolates composed of 10 single-spore isolates each of *A. cepistipes, A. sinapina,* NABS X, and NABS XI, when paired in all combinations[a]

Species	Species: *cepistipes*	*sinapina*	NABS X	NABS XI
cepistipes	58	6.5	9	64
sinapina	—	69	3.5	5.5
NABS X	—	—	71	5.5
NABS XI	—	—	—	55

[a]Percentages are based on 110 intraspecies and 200 interspecies pairs.

DISCUSSION

Most of the RFLP patterns observed for the *Alu* I digests of the PCR-amplified IGS region correspond to those reported earlier (Harrington and Wingfield, 1995; Banik et al., 1996). Pattern C from *A. cepistipes* and Pattern D from NABS XI have not been previously reported. Only one pattern was present in NABS X, and thus RFLP analysis could not be used to determine nuclear combination of pairings within this species.

Based on the primary morphological ratings, the amount of intraspecies compatibility obtained was lower than expected, as well as lower than that re-

ported by Bérubé et al. (1996). This could have been due to differences in media, interpretations of morphologies, or isolates used in the pairings. In addition, our calculations of percentage of compatibility included all self-crosses, which are predisposed to be negative. This reduced the level of compatibility by about 10% compared to calculations in which self-crosses are excluded. The amount of intraspecies compatibility in Set A isolates was similar for all species across both replicates. However, Set B isolates showed a great range in intraspecies compatibility and, except for *A. sinapina,* less compatibility than that in Set A. This was especially noticeable for Set B NABS XI and *A. cepistipes* pairings. One reason for low compatibility in Set B *A. cepistipes* is that two of the isolates did not appear to be compatible with any other isolates or with each other. Similarly, one NABS XI isolate was compatible with only one other NABS XI isolate. This loss of compatibility could be due to degeneration of the isolates in culture.

Based on the primary ratings, all interspecies pairings, except those between NABS XI and *A. cepistipes,* exhibited much lower rates of compatibility than those observed within species. Relative to *A. sinapina* and *A. cepistipes,* these results are comparable to those of Bérubé et al. (1996). The compatibility between NABS XI and *A. cepistipes* was as high or higher than that observed within each of these species.

Nuclear combination was confirmed, by identification of RFLP combined patterns in sectors from the resulting subcultures, in only 62% of the pairings with positive primary readings for both replicates. The lack of detection of combined patterns in every pairing rated as positive was unexpected but may have several explanations: (i) the subculture mycelium could be composed of hyphae of differing nuclear condition, (ii) there may be a lack of nuclear exchange in apparently positive pairings, and (iii) the pairings may have been misread. It is beyond the scope of this study to completely address these possibilities, but there is evidence to support the first hypothesis. It was observed that sibling hyphal tip cultures, taken from several subculture sectors, possessed different RFLP patterns. In these cases, it appears that a given sector was composed of a mosaic of hyphae, some expressing RFLP patterns indicative of haploid nuclei and others exhibiting the combined RFLP patterns of a multinucleate or diploid state. Mosaics of this type have been reported for *A. ostoyae* (Rizzo and May, 1994; Rizzo and Harrington, 1992). Haploid mycelia have also been reported in heterokaryotic isolates of *Heterobasidion annosum* (Fr.) Bref. (Hansen et al., 1993).

Nuclear combination was also observed in a small number of the pairings with negative primary readings for both replicates. All but one of these pairings involved confrontations either within or between NABS XI and *A. cepistipes.*

In pairings with nonagreeing primary readings between replicates, the ultimate decision as to their compatibility was based on morphological readings of the subcultures from those pairings. However, nuclear combination in sectors from subcultures rated positive could only be confirmed with RFLP data 46% of the time, which was slightly less than the combined pattern detection rate in negatively rated subcultures (49%). Thus, it appears that the subculture readings do not correspond well with the RFLP data, and this method is less representative of nuclear combination. Some possible reasons for these discrepancies have already been discussed. It is also possible that many of the pairs with negatively rated subcultures that exhibited combined RFLP patterns are slow to convert to a crustose-type morphology. The high incidence of combined patterns in negatively rated subcultures may represent a situation similar to that described in *Phellinus weirii* (Murr.) Gilbertson by Angwin and Hansen (1993), in which secondary mycelium formation occurred between two homokaryons that displayed intense line formation, usually indicative of sexual incompatibility. Almost half (16 of 36) of the negatively rated subcultures that possessed combined RFLP patterns exhibited three or more sectors, with the middle sector solely possessing a combined pattern in 9 of these subcultures. In these pairings it seems likely that the somatic incompatibility was expressed phenotypically as a negative reaction, but sexual compatibility still occurred. In addition, it should be remembered that the pairings in question are more ambiguous by nature than those in which both replicates agree, and thus a greater deviation from the RFLP results might be expected. It is interesting to note that subcultures from pairings between taxa with high compatibility based on the primary readings were more likely to exhibit combined RFLP (36 of 54) than subcultures from pairings between taxa with low compatibility in the primary readings (16 of 65). This may suggest that a substantial number of the subcultures rated positive from pairings of species with low primary compatibility are in fact negative. Since 40 of the 60 pairs designated positive in pairings between these taxa were assigned based on subculture readings this could reduce the overall compatibility between these groups.

Overall, nuclear combination, as indicated by combined RFLP patterns, was observed in pairings between all of the species tested. However, except for pairings between *A. cepistipes* and NABS XI, the incidence of recovery of combined patterns from in-

terspecies pairings was insignificant compared to that from within species pairings. Pairings between *A. cepistipes* and NABS XI exhibited a rate of combined RFLP pattern recovery similar to that observed within species pairings.

Despite the discrepancies noted between the primary morphological pairing and subculture readings and the RFLP nuclear combination data, all methods support the original observation by Morrison et al. (1985) on the compatibility between NABS XI and *A. cepistipes*. We believe that vernacular NABS XI should be dropped and *A. cepistipes* be used to refer to this taxon.

However, the relationship among NABS X, *A. sinapina*, and *A. cepistipes* is not as well defined. The RFLP and culture morphology data indicate a small amount of compatibility between each of these species. However, even though the RFLP data show that nuclei from these species can reside simultaneously in the same thallus, the prospects for true interfertility between them is certainly much less than for NABS XI and *A. cepistipes*. Further molecular biological information is required to adequately assess the relationships in this group. Anderson and others (Anderson et al., 1989; Anderson and Stasovski, 1992) have demonstrated that these species are closely related in terms of evolution of rDNA. Perhaps other gene systems, such as lactase, which are more related to function of an organism in nature, would be more enlightening on the taxonomic status of these groups. Furthermore, a reappraisal of the compatibility within the genus, using a technique similar to that employed here, is needed to place the current data in perspective.

At this time, we suggest that *A. sinapina*, *A. cepistipes*, and NABS X remain as separate species. In addition, we believe that NABS X should be formally described as a new species. Based on the evidence available, we believe that these species are at the point in evolution where a low level of interbreeding may be possible but is no longer significant to the genomic make-up of each species as a whole. Unless new data arise that specifically address the combination of these three species in situ, we contend that maintaining their individual species status is the best course of action and is in keeping with the current taxonomic trend in the genus *Armillaria*.

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