

Somatic incompatibility in dikaryotic–monokaryotic and dikaryotic pairings of *Echinodontium tinctorium*¹

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Somatic incompatibility in dikaryotic–monokaryotic (di–mon) and dikaryotic pairings of *Echinodontium tinctorium* was investigated *in vitro* on 4.5% malt agar. Antagonistic reactions of varying intensity occurred in all pairings between 12 allopatric dikaryons from Idaho and Arizona, between 14 sib-composed dikaryons from two Idaho sites, and in over 95% of pairings between sympatric dikaryons from separate trees at each location. Antagonistic reactions in dikaryotic pairings macroscopically appeared as dark reaction lines in the agar and aversion zones (barrage reactions) with hyphal massing on each side of the aversion zones. Self-crosses of dikaryons were somatically compatible, and hyphal anastomoses were common. Hyphal anastomoses were rare in the aversion zones between somatically incompatible dikaryons. Somatic incompatibility occurred in 51% of 89 di–mon pairings between 29 monokaryotic isolates and 7 sib-composed dikaryons from the same parent. Somatically incompatible di–mon pairings were characterized by hyphal massing in the contact zone and reaction lines on the reverse, but they lacked barrage reactions. Clamp connections formed in 54% of all di–mon crosses and at similar frequencies when no antagonistic reactions were present (55%) or when only hyphal massing occurred (53%), but they formed less frequently (33%) when dark reaction lines were present. The potential applications of these findings to epidemiological studies are discussed.

Key words: *Echinodontium*, somatic incompatibility, epidemiology, Buller phenomenon, dikaryotic–monokaryotic, wood decay.

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L'auteur a étudié l'incompatibilité somatique chez des paires de dicaryon–monocaryon (di–mono) et de dicaryon de l'*Echinodontium tinctorium* en les cultivant *in vitro* sur malt à 4,5%, gélosé. On observe des réactions antagonistes de différentes intensités chez toutes les paires obtenues entre les 12 dicaryons allopatriques provenant de l'Idaho et de l'Arizona, entre les 14 dicaryons formés de frères provenant de divers endroits de l'Idaho et chez plus de 95% des paires obtenues entre des dicaryons sympatriques provenant d'arbres séparés dans chaque localité. Les réactions antagonistes chez les paires de dicaryons se manifestent macroscopiquement sous forme de lignes de réaction sombres dans l'agar et par des zones d'exclusion (réaction de barrage) avec une accumulation d'hyphes de chaque côté des zones d'exclusion. Les auto-croisements de dicaryons sont somatiquement compatibles et les anastomoses d'hyphes sont fréquente. Ces anastomoses d'hyphes sont rares dans les zones d'exclusion entre les dicaryons somatiquement incompatibles. L'incompatibilité somatique se manifeste chez 51% des 89 paires di–mono obtenues à partir de 29 isolats monocaryotiques et 7 dicaryons composés de frères provenant du même parent. Les paires di–mono somatiquement incompatibles sont caractérisées par l'accumulation d'hyphes dans la zone de contact et de lignes de réactions sur le côté inverse de la gélose, mais ne montrent pas de réaction de barrage. Les anastomoses se sont formées chez 54% de tous les croisements di–mono et à des fréquences similaires en absence de réactions antagonistes (55%) où encore lorsque seulement une accumulation d'hyphes prend place (53%), mais se forment moins fréquemment (33%) lorsque des lignes de réactions foncées sont présentes. Les applications potentielles de ces résultats aux études épidémiologiques font l'objet d'une discussion.

Mots clés : *Echinodontium*, incompatibilité somatique, épidémiologie, phénomène de Buller, dicaryo–monocaryon, dégradation du bois.

[Traduit par la rédaction]

Introduction

Somatic incompatibility (SI) commonly occurs between intraspecific heterokaryons of lignicolous basidiomycetes with different mating genotypes (3, 8, 27, 29), restricting plasmogamy and exchange of genetic information between them. Antagonistic reactions indicative of SI *in vitro* are useful for identifying common genotypes of individuals (clones) and for determining the genetic similarity between separate strains within basidiomycete populations. Studies of SI systems in wood decay fungi have provided means for tracing the movement, distribution, and dispersal mechanisms of these fungi within forest stands. SI systems have been reported in impor-

tant wood decay fungi including *Armillaria mellea* (Vahl:Fr.) P. Kumm. (1, 30), *Fomitopsis cajanderi* (P. Karst.) Kotlaba & Pouzar (2), *Fomitopsis pinicola* (Sw.:Fr.) P. Karst. (27), *Ganoderma lucidum* (Curtis:Fr.) P. Karst. and *Ganoderma tsugae* Murrill (3), *Heterobasidion annosum* (Fr.:Fr.) Bref. (31), *Inonotus arizonicus* R.L. Gilbertson (17), *Phaeolus schweinitzii* (Fr.:Fr.) pat. (4), *Phellinus weirii* (Murrill) R. L. Gilbertson (8, 18), and *Trametes versicolor* (L.:Fr.) Pilát (28).

The Indian paint fungus *Echinodontium tinctorium* (Ellis & Everh.) Ellis & Everh. is an important hydneaceous hymenomycete because it causes a true physiological white heartrot principally in living true firs (*Abies* spp.) and hemlocks (*Tsuga* spp.) throughout western North America. Heartwood volume losses attributed to *E. tinctorium* decay in conifer stems are possibly second only to *Phellinus pini* (Thore:Fr.) A. Ames in the Pacific Northwest (5, 9, 14, 15, 25). The fungus usually

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causes insignificant losses in *Picea glauca* (Moench) Voss, *Picea engelmannii* Parry, and *Pseudotsuga menziesii* (Mirb.) Franco. Estimates of losses caused by *E. tinctorium* in interior hemlock stands of British Columbia have exceeded 30% of standing gross volume (13). Most damage occurs in mature and overmature trees that have exceeded the 120- to 150-year pathological rotation age (25), although significant losses can occur in younger trees (25, 26). Since most old-growth forests in western North America have been cut and pathological rotations are now commonly practiced (14, 16), future losses caused by *E. tinctorium* should decline.

