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Neurospora in temperate forests of western North America

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Abstract: The fungal genus *Neurospora* has a distinguished history as a laboratory model in genetics and biochemistry. The most recent milestone in this history has been the sequencing of the genome of the best known species, *N. crassa*. The hope and promise of a complete genome sequence is a full understanding of the biology of the organism. Full understanding cannot be achieved, however, in the absence of fundamental knowledge of natural history. We report that species of *Neurospora*, heretofore thought to occur mainly in moist tropical and subtropical regions, are common primary colonizers of trees and shrubs killed by forest fires in western North America, in regions that are often cold and dry. Surveys in 36 forest-fire sites from New Mexico to Alaska yielded more than 500 cultures, 95% of which were the rarely collected *N. discreta*. Initial characterization of genotypes both within a site and on a single tree showed diversity consistent with sexual reproduction of *N. discreta*. These discoveries fill important gaps in knowledge of the distribution of members of the genus on both large and small spatial scales and provide the framework for future studies in new regions and microhabitats. The overall result is that population biology and genetics now can be combined, placing the genus *Neurospora* in a unique position to expand its role in experimental biology as a useful model organism for ecology, population genetics and evolution.

Key words: ecology, fire, fungi, natural history, Neurospora, temperate forests

INTRODUCTION

Species of *Neurospora* possess life cycles adapted to respond to fire. Beginning with the earliest reports, most isolates have been obtained from vegetation killed by fire. The importance of fire is twofold. First, fire produces a sterile environment rich in nutrients derived from dead plant tissue, a particularly suitable substrate for *Neurospora*. Second, fire provides heat and chemical byproducts necessary for ascospore germination, a requirement mirrored in the standard laboratory practice of treating ascospores with either heat or furfural to effect germination (Davis and de Serres 1970, Sussman 1969).

One of the earliest accounts of *Neurospora* was from burned pines after the Tokyo fire of 1923 (Kitazima 1925, Perkins 2002). This early report, however, contrasts with the distribution of subsequent collections in terms of both substrate and latitude. Although circumglobal in distribution, nearly all isolates have come from tropical and subtropical regions. In the United States, most isolates have been obtained from southeastern states, particularly Florida and Louisiana. Globally, the majority of isolates have been collected from burnt grasses, but collections from scorched woody shrubs and cooked food are not uncommon (Turner et al 2001, Perkins and Turner 1988).

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During a trip to the Florida Everglades after highly publicized fires in the spring of 1999, we made numerous observations of extensive growth of species of *Neurospora* and other fungi beneath the bark of shrubs that had been killed by fire (Powell et al 2003). The dead phloem and cambium tissues beneath bark provide early saprophytic colonizers with a microenvironment devoid of competitor microorganisms and rich in nutrients and moisture. A series of fires in the cottonwood-dominated forest (bosque) along the Rio Grande in central New Mexico during the spring of 2000 presented an opportunity to examine this microenvironment on diverse tree and shrub species. Our survey led to the surprising discovery of species of *Neurospora* in the Rio Grande bosque, a riparian habitat in an otherwise arid, semidesert environment. Subsequent surveys from 2000 through 2002 across western North America revealed that *Neurospora* is a common primary colonizer of trees and shrubs killed by forest fires.

The observations reported here are noteworthy in the context of the historic role *Neurospora* plays in experimental biology. They point to the need for more complete knowledge of the fundamental biology of members of the genus, in part to fulfill the potential of the recently acquired complete genome sequence of *N. crassa* (Galagan et al 2003).

MATERIALS AND METHODS

Forest-fire sites.—Selection of sites was somewhat arbitrary, depending on timing and accessibility. A concerted effort was made to survey a large geographic range to discover the distribution limits of *Neurospora*. Major forest fires were located and conditions monitored with the aid of a Website (www.nifc.gov/fireinfo/nfn.html) and links therein. Sites first were surveyed anywhere from 6 d to 6 wk after a fire.

Isolate collection, culturing and identification.—*Neurospora* species were collected in the field as described by Perkins and Turner (1988). A small piece of sterile filter paper, prepackaged in a sterile envelope, was pressed against a conidiating colony, resulting in a smear of conidia. The filter paper then was resealed into the envelope. Isolates were obtained from beneath the bark of fire-killed trees and shrubs. Colonies were revealed by peeling the bark (FIG. 1B, C, D). Collections generally were made along a linear transect through a burn site. Each isolate listed in TABLE I was collected from a separate tree or shrub.

