Global distribution and molecular diversity of *Didymium difforme*

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Fifty-six collections of *Didymium difforme* from three different geographically distant regions of the world were examined for intraspecific variation using DNA sequences. An approximately 400 base pair region of the mitochondrial small subunit was sequenced for each collection. The analysis of the sequences did not resolve the collections from each geographic region into separate groups. Instead, all but one of the six major groups included sequences from collections originating in at least two different regions. The one group with sequences from one region (Kenya) to the exclusion of the other two regions was made up of sequences from collections made at different localities within the region. Other sequences from the same localities were, however, found in the other major groups most closely related to sequences from the other two regions.

Key words – cosmopolitan species – distribution – intraspecific variation – mitochondria – molecular diversity – myxomycete

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Introduction

The morphological species concept in myxomycetes lends itself to the false description of variants from the norm as new species (Clark 2000, 2004). Without knowing the mechanism by which the morphological characters are affected by environmental conditions or some experimental data on the difference between morphological variants and "normal" isolates, assumptions cannot be made as to the classification of these collections. It has been recognized that variation under natural conditions may play a part in the development of typical or atypical fruiting bodies of particular species of myxomycetes. As early as 1901, culturing experiments were used to determine the stability of morphological variation in collections of *Didymium difforme* (Pers.) Gray (Lister 1901). Variations in culture conditions, including abiotic factors such as moisture and biotic factors such as the assemblages of bacteria and fungi present in the same culture have been shown to produce morphological variation in *D. difforme* (Cayley 1929). These factors represent only a very small part of the natural environment in which myxomycetes are found, which suggests the possibility that many different conditions and combinations of environmental characteristics could be important in the normal or abnormal development and growth of the species.

It cannot be ruled out that genetic variation plays a part in the morphological variation observed within *D. difforme* or any other species of myxomycete, because as yet no data exist to describe the intraspecific molecular variation within *D. difforme*. Early isozyme studies (Franke et al. 1968, Franke and Berry 1972, Franke 1973, Berry and Franke 1973, Betterley and Collins 1983) were able to

provide general conclusions that intraspecific variation exists within other species but did not reveal any correlation between morphological variation and enzyme or protein variation.

There are not enough data at this point to determine if the morphological variation observed for a particular species can be attributed to an accumulation of genetic variation. It is also possible that the variation noted in fruiting bodies can be attributed to plasticity in the phenotype where variation is due to differences in microhabitats or microclimate (Clark 2000, 2004).

In other species of the genus *Didymium*, e.g., *Didymium iridis* (Ditmar) Fr., *D. megalosporum* Berk. & M.A. Curtis, *D. ovoideum* Nann.-Bremek., and *D. squamulosum* (Alb. & Schwein.) Fr., for which more isolates have been examined, both heterothallic and non-heterothallic lines were shown to exist, thus indicating that myxomycete morphospecies may be complexes of heterothallic (sexual) lines associated with a number of non-heterothallic (presumed apomictic) clonal lines (El Hage et al. 2000, Clark 2004). Clonal lines would evolve independently of other lines, meaning they can independently accrue variation, which may ultimately affect morphology. The isolates for which the reproductive system has been studied were found to be non-heterothallic–either homothallic or apomictic–wherein cultures started from single spores went through the entire life cycle spore to spore (Cayley 1929, Clark 2004). For these isolates, it is possible that any morphological variation could be the result of independent evolution within the strain, however, no experimental evidence exists to understand the cause or stability of any morphological variation found within the morphospecies.

Didymium difforme is a cosmopolitan species. Its distribution appears to be centered in temperate regions of the northern hemisphere. However, where significant studies of myxomycete biodiversity have been carried out, the species usually has been isolated. The habit of *D. difforme* is not limited to any particular substrate and the species has been found on bark and dung as well as aerial litter and ground litter.

The Global