Many details in the life history and epidemiology of *E. tinctorium* are unknown. For example, knowledge of its infection courts, mode of infection, and mechanism(s) of spread within forest stands is fragmentary. Etheridge et al. (10, 11, 12) provide the most recent hypothesis to explain the infection process and events leading to decay. Some details in its nuclear cycle and life history were recently reported (34, 35). Wilson (35) demonstrated that *E. tinctorium* has a bifactorial (tetrapolar) mating system with multiallelic incompatibility factors. The present study was conducted to determine if *E. tinctorium* has an SI system applicable to epidemiological studies, to document differences in SI reactions of dikaryotic-monokaryotic (di-mon) and dikaryotic-dikaryotic (di-di) pairings, and to determine the effects of SI interactions in di-mon pairings on occurrence of the Buller phenomenon.

Materials and methods

Fruiting body collections and isolations

Twelve basidiocarps of *E. tinctorium*, 6 (ADW-LP (15, 18, 30, 33, 47, 48) ID) from Idaho and 6 (ADW-SH (1, 2, 3, 4, 5, 6) AR) from Arizona, yielded dikaryotic isolates for di-di pairings. Two additional basidiocarps (ADW-LP 160 ID and ADW-BP 230 ID) from Idaho provided homokaryons for di-mon pairings and for the synthesis of sib-composed heterokaryons for di-mon and di-di pairings. Basidiocarps were collected during peak sporulation periods (23, 24) on separate living trees from the following hosts and locations: ADW-LP (15, 18, 30, 33, 47, 48) ID and ADW-LP 160 ID from *Abies grandis* (Dougl.) Lindl. and *Tsuga heterophylla* (Raf.) Sarg., near Disalto Creek Trail at the base of Strychnine Ridge and Sandy Mountain, adjacent to Laird Park, St. Joe National Forest, Latah Co., Idaho; ADW-BP 230 ID on *A. grandis*, at Black Pine Cabin Picnic Area near Waha, Craig Mountains, Nez Perce Co., Idaho; and ADW-SH (1, 2, 3, 4, 5, 6) AR on *Abies concolor* (Gordon & Glend.) Lindl., near Summerhaven, Mount Lemmon, Santa Catalina Mountains, Coronado National Forest, Pima Co., Arizona.

Basidiocarps were placed in moist chambers at 4°C to induce mycelial growth from freshly exposed contextual tissues and to induce sporulation. Dikaryotic isolates were obtained from basidiocarps by plating small masses of hyphae from fresh mycelial mats or pieces of red contextual tissues on 4.5% Difco malt agar (MA) incubated at 21°C. Portions of spore prints were collected on Saran Wrap and frozen at -20°C under low relative humidity for 9-10 weeks. Homokaryons were isolated using single-spore methods described previously (35).

Dikaryotic pairings

Six dikaryotic isolates from Idaho were paired in all possible combinations with six Arizona isolates possessing different mating incompatibility genotypes. Mycelia were placed 4-7 cm apart on 4.5% MA in 9-cm Petri dishes. In addition, 14 sib-composed dikaryons with different genotypes, 7 from each of two Idaho sites, were paired in 49 combinations. The isolates from each location also were paired in all possible combinations. Pairings of isolates with themselves (self-crosses) were used as controls. Macroscopic hyphal interactions observed on the front and reverse of each plate were recorded 8-10

weeks after incubation at 21°C under 8 h of daily fluorescent illumination. Macroscopic somatic interactions of dikaryotic pairings were compared with di-mon and incompatible pairings of monokaryotic (single-spore) isolates. Mycelium in the zone between paired strains was examined microscopically (400× magnification).

Di-mon pairings

Seven sib-composed dikaryotic isolates (designated A-G, all with the genotype $A_3A_4B_3B_4$) were paired in 89 combinations with 29 monokaryotic isolates (MK 61-76, 78-90) with mating types A_3B_3 , A_3B_4 , A_4B_3 , A_4A_4 . Incubation was as above. The monokaryotic colony in each pairing was examined microscopically for clamp connections 1 cm behind the line of contact with the opposing dikaryon. Macroscopic and microscopic observations of interactions between colonies on the front and reverse of each plate were recorded as for dikaryotic pairings.

Results

Dikaryotic pairings

SI reactions of varying intensity formed between dikaryotic isolates with different mating incompatibility genotypes regardless of the host or geographical location. Antagonistic reactions occurred in all pairings between Idaho and Arizona isolates. Antagonism also occurred in over 95% of pairings between isolates from separate trees at one location and between sib-composed dikaryons from different parents. Isolates from separate trees at each location were rarely somatically compatible. Antagonistic reactions macroscopically appeared as moderate to strong aversion zones (barrage reactions) on the front (Fig. 1A) and a single, broad, dark brown reaction line or two thinner parallel reaction lines in the agar, as seen on the reverse beneath the aversion zone (Fig. 1B). Aversion interactions caused a dense buildup of aerial mycelium from each colony on each side of the aversion zone. Aerial mycelium was sparse, and little intermingling of hyphae occurred between isolates in the aversion zone. Dark reaction lines and aversion zones were similar to but broader and more intense than reactions lines and barrage zones observed in previous common B pairings ($A \neq B =$) of monosporous isolates (35). Pairings between dikaryons from basidiocarps with identical mating genotypes showed no apparent SI on the front or reverse (Fig. 2), and the mycelia grew together in a manner similar to that of compatible matings ($A \neq B \neq$) of monokaryons with no aversion zones or reactions lines.