Collection envelopes were frozen at −20 C for at least 24 h to kill arthropods. A primary culture was made by cutting a small piece of filter paper containing conidia and placing it in a 10 × 75 mm tube containing 1 mL Vogel's minimal medium N (Vogel 1964) with 200 μg/mL chloramphenicol added to prohibit bacterial growth. Cultures were incubated 2 d at 34 C or until good conidiation was observed. To ensure that single genetic individuals would be character-

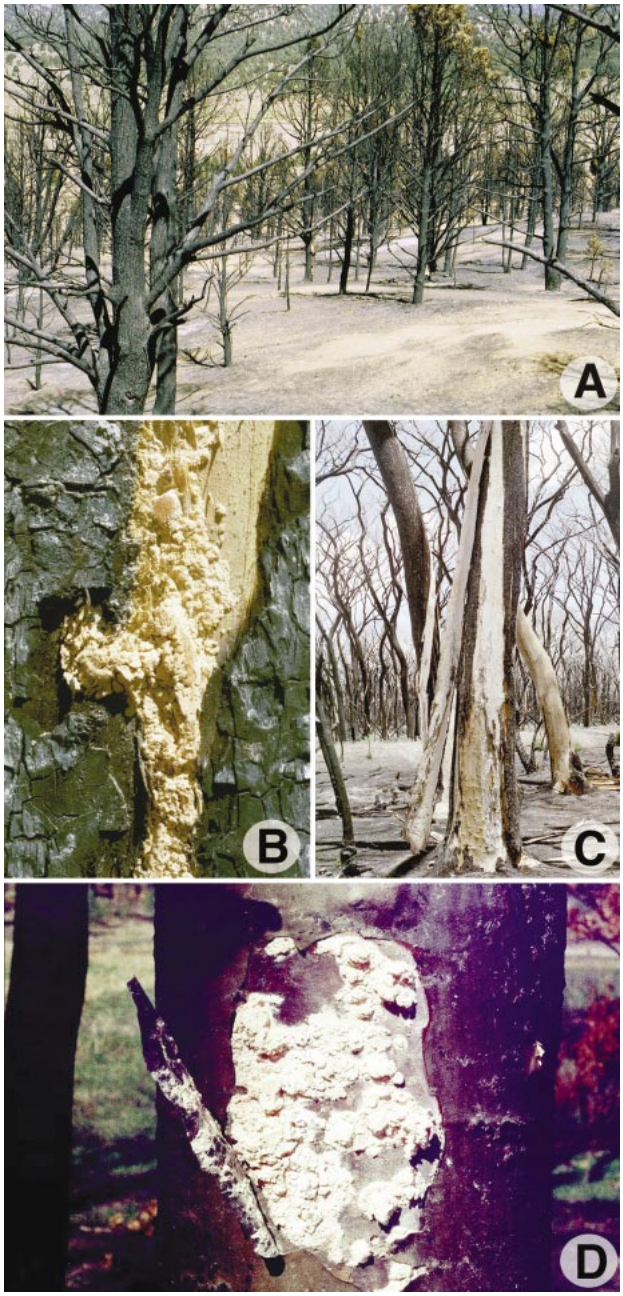

FIG. 1. *Neurospora* habitats in western North America. A. Kennedy Meadows, California. Typical wildfire site where collections were made. The predominate tree species here was single-leaf piñon pine (*Pinus monophylla*). Note that charred bark on most trees is intact. *Neurospora* was sporadic but common at this site. B and C. On eastern cottonwood (*Populus deltoides*), Los Lunas site, New Mexico. B. *Neurospora* sp. was observed to grow through intact bark, pushing out scales of the charred, outermost bark 6 wk after the fire. C. Bark was peeled to reveal growth to greater than 6 m above ground, 3 wk after the fire. D. On quaking aspen (*Populus tremuloides*), 3 wk after the fire, Perma site 1, Montana.