Microscopic observations indicated that hyphal anastomoses commonly formed between self-paired isolates, but anastomoses were rarely observed between somatically incompatible dikaryons with different genotypes. The dark reaction lines, forming beneath aversion zones were due to accumulations of pigment within the agar, not within interacting mycelia. SI had no apparent effects on the morphology of interacting mycelia, although aerial mycelia were more compacted adjacent to the aversion zones.

Di-mon pairings

SI interactions occurred in various combinations (patterns) in 51% of di-mon crosses (Table 1). Interactions were absent in 22% of the di-mon pairings, whereas 27% of di-mon crosses resulted in clamp connection formation in the absence of SI interactions. Hyphal massing in the contact zone and formation of dark reaction lines on the reverse were characteristic of di-mon SI (Fig. 3), but aversion zones were absent. Mycelia of opposing colonies appeared to push against each other, forcing an upward accumulation of aerial mycelia

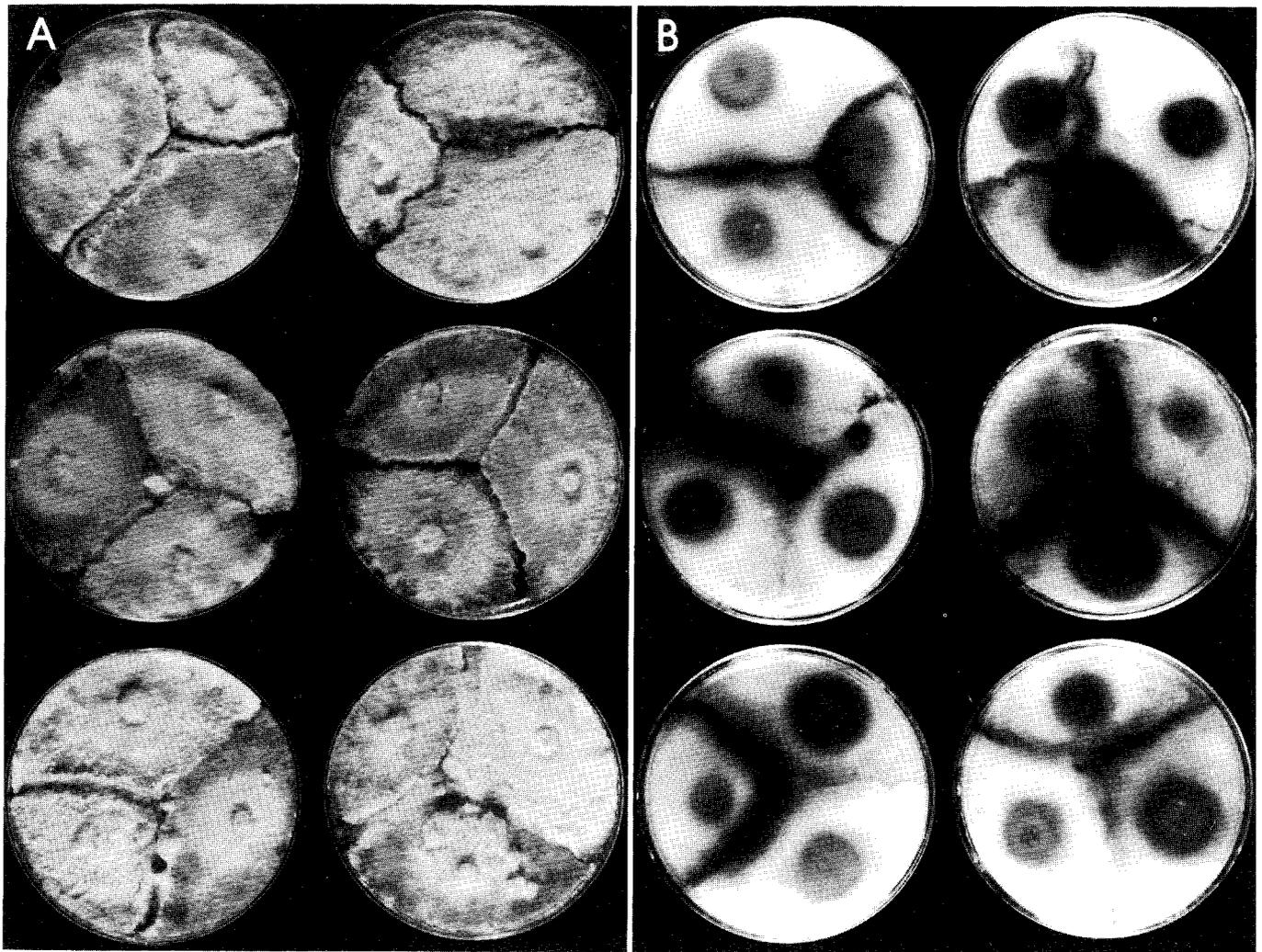


FIG. 1. Somatic incompatibility between heterogenic dikaryons of *E. tinctorium* on 4.5% malt agar after 8–10 weeks of incubation at 21°C. (A) Aversion zones (barrage reactions) between genotypically unrelated dikaryons (front view). (B) Dark reaction lines beneath the aversion zones in the same plates (reverse view).

(Fig. 3A). This dense buildup of raised mycelial growth was similar to barrier reactions observed in common A and B pairings ($A = B =$) between monokaryotic isolates (35), but greater and higher mycelial masses formed above the agar surface in di–mon pairings. A single dark brown reaction line often discolored the agar beneath these mycelial masses (Fig. 3B). Reaction lines of di–mon pairings were thinner and weaker than those forming between dikaryons with heterozygous genotypes but wider and more intense than those observed in common B ($A \neq B =$) matings of monokaryons. In general, formation of specific combinations (patterns) of di–mon interactions did not appear to be associated with particular monokaryon or dikaryon genotypes, since monokaryons and dikaryons of a given genotype were associated with several interaction combinations. However, specific interaction patterns appeared to be determined more by the specific monokaryon and dikaryon paired.