TABLE I. Geographic and mating-type distributions of isolates of *Neurospora* collected on trees and shrubs in western North America from 2000 through 2002[a]

State	Site	Latitude	Elevation	Number of isolates[b]				
				N. discreta		*N. sitophila*		*N. crassa*
				mat A	*mat a*	*mat A*	*mat a*	*mat a*
New Mexico	La Joya	34°25′	1430 m	9	9			
	Belen	34°38′	1445 m	13	12	7	0*	
	Los Lunas	34°46′	1450 m	16	5*	13	2*	
	Bernalillo	35°18′	1553 m	10	14	0	1	
	Los Alamos 1	35°35′	2326 m	0	1	1	0	
	Los Alamos 2	35°35′	2327 m	4	7			
	Pecos	35°39′	2485 m	9	2*			
California	Manter Meadow	35°53′	2196 m	5	2			
	Kennedy Meadow	36°0′	1906 m	28	5*			
	Summit Rd.	37°3′	750 m	0	3			
	Chiquita Loma	37°6′	739 m	6	5			
	Coleville	38°32′	1778 m	14	20			
	Murphy Meadows	39°19′	2399 m	21	13			
	Manzanita Slope	39°21′	2160 m	3	3			
	Iceland Rd	39°22′	1681 m	21	15			
	Plumas	40°0′	701 m	0	3			
	Susanville	40°24′	1194 m	7	2			
	Hayfork	40°33′	620 m	0	2			
	Weaverville	40°43′	709 m	17	12			
Nevada	Wells	41°12′	1952 m	17	11			
Wyoming	Yellowstone	44°30′	2450 m	1	0			
Idaho	Yankee Fork	44°21′	2147 m	5	1			
	Blackbird Creek	45°5′	1647 m	19	4*			
	Napais Creek	45°8′	1485 m	4	1			
	Panther Creek Rd	45°18′	1002 m	1	0			
Washington	Chelan Lake	47°57′	680 m	23	7*			
Montana	Laird Creek	45°52′	1366 m	3	3			
	Sula 18 mile	45°52′	1302 m	4	0			
	Skalkaho	46°9′	1220 m	0	1			
	Blodgett Trailhead	46°14′	1357 m	5	0			
	Perma site 1	47°21′	780 m	3	10*			
	Perma site 2	47°23′	930 m	3	22*			2
	Rexford	48°49′	945 m	0	0	1	0	
	Turner Creek Rd	48°52′	1247 m	1	0			
	Northwest Peaks	48°59′	1912 m	1	0			
Alaska	Tok	63°21′	515 m	21	29			
Total				294	224	22	3	2

[a] Partial host list (locations given in parentheses by US state abbreviation): *Abies concolor* (white fir, CA), *Acer* sp. (maple, CA, WA), *Alnus rhombifolia* (white alder, CA), *Alnus incana* (mountain alder, MT, WA), *Amelanchier alnifolia* (service berry, ID, MT, WA), *Arbutus menziesii* (Pacific madrone, CA), *Arctostaphylos* sp. (manzanita, CA), *Cercocarpus ledifolius* (curlleaf Cercocarpus, CA), *Chilopsis linearis* (desert willow, NM), *Chrysolepis chrysophylla* var. *minor* (golden chinquapin, CA), *Crataegus douglasii* (black hawthorne, ID), *Cystisus scoparius* (Scotch broom, CA), *Juniperus osteosperma* (Utah juniper, NV), *Juniperus scopulorum* (Rocky Mountain juniper, NM, MT), *Picea* sp. (spruce, NM), *Picea mariana* (black spruce, AK), *Pinus attenuata* (knobcone pine, CA), *Pinus contorta* (lodgepole pine, CA, WY, MT), *Pinus jeffreyi* (Jeffrey pine, CA), *Pinus monophylla* (singleleaf pinyon pine, CA, NV), *Pinus ponderosa* (ponderosa pine, NM, MT), *Populus deltoides* (cottonwood, NM), *Populus tremuloides* (aspen, NM, CA, ID, MT, WA), *Populus trichocarpa* (black cottonwood, CA, ID, WA), *Prunus emarginata* (bitter cherry, CA), *Pseudotsuga menziesii* (Douglas fir, ID, MT), *Quercus grisea* (gray oak, NM), *Salix* sp. (willow, NM, CA, ID, MT, WA), *Thuja plicata* (western red cedar, MT).

[b] An asterisk (*) denotes instances where the mating type *mat A:mat a* numerical ratio deviated significantly ($P < .05$) from 1:1 (Perkins 1994). Only one sample per tree or shrub is included in this table. Additional *N. discreta* isolates collected but not listed in this table included 9 from a single tree in Tok, AK (5 *mat A*, 4 *mat a*; see TABLE II), 8 from a single tree at Chelan Lake, WA (0 *mat A*, 8 *mat a*), 7 from a single tree at Chiquita Loma, CA (4 *mat A*, 3 *mat a*), 8 from two trees at Iceland Rd., CA (6 *mat A*, 2 *mat a*), and 15 from two shrubs at Weaverville, CA (11 *mat A*, 4 *mat a*).

ized, single conidium subcultures were isolated from each of the primary cultures, essentially as described by Jacobson (1995). Mass transfers of hyphae of each single conidium subculture was made to new tubes of complete medium for storage at −20 C.