The influences of each di–mon interaction on dikaryotization of monokaryons by dikaryons (the Buller phenomenon) and on occurrence of somatic antagonism were examined by

comparing the formation frequencies of each interaction under all combinations of pairing conditions (Table 2). Clamp connections were found in what were originally monokaryotic mats in 54% of di–mon pairings. Similar frequencies of clamp formation resulted when no SI reactions were present or when only hyphal massing occurred. However, clamp connections formed at a lower frequency (33%) when dark reaction lines were present. Intrusive growth of dikaryotic hyphae was never observed in monokaryotic mats. Dikaryotization of monokaryons occurred at a slow rate and required over a year in some cases. Collectively, SI reactions developed in 51% of di–mon pairings and at comparable rates in the absence and presence of clamp connections. Similarly, hyphal massing did not appear to be influenced by the presence of clamp connections, although dark reaction lines formed less frequently when clamp connections were present. Hyphal massing always occurred in the contact zone when dark reaction lines formed on the reverse, yet reaction lines were present only in 53% of pairings in which hyphal massing occurred. No interactions occurred in 23% of di–mon pairings.

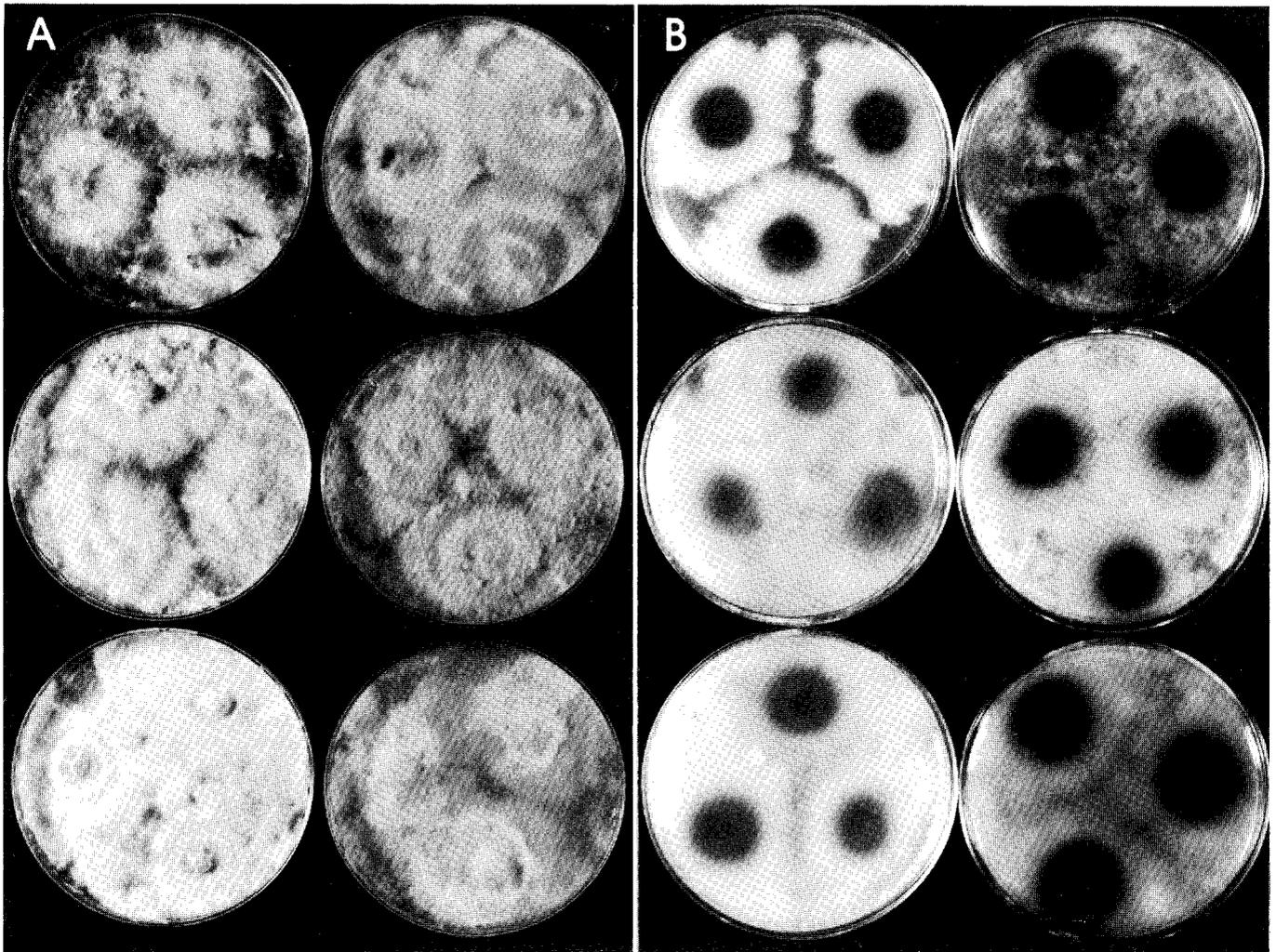


FIG. 2. Somatic compatibility between homogenic dikaryons of *E. tinctorium* on 4.5% malt agar after 8–10 weeks of incubation at 21°C. Pairings between genotypically related (identical) dikaryons showed no apparent somatic incompatibility interactions in the (A) front view, or (B) reverse view.