Strains of *Neurospora* were assigned to species based on mating success with species tester strains (Perkins and Turner 1988). The isolates from New Mexico initially were crossed sequentially with species testers. As it became evident that most isolates were *N. discreta*, isolates first were crossed with *N. discreta* tester strains. Only if that test was negative were crosses to other testers made. Representative isolates of each species found at each site (including both mating types when possible) were deposited at the Fungal Genetics Stock Center (FGSC), University of Kansas Medical Center, Kansas City, Kansas 66160 (www.FGSC.net), accession numbers 8548–8591, 8978–8995 and 8999–9000.

Characterization of frq *and* het-c.—The autosomal loci *frq* (linkage group VII) and *het-c* (linkage group IIL) have proven valuable in characterizing the relationship among natural isolates of *Neurospora* (Powell et al 2003, 2001, Wu et al 1998). Previous surveys of *frq*, an essential component of the circadian oscillator in *Neurospora* (Dunlap 1999), have demonstrated that the upstream portion exhibits substantial polymorphism, even among closely related members of the same species (Gallegos et al 2000). *het-c* is one of 11 heterokaryon incompatibility genes in *N. crassa*, which function in somatic self recognition during vegetative growth (Glass et al 2000). Previous studies have offered insight into the population dynamics of *het-c* (Powell et al 2001, Wu et al 1998). In the present study, partial sequences of both loci were obtained from 33 isolates of *N. discreta*. Twenty-four of the isolates were collected in Bernalillo, New Mexico, each from a different tree, and nine were collected in Tok, Alaska, sampled vertically from a single tree (TABLE II).

Portions of *het-c* and *frq* were amplified by polymerase chain reaction (PCR) followed by direct sequencing of amplicons. Genomic DNA was isolated following standard procedures (Gallegos et al 2000). PCR products were prepared for sequencing using Amicon® Microcon®-PCR Centrifugal Filter Devices, per the manufacturer's specifications. Nucleotide sequences were obtained using dideoxy dye-terminator chemistry (ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA polymerase, FS) and a model 377 automated sequencer, in accordance with instructions supplied by the manufacturer (Perkin-Elmer). Sequence data were processed and analyzed with Sequencher™ software (version 3.1.1) and Clustal W, a Web-based alignment tool (Thompson et al 1994). Alignments were refined via visual inspection.

The target region of *frq* was amplified with primers 5′ TCT CTC CTC AAT TTT GGC CTG G 3′ and 5′ GCG AGA TAC TAG CAG CAG AG 3′. With respect to the *N. crassa* GenBank entry (accession U17073), these primers correspond respectively to nucleotides 444–465 and 1561–1542. The product produced using this primer pair was 1117 nucleotides in length. Purified PCR products were sequenced using the first primer listed above and a third primer with this sequence: 5′ CTT CTG ACT CGA CCC TTT 3′. This latter sequence corresponds to nucleotides 774–791 in the GenBank entry.

TABLE II. Mating type, *het-c* and *frq* alleles of isolates of *N. discreta* from two populations

Isolate number	Mating type	*het-c* allele[a]	*frq* allele[b]
Bernalillo, New Mexico (one isolate per tree)			
w787	*mat A*	Groveland	*frq1*
w790	*mat A*	Groveland	*frq1*
w782	*mat A*	Oak Ridge	*frq1*
w783	*mat A*	Oak Ridge	*frq1*
w784	*mat A*	Oak Ridge	*frq1*
w789	*mat A*	Oak Ridge	*frq1*
w791	*mat A*	Oak Ridge	*frq3*
w795	*mat A*	Oak Ridge	*frq1*
w799	*mat A*	Oak Ridge	*frq1*
w800	*mat A*	Oak Ridge	*frq1*
w777	*mat a*	Groveland	*frq2*
w778	*mat a*	Groveland	*frq1*
w780	*mat a*	Groveland	*frq1*
w781	*mat a*	Groveland	*frq1*
w785	*mat a*	Groveland	*frq3*
w793	*mat a*	Groveland	*frq1*
w796	*mat a*	Groveland	*frq1*
w801	*mat a*	Groveland	*frq1*
w802	*mat a*	Groveland	*frq1*
w779	*mat a*	Oak Ridge	*frq1*
w786	*mat a*	Oak Ridge	*frq1*
w792	*mat a*	Oak Ridge	*frq1*
w794	*mat a*	Oak Ridge	*frq1*
w798	*mat a*	Oak Ridge	*frq1*
Tok, Alaska (vertical sample up a single tree)			
w854	*mat A*	Groveland	*frq2*
w855	*mat A*	Groveland	*frq2*
w856	*mat A*	Groveland	*frq2*
w860	*mat A*	Groveland	*frq2*
w861	*mat A*	Groveland	*frq4*
w857	*mat a*	Groveland	*frq2*
w858	*mat a*	Groveland	*frq2*
w859	*mat a*	Groveland	*frq2*
w853	*mat a*	Oak Ridge	*frq2*