Discussion

Echinodontium tinctorium possesses a strong SI system that operates between heterogenic dikaryons and less frequently between monokaryons and sib-composed dikaryons with common parents. Although the genetic system controlling SI in *E. tinctorium* was not investigated, this study has shown differences that distinguish the *in vitro* SI interactions of di–mon pairings from pairings of dikaryotic isolates. Di–mon pairings usually resulted in hyphal massing in the contact zone with no zones of aversion, whereas di–di pairings produced strong barrage reactions with hyphal massing on each side of the aversion zones. However, both di–mon and dikaryotic pairings formed dark reaction lines, seen within the agar on the reverse, which are characteristic of SI in higher Basidiomycetes. Reaction lines were typically broader, darker, and apparently stronger in dikaryotic pairings than in di–mon pairings. Previous studies (2, 33) have indicated that the strength of heterogenic SI interactions between dikaryons reflects the genetic dissimilarity between interacting strains. This could explain why di–mon SI interacting were weaker than those of di–di pairings. The genotypes (mating alleles) of dikaryons paired from separate localities differed markedly, whereas the mono-

karyons had mating alleles in common with sib-composed dikaryons in di–mon pairings. The difference in SI interactions also might be explained by the numbers of nuclei involved in the interaction. If SI interactions are under nuclear control, somatic incompatibility between dikaryons would result from SI determinants on two pairs of interacting nuclei. In contrast, SI interactions in di–mon pairings would result from interactions between a single nucleus of the monokaryon and a pair of nuclei in the dikaryon. Gene products of SI alleles theoretically may be produced more abundantly as the number of interacting nuclei increases. An increase in gene products should yield stronger reactions from the concomitant activation of multiple copies of somatic incompatibility factors in more nuclei. The possible role of cytoplasmic DNA constituents in controlling SI also should be investigated. The barrage reaction of dikaryotic pairings would seem to be a stronger indicator of SI than hyphal massing in di–mon pairings since aversion zones often prevent contact and anastomoses between opposing hyphae. Nevertheless, dark reaction lines on the reverse appeared to be more consistent indicators of SI in *E. tinctorium*.

Antagonistic SI reactions were observed between heterogenic dikaryotic isolates from common collection sites (sym-

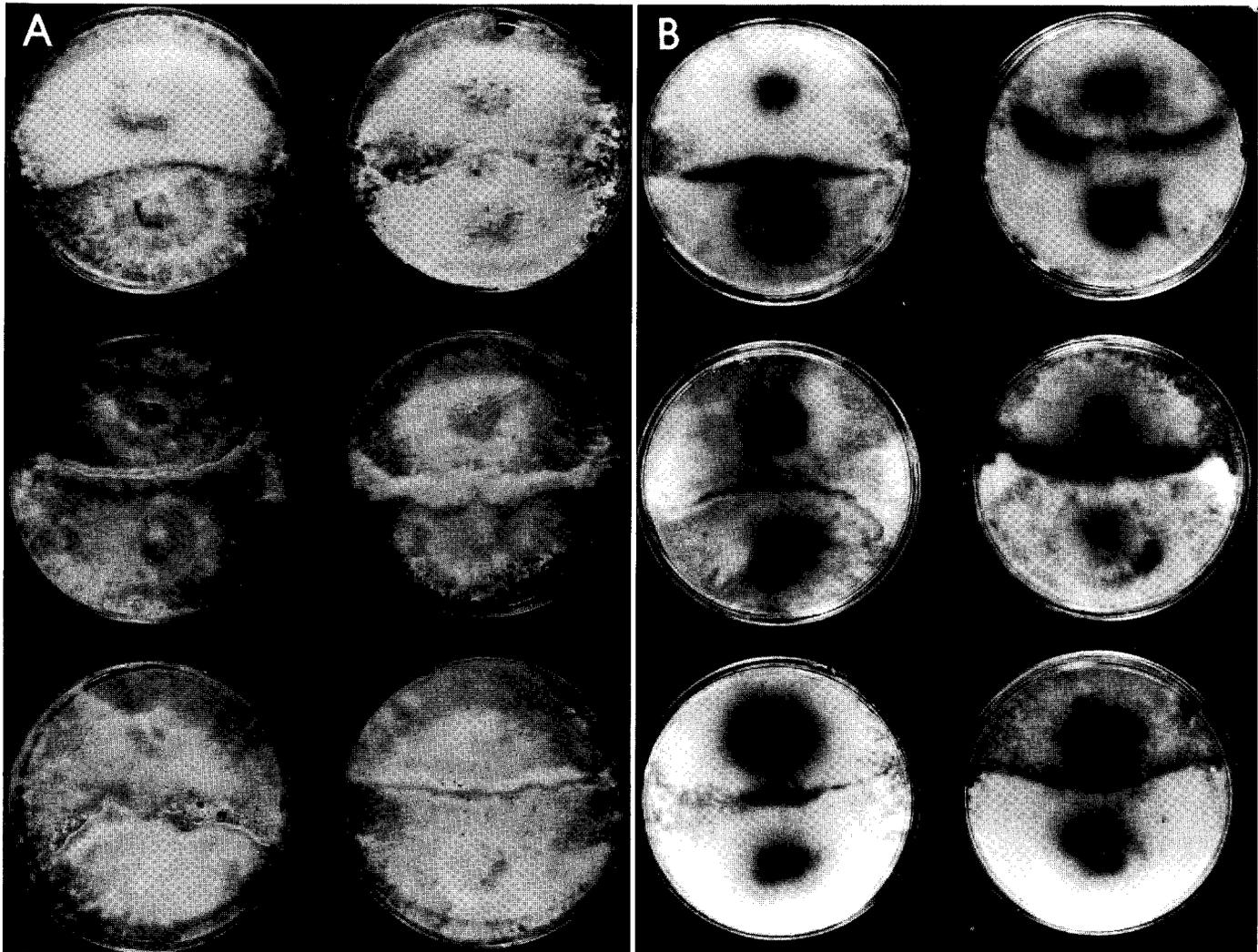


FIG. 3. Somatic incompatibility in di-mon pairings of *E. tinctorium* on 4.5% malt agar after 8–10 weeks of incubation at 21°C. (A) Hyphal massing between genetically related monokaryons and sib-composed dikaryons from the same parent (front view). (B) Dark reaction lines beneath the contact zones in the same plates (reverse view).