[a] Assignment of *N. discreta het-c* alleles to functional classes (Groveland or Oak Ridge) is based on sequence similarity with *N. crassa* alleles (Wu et al 1998). Functional specificity has not been determined experimentally.

[b] Allelic variation in the region upstream of the *frq* gene is represented by numbers that indicate sequence variants. All *frq* variation was represented by minor insertion/deletion events and base substitutions in non-coding regions, confirmed by sequencing in forward and reverse directions.

The *het-c* region amplified included the region of functional specificity and flanking sequences. The *N. crassa het-c*^{OR} sequence (strain 74-OR23-IV*A*, GenBank accession L77234) served as template for the design of primers: 5′ CAC CAG TGC CGG CTA TAT TCG 3′ and 5′ CTA GCA ACG ATG GAG ACT TTA TC 3′, which correspond respec-

tively to nucleotides 1087–1107 and 1606–1584. The precise product size was a function of the particular functional allele amplified. Nucleotide sequence data for the *het-c* purified PCR products were generated with the primers used for amplification.

The partial *frq* and *het-c* sequences reported here have been archived at GenBank under accession AY254004–AY254036 and AY251910–AY251942, respectively.

RESULTS

Distribution of Neurospora *spp.*—In 2000 and 2001, we observed species of *Neurospora* that had colonized beneath the bark of trees at four bosque sites over a distance of approximately 110 km. In some stands of fire-killed, mature cottonwoods, every tree was heavily colonized (FIG. 1). Our initial discovery of *Neurospora* in New Mexico raised the possibility that the Rio Grande bosque might be a refuge for subtropical fungal species. The riparian environment near the river extends to the Gulf of Mexico and stands in contrast to the surrounding scrub and grasslands. However, surveys of 36 burn sites throughout the western United States from 2000 to 2002, including sites in California, Nevada, northern New Mexico, Idaho, Washington, Montana and Alaska, revealed the presence of *Neurospora* species in conifer forests and riparian woodlands over this entire range of greater than 4000 km (TABLE I, FIG. 2). We observed *Neurospora* at most of the burn sites that were surveyed (36 of 41). The sites sampled ranged in elevation from 515 m to >2400 m. Isolates collected in these forests therefore represent new records for species of *Neurospora* in terms of both latitude and elevation.

Despite extensive efforts to find *Neurospora* species on scorched grasses and herbaceous plants, known to be suitable substrates in the tropics and subtropics, all colonies observed in western forests were found beneath the bark of woody species. In contrast, we observed little or no host specialization. Species were observed beneath the bark of at least 29 species of conifers and angiosperms, trees and shrubs (TABLE I).

Of the 545 individuals collected from separate plants and identified to species, 518 (95%) were *N. discreta*, based on mating behavior (TABLE I). Twenty-five individuals of *N. sitophila* were collected, 24 of them from four sites in New Mexico. Only two individuals of *N. crassa* were identified from a single site in Montana (Perma site 2), but they were collected at different times, Sep 2000 and Jul 2001. Three other isolates of *N. discreta* also were collected from this site 9 mo apart. Although the site sustained a single fire event, vegetative structures of *Neurospora* persisted and remained viable during the harsh winter. The

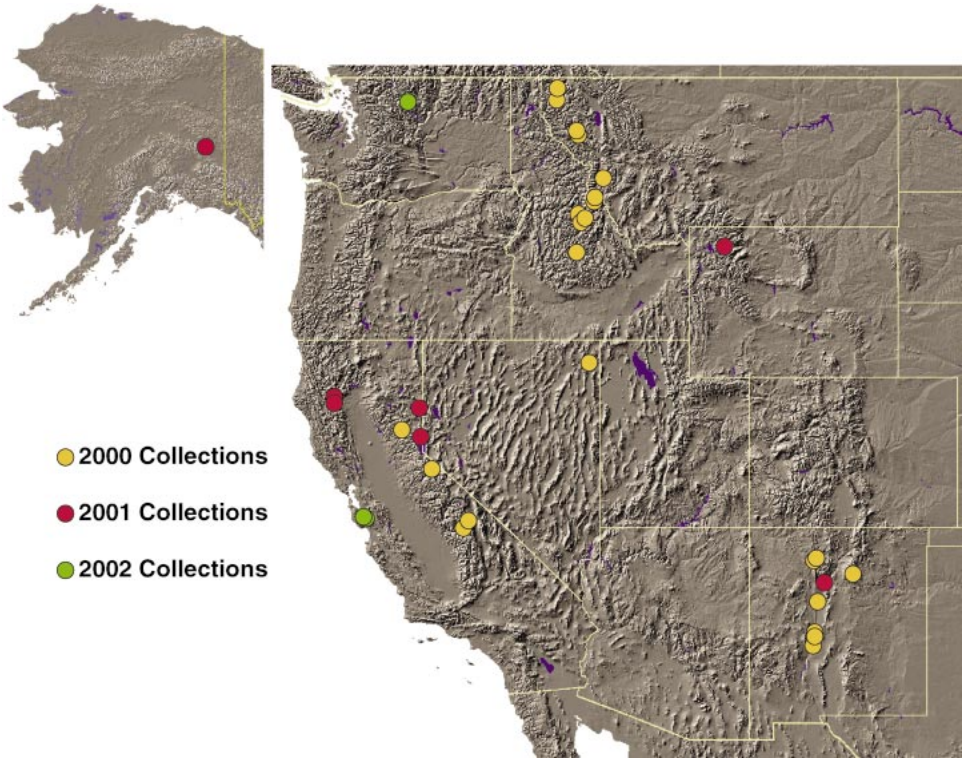

FIG. 2. Sites where *Neurospora* was collected in western North America from 2000 through 2002. Each collection site is represented by a colored circle; details of each collection and site are listed in TABLE I.

Weaverville, California, site also was visited after a winter had passed and again vegetative *Neurospora* colonies were observed (data not shown).

Population genetics.—Both mating types (*mat A* and *mat a*) were present in forest habitats at most sites, although the ratio of *mat A* to *mat a* occasionally was skewed from 1:1 (TABLE I). The presence of both mating types suggests a potential for sexual reproduction, as has been noted for tropical areas. In extensive examinations of several burn sites ca 6 wk after the fire, we observed sexual fruiting bodies (perithecia) only once.

To further examine the possibility of sexual reproduction and to give a preliminary assessment of genetic diversity among *N. discreta* isolates, two additional markers were characterized for a subset of individuals. Genetic diversity within and among collection sites, and even among isolates from a single tree, was demonstrated by sequence analysis of the specificity domain of the *het-c* incompatibility locus (Wu et al 1998) (TABLE II). As has been observed in studies of other species from other locations (Wu et al 1998, Powell et al 2001), isolates of *N. discreta* from sites in western North America possessed different ancient alleles at the *het-c* locus (TABLE II). Moreover, isolates of *N. discreta* of the same mating type from a given location also possessed different functional *het-c* alleles (TABLE II), indicating that isolates of a given mating type were not all derived clonally from a single individual. Analysis of DNA sequences upstream of the *frq* gene, which is involved in circadian control and therefore independent of heterokaryon incompatibility (Loros and Dunlap 2001), revealed additional variation among *N. discreta* isolates examined from New Mexico and Alaska, even on small spatial scales (TABLE II). At the same time, the *frq* sequences confirmed that isolates of *N. discreta* were closely related relative to isolates from other species (analysis not presented). Taken together, the results from mating type, *het-c* and the *frq* upstream region support the conclusion that *N. discreta* isolates from western North America represent a monophyletic group within which local sexually reproducing populations exist.