patric), between dikaryons from geographically separated sites (allopatric), and between sib-composed dikaryons with different genotypes. Similar results have been reported for pairings of heterogenic dikaryons of other wood decay fungi (3, 4, 28). The typical formation of dark reaction lines in culture between somatically incompatible dikaryons (carrying different mating alleles) appears to be synonymous with the formation of zone lines in wood between the decay columns of genotypically distinct strains of the same species and between different species. Visual signs of SI interactions, as zone lines in wood, have been observed with wood rotters such as *T. versicolor* (28) and *Armillaria* species (20). Several studies of white rot fungi have provided evidence to support the link between reaction lines in culture and zone lines in wood (3, 17). Zone lines delimit territorial boundaries between decay zones of fungi. The dark pigment likely represents accumulations of fungistatic secondary metabolites that prevent intrusive growth across the boundary between the decay columns of different fungi or between genetically different strains of the same species. The interaction may be a mechanism that allows the fungus in each decay column to secure a territory of its own within which it can carry on its activities unimpeded by competition,

allowing each fungus to coexist within the same tree. In cases where a fungus has already become well established in a tree, SI may prevent entry of other fungi. Many white rot fungi produce phenol oxidases and other oxidases that yield dark pigments, through oxidation of secondary metabolites, that may be toxic to fungi and account for the formation of dark reaction lines or zone lines.

The Buller phenomenon occurred in di-mon pairings of *E. tinctorium*, but it usually required long periods to develop, perhaps owing to the slow growth and SI interactions of the fungus. The slow dikaryotization of monokaryons might explain why only 54% of di-mon pairings were successful. Hyphal massing in the contact zone did not appear to preclude dikaryotization of monokaryons, although clamp connections formed less frequently (33%) when reaction lines were present. However, SI was more sporadic in di-mon pairings due to the relatively infrequent occurrence (27%) of reaction lines compared with dikaryotic pairings.

Di-mon pairings are generally expected to be compatible, resulting in dikaryotization of the monokaryon when the isolates are interfertile or share alleles for incompatibility. In the absence of SI, natural di-mon pairings could provide some

TABLE 1. Somatic incompatibility interactions in di-mon pairings of *Echinodontium tinctorium*

Monokaryotic isolates	No. of each genotype				Dikaryotic isolates ^a	Interactions ^b			Formation frequency ^c (%)
	A ₃ B ₃	A ₄ B ₄	A ₃ B ₄	A ₄ B ₃		CL	HM	RL	
61,66,70,75,76,67,66,67	8				A,A,B,B,B,E,G,G	-	-	-	22.5
72,80,65,74		4			A,A,B,B	-	-	-	
84			1		D	-	-	-	
64,71,68,71,71,71,81				7	A,B,D,D,E,F,F	-	-	-	
61	1				D	+	-	-	10.1
69		1			C	+	-	-	
78,63,87,88,63,63			6		A,B,B,B,C,E	+	-	-	
62				1	E	+	-	-	
66,67,75	3				B,B,F	++	-	-	16.9
73,80		2			D,G	++	-	-	
84,85,86,87,88,90,78,88,86,90			10		A,A,A,A,A,E,E,G,G	++	-	-	
67,67	2				A,D	-	+	-	5.6
69,65		2			D,E	-	+	-	
71				1	A	-	+	-	
61	1				B	-	+	+	17.9
83,73,83,73,83		5			D,E,E,F,F,	-	+	+	
90,84,87,89			4		D,E,E,F	-	+	+	
82,81,89,82,62,62				6	A,B,B,E,F,G	-	+	+	
79,78			2		E,F	+	+	-	4.5
64,82			2		B,B	+	+	-	
65,73		2			A,B	++	+	-	13.5
86,86,85,90,78,84			6		B,E,F,F,G,G	++	+	-	
89,81,82,71				4	A,E,F,G	++	+	-	
63,88,78			3		D,D,G	+	+	+	3.4
69,72		2			E,E,	++	+	+	5.6
87,85			2		D,G	++	+	+	
64				1	E	++	+	+	

^aDikaryotic isolates A-G had a common genotype, A₃A₄B₃B₄. The isolates are listed in the same order as the monokaryotic isolates with which they were paired.

^bCL, clamp connections; HM, hyphal massing in the contact zone; RL, reaction line(s); -, absent; +, weak to moderate reaction or formation of small to moderate numbers of clamp connections; ++, formation of abundant clamp connections.

^cFormation frequencies indicate the percentage of di-mon pairings with the indicated combination (pattern) of pairing interactions. The sum of formation frequencies for all interaction patterns is equal to 100%.

TABLE 2. Effects of pairing conditions on success of di-mon pairings and occurrence of somatic incompatibility interactions in *Echinodontium tinctorium*

Interaction type	Pairing conditions ^a	No. of pairings with interaction ^b	Total no. of pairings with conditions ^c	Frequency of occurrence ^d (%)
Clamp connections	All	48	89	53.9
	No SI	24	44	54.5
	HM	24	45	53.3
	RL	8	24	33.3
	HM or RL	24	45	53.3
	HM and RL	8	24	33.3
Somatic incompatibility	All	45	89	50.6
	CL	24	48	50.0
Hyphal massing	All	45	89	51.2
	CL	24	48	50.6
	RL	24	24	100.0
Reaction lines	All	24	89	27.0
	CL	8	48	16.7
	HM	24	45	53.3

^aAll, all pairing conditions; No SI, no somatic incompatibility interactions present; HM, hyphal massing present; RL, reaction lines present; HM or RL, either hyphal massing or reaction lines were present; HM and RL, both hyphal massing and reaction lines were present; CL, clamp connections present.