DISCUSSION

Western forests in temperate North America do not simply represent a newly recognized habitat for members of the genus *Neurospora*. Isolates from these forests also dramatically expand the geographical distribution of the heretofore rarest species, *N. discreta* (Turner et al 2001, Perkins and Raju 1986). Ninety-five percent of them have been assigned to this species, based on mating behavior and molecular-genetic analyses. The first collections of *N. discreta* had been made in Jasper County, Texas, in 1977 (Perkins and Raju 1986). Before the work reported here, only 143 of 4666 total isolates (3%) obtained globally were *N. discreta* (Turner et al 2001). In contrast, the two heterothallic species observed most often in the southeastern United States and globally, *N. tetrasperma* and *N. intermedia*, have not yet been collected from western forests; while *N. sitophila* and *N. crassa*, also common among global isolates, were collected only rarely in the current survey (TABLE I).

Our study raises two questions for which only partial answers exist: (i) What are the reservoirs of spores and/or mycelia in the habitats examined, when fire has been excluded for decades? and (ii) What are the mechanisms by which dispersal and colonization take place so quickly after a fire? In being both ubiquitous and at the same time unseen in the absence of fire, species of *Neurospora* in western forests present a paradox in distribution. *Neurospora* was present at most of the burn sites that were surveyed (36 of 41). Many sites were visited within 7–10 d after the fire. The rapid appearance and frequency of these fungi imply at least one local reservoir of vegetative and/or reproductive structures (mycelium and spores). One such reservoir appears to be soil (Pandit and Maheshwari 1996). We have recovered *Neurospora* from soil from burned and unburned sites in the Rio Grande bosque (data not shown). It is difficult to understand, however, how soil-borne ascospores could serve as the only source of inoculum after fires, particularly in forest stands where fire has been excluded for decades or even hundreds of years.

Although the recovery of *Neurospora* species from soil suggests a reservoir of ascospores, the growth we have observed in the field is almost entirely asexual, with abundant production of mycelia and conidia but not ascospores. As mentioned, direct observation of sexual fruiting bodies (perithecia) occurred only once. Nevertheless, we have obtained ascospores from crosses in the laboratory, indicating the existence of viable mating populations (data not shown) and there is strong circumstantial evidence that these populations reproduce sexually and produce viable ascospores (see Results).

The observed distribution of *Neurospora* within sites suggests rapid primary colonization, possibly involving ascospores, followed by the rapid production of conidia and extensive secondary colonization resulting from conidial dispersal. The mode of primary colonization remains a particular mystery. At some Rio Grande bosque sites, for instance, nearly every cottonwood killed by fire had extensive growth under

the bark (FIG. 1) within 7–10 d. If primary colonization resulted from soil-borne ascospores, which were sufficiently deep to survive a burn, it is hard to conceive a mechanism by which such ascospores were dispersed above ground so quickly. It is even more problematic to suppose that colonization resulted from ascospores or other fungal propagules already present on tree surfaces. Fire temperatures at the sites surveyed were hot enough to burn outer layers of the bark, as well as kill tissues below the bark, resulting in tree death. It is likely that fungal structures present on or in the bark also would have been killed. Although ascospores can survive temperatures of up to 67 C for 200 min, and desiccated conidia can survive temperatures above 100 C (Lindegren 1932, Fahey et al 1978), it is likely that neither can withstand high temperatures associated with fires (Whelan 1995). Endophytic hyphae or conidia in tissues below the bark possibly could survive a fire. There is to date, however, no evidence for species of *Neurospora* growing as endophytes.

Mode of primary colonization aside, the rapid spread of *Neurospora* species at a given site suggests secondary colonization by conidia. Several observations point to dispersal by microfauna, as opposed to air currents alone. First, colonization often was observed under sections of intact bark, apparently shielded from airborne spores in the vicinity of colonization. Second, on many trees, we observed a continuous mass of mycelium under bark from ground level to more than 7 m within 10 d of fire (FIG. 1). The maximum growth for *Neurospora* in the laboratory is 4 mm/h (Perkins and Pollard 1986), which in 10 d would produce linear growth of only ca 1 m. Finally, the moist environment beneath charred bark often teems with larvae, small insects, mites and isopods, suggesting substantial opportunity for dispersal by faunal vectors.

These results highlight the potential for exploiting the genus *Neurospora* in addressing questions in population biology, evolution and ecology. Our studies, in time, overlapped efforts to acquire the complete sequence of the *N. crassa* genome (Galagan et al 2003). While the acquisition of this genome sequence makes an important step toward the goal of developing species of *Neurospora* as complete model organisms, it is clear that much remains to be learned about fundamental aspects of these organisms.