^bThe number of pairings in which the interaction occurred (under the indicated conditions).

^cTotal number of pairings in which the indicated condition(s) occurred.

^dThe percentage of total pairings in which the interaction occurred (under the indicated conditions).

genetic alternatives to the genotypes of dikaryotic mycelium (long established in decay columns) through anastomosis with monokaryotic hyphae (bearing new traits) that subsequently infect the tree. If newly infecting monokaryons become dikaryotized by the established dikaryon to form a new dikaryon that proliferates and forms basidiocarps, then the propagules of these new basidiocarps could perpetuate the traits in the population. By this mechanism, di-mon compatibility may provide an alternative means of introducing new traits into a population via dikaryotic thalli established in decay columns for many years. The mechanism would allow genetic changes to occur without sexual reproduction, and thus it could serve as a survival advantage to a fungus such as *E. tinctorium* that has a long reproductive cycle requiring an extensive decay column before basidiocarps can form and sexual recombination can occur.

Dikaryotic isolates of *E. tinctorium* from the Idaho and Arizona collection sites were previously shown to represent populations that have different sexual incompatibility genotypes (35). Somatic antagonism between heterogenic dikaryons appears to be a common feature of fungi possessing homogenic mating systems (2, 4, 8, 17, 28). The occurrence of SI between allopatric isolates with different mating alleles and the absence of SI in pairings of homogenic dikaryons suggests that some SI alleles may be linked to mating alleles. This hypothesis is supported by the occurrence of reactions resembling SI reactions in monokaryotic pairings of *E. tinctorium* (35) and in *F. pinicola* (27). However, sexual incompatibility reactions in mating studies, as in di-mon pairings, occurred between isolates sharing common mating alleles. Such antagonistic reactions may not be caused by mating alleles but by separate distinct genes associated with or identical to SI genes. The association between SI and mating alleles is further supported by the commonly observed intensification of antagonistic reactions as the genotypic similarity between mating types decreases (2, 3, 31, 32, 33). Brasier (6) and Rayner et al. (29) have suggested that the variability in SI reaction intensities can be explained by a polygenic control system. If SI alleles were shown to be linked to mating alleles, this would demonstrate that SI is under nuclear control. The mechanism and genetic basis of SI regulation in wood decay fungi and the role of cytoplasmic constituents are unknown.

Somatic interactions *in vitro* were shown here to be potentially useful in epidemiological studies of *E. tinctorium* to identify genetic similarities between dikaryons in living trees. The method is applicable to population studies for determinations or estimations of (i) the origin of the inoculum, (ii) the number of infections leading to decay in individual trees, (iii) the number of infection foci, (iv) the size of infection centers, (v) the distribution of individual clones (mating types) within infection centers, (vi) the mechanism(s) of dispersal within forest stands, and (vii) the rate of spread within individual trees and the expansion rate of infection centers (when populations are sampled over time). Some of these applications have been utilized in epidemiological studies of other wood decay fungi (1, 8, 21, 22, 30, 31, 32).

Studies comparing somatic and sexual incompatibility methods for determining population structure in the *A. mellea* complex have indicated that the two methods produce consistent results (21, 22). SI studies of intraspecific antagonism in bifactorial (tetrapolar) species provide good approximations of the genetic similarity between dikaryotic genotypes, but unlike mating studies, they do not provide detailed information on

the specific genotypes involved without prior identification of mating alleles in reference strains from the population. One can often determine clonal size and detect differences in dikaryotic genotypes using SI studies, but one cannot determine with any certainty which and how many alleles are different without mating studies. However, both methods often can be used equally well to identify genotypes in unifactorial (bipolar) species. Despite the limitations in bifactorial fungi, somatic studies are usually less time consuming and sometimes less ambiguous than mating studies since microscopic examinations are not necessary. Studies of somatic antagonism also are more feasible than mating studies for large-scale surveys of clonal (genet) distributions.

The potential benefits of heterogenic SI interactions to wood decay fungi, such as protection against infectious agents (mycoviruses) and maintenance of successful (adapted) genotypes, have been discussed previously (7, 19). SI systems also restrict outbreeding and have the opposite effect of multi-allelic, homogenic mating systems (16). Perhaps the common close association of heterogenic SI systems with homogenic mating systems provides a balance between the enhancement of outbreeding and inbreeding. The operation of opposing systems may optimize the survival potential of a fungus by reducing the rate of speciation and increasing adaptability over a wider region (through multiple allelomorphs) yet perpetuate the genotypes of populations successfully adapted to localized environments (through heterogenic SI systems).

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1. ADAMS, D. H. 1974. Identification of clones of *Armillaria mellea* in young-growth ponderosa pine. *Northwest Sci.* **48**: 21–28.
2. ADAMS, D. H., and ROTH, L. R. 1967. Demarcation lines in paired cultures of *Fomes cajanderi* as a basis for detecting genetically distinct mycelia. *Can. J. Bot.* **45**: 1583–1589.
3. ADASKAVEG, J. E., and GILBERTSON, R. L. 1987. Vegetative incompatibility between intraspecific dikaryotic pairings of *Ganoderma lucidum* and *G. tsugae*. *Mycologia*, **79**: 603–613.
4. BARRETT, D. K., and USCUPPIC, M. 1971. The field distribution of interacting strains of *Polyporus schweinitzii* and their origin. *New Phytol.* **70**: 581–598.
5. BOYCE, J. S. 1923. A study of decay in Douglas fir in the Pacific Northwest. U.S. Dep. Agric. Bull. No. 1163. pp. 1–19.
6. BRASIER, C. M. 1984. Inter-mycelial recognition systems in *Ceratocystis ulmi*: their physiological properties and ecological importance. In *The ecology and physiology of the fungal mycelium*. Edited by V. H. Jennings and A. D. M. Rayner. Cambridge University Press, Cambridge. pp. 451–497.
7. CATEN, C. E. 1972. Vegetative incompatibility and cytoplasmic infection of fungi. *J. Gen. Microbiol.* **72**: 221–229.
8. CHILDS, T. W. 1963. *Poria weirii* root rot. *Phytopathology*, **53**: 1124–1127.
9. ETHERIDGE, D. E. 1972. True heartrots of British Columbia. *Can. For. Serv. Pac. For. Res. Cent. For. Pest Leaflet*. No. 55.
10. ETHERIDGE, D. E., and CRAIG, H. M. 1976. Factors influencing infection and initiation of decay by the Indian paint fungus (*Echi-*