Among complex eukaryotes, the genus *Drosophila* has been a versatile model not only for experimental molecular biology and genetics but also for organismal and population biology. Certain fungi, likewise, have excellent potential as models for work at diverse levels. Fungi offer the same potential for the manipulation of experimental evolution as do bacteria but with the advantage that they are complex, multicellular eukaryotes with genomes one or two orders of magnitude smaller than plants or animals. With their sophisticated genetic tools and publicly available genome sequences (Galagan et al 2003, Wood et al 2002, Goffeau et al 1996), the leading fungal models are *Saccharomyces*, *Schizosaccharomyces* and *Neurospora*. In the context of evolutionary biology and ecology, what has been lacking is access to natural populations. For *Neurospora*, it is now clear that large numbers of individuals can be sampled in a predictable manner in diverse ecosystems, providing an important component needed for a complete model system. However, additional studies of species distributions, microhabitat preferences and local and global variation are needed, even to fulfill the promise of *Neurospora* in experimental realms where it has traditional strengths as a model organism, such as regulation and function of metabolic pathways, signal transduction, circadian rhythm and the genetics of recombination.

In contrast, the use of other recognized model fungi for studies of natural populations has been extremely limited. Although *S. cerevisiae*, *Candida albicans* and *Aspergillus* species have been used for experimental evolution (Zeyl and Bell 1997, Cowen et al 2001, deVisser et al 1997), collections of natural isolates comparable to those for *Neurospora* are not available and would be difficult to obtain in a predictable manner. *S. cerevisiae* is difficult to find in natural environments, and isolates from vineyards are likely to have escaped from domestic settings (Naumov et al 2000a). Similarly, *A. flavus* has been collected in nature, but its distribution might be influenced by agriculture (Geiser et al 1998). Certain species, such as the yeast *S. paradoxus*, resident under the bark of oaks (Naumov et al 2000b), or *A. nidulans*, collected away from agricultural fields (Geiser et al 1996), might hold promise, but sufficient survey work is lacking to date.

Although tropical and subtropical *Neurospora* species might have been subjected to human intervention associated with agriculture, it seems certain that the populations in forests of western North American are relatively undisturbed by human influence. In addition, colonies of *Neurospora* are easily recognized in the field and can be large enough to provide sufficient material for bulk analysis (FIG. 1), such as employing microarray expression assays. Other model microfungi either are impossible to find in a predictable manner or difficult to identify in the field, or both, and obtaining sufficient biomass for experimentation requires laboratory cultivation.

Our results provide a reminder that much remains to be learned regarding the distribution and role of

fungi in natural settings. Previous reports of *Neurospora* from temperate regions have been anecdotal or have not involved natural sites and therefore have failed to signal that members of the genus might be common in temperate forests. Examples include burned trees in Tokyo (Kitazima 1925, Perkins 2002), Parisian bakeries (Perkins 1991), logs used for lumber (Shaw 1993, Ridley 1994), soil (Werner 1969, cited in Wicklow 1975), and the occasional observation on compost (D. D. Perkins, Stanford University, Palo Alto, California, and A. M. Rossman, USDA ARS, Beltsville, Maryland, pers comm) or wood (a single isolate of *N. crassa*, FGSC 3885, was collected from wood near Mount Wilson, California, in 1965, however, details as to the plant species and the condition of the substrate are unknown [N. H. Horowitz, pers comm to D. D. Perkins]).

Neurospora is one of the most easily recognized of fungal genera, with species having been common subjects in classroom exercises as well as research laboratories for more than half of the past century (Perkins 1992, Davis 2000, Perkins and Davis 2000, Davis and Perkins 2002). The fact that species have been reported so infrequently from temperate regions therefore contrasts sharply with the abundance of colonies observed at burn sites in western forests.

Of more general significance, the serendipitous nature of our initial discovery of *Neurospora* in forest habitats indicates that inadequate attention has been paid to the succession of decomposing microorganisms in forests after disturbance in general and fires in particular. In our surveys of *Neurospora*, we have observed dozens of other fungal species, often in abundance. For none of these species is there a clear picture regarding either the importance of fire to the biology of the organism or the importance of the organism to forest ecology. Some literature exists on so-called pyrophilous, carbonicolus or phoenicoid fungi that grow and/or fruit after fires (e.g., Wicklow 1975, Johannesson et al 2000, Carpenter and Trappe 1985 and references therein), but none, to our knowledge, that specifically examined fungi on freshly fire-killed vegetation. Therefore, while expanding our knowledge of the genus *Neurospora* in important respects, the observations reported here also symbolize the many frontiers in microbial ecology.

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