- nodontium tinctorium*) in western hemlock. Can. J. For. Res. **6**: 299–318.
11. ETHERIDGE, D. E., CRAIG, H. M., and TAYLOR, L. D. 1970. Factors affecting suitability of western hemlock as a substrate for spore germination and growth of the Indian paint fungus. Northwest Sci. **44**: 244–252.
 12. ETHERIDGE, D. E., CRAIG, H. M., and FARRIS, S. H. 1972. Infection of western hemlock by the Indian paint fungus via living branches. Can. For. Serv. Bi-mon. Res. Notes, **28**(1): 3–4.
 13. FOSTER, R. E., CRAIG, H. M., and WALLIS, G. W. 1954. Decay of western hemlock in the upper Columbia region, British Columbia. Can. J. Bot. **32**: 145–171.
 14. GILBERTSON, R. L. 1980. Wood-rotting fungi of North America. Mycologia, **72**: 1–49.
 15. GILBERTSON, R. L. 1981. North American wood-rotting fungi that cause brown rots. Mycotaxon, **12**: 372–416.
 16. GILBERTSON, R. L., and RYVARDEN, L. 1986. North American polypores. Vol. 1. Fungiflora, Oslo, Norway.
 17. GOLDSTEIN, D., and GILBERTSON, R. L. 1981. Cultural morphology and sexuality of *Inonotus arizonicus*. Mycologia, **73**: 167–180.
 18. HANSEN, E. M. 1979. Sexual and vegetative incompatibility reactions in *Phellinus weirii*. Can. J. Bot. **57**: 1573–1578.
 19. HARTIL, D. L., DEMPSTER, E. R., and BROWN, S. W. 1975. Adaptive significance of vegetative incompatibility in *Neurospora crassa*. Genetics, **81**: 553–569.
 20. HOOD, I. A., and MORRISON, D. J. 1984. Incompatibility testing of *Armillaria* isolates in a wood substrate. Can. For. Serv. Res. Notes, **4**: 8–9.
 21. KILE, G. A. 1983. Identification of genotypes and the clonal development of *Armillaria luteobubalina* Watling & Kile in eucalypt forests. Aust. J. Bot. **31**: 657–671.
 22. KORHONEN, K. 1978. Interfertility and clonal size in the *Armillariella mellea* complex. Karstenia, **18**: 31–42.
 23. MALOY, O. C. 1961. Sporulation and sporophore survival studies on *Echinodontium tinctorium*. Northwest Sci. **35**: 160–161.
 24. MALOY, O. C. 1963. Sporulation and sporophore survival of *Echinodontium tinctorium*. Plant Dis. Rep. **47**: 627–631.
 25. MALOY, O. C. 1967. A review of *Echinodontium tinctorium* Ell. & Ev., the Indian paint fungus. Wash. Agric. Exp. Stn. Bull. No. 686.
 26. MALOY, O. C., and GROSS, H. L. 1963. Decay in young grand fir. J. For. **61**: 850–853.
 27. MOUNCE, I. 1929. Studies in forest pathology. II. The biology of *Fomes pinicola* (SW) Cooke. Can. Dep. Agric. Bull. No. 111. pp. 1–56.
 28. RAYNER, A. D. M., and TODD, N. K. 1978. Polymorphism in *Coriolus vesicolor* and its relation to interfertility and intraspecific antagonism. Trans. Br. Mycol. Soc. **71**: 99–106.
 29. RAYNER, A. D. M., COATES, D., AINSWORTH, A. M., ADAMS, J. J. H., WILLIAMS, E. N. D., and TODD, N. K. 1984. The biological sequences of the individualistic mycelium. In The ecology and physiology of the fungal mycelium. Edited by V. H. Jennings and A. D. M. Rayner. Cambridge University Press. Cambridge. pp. 509–540.
 30. SHAW, C. G., III, and ROTH, L. F. 1976. Persistence and distribution of a clone of *Armillaria mellea* in a ponderosa pine forest. Phytopathology, **66**: 1210–1214.
 31. STENLID, J. 1985. Population structure of *Heterobasidium annosum* as determined by somatic incompatibility, sexual incompatibility, and isoenzyme patterns. Can. J. Bot. **63**: 2268–2273.
 32. THOMPSON, W., and RAYNER, A. D. M. 1982. Spatial structure of a population of *Tricholomopsis platyphylla* in a woodland site. New Phytol. **92**: 103–114.
 33. TODD, N. K., and RAYNER, A. D. M. 1978. Genetic structure of a natural population of *Coriolus versicolor* (L. ex Fr.) Quéf. Genet. Res. **32**: 55–65.
 34. WILSON, A. D. 1988. Advances in the life history of the Indian paint fungus, *Echinodontium tinctorium* (Ell. & Ev.) Ell. & Ev. Ph.D. dissertation, Department of Plant Pathology, Washington State University, Pullman.
 35. WILSON, A. D. 1990. The genetics of sexual incompatibility in the Indian paint fungus, *Echinodontium tinctorium*. Mycologia, **82**: 332–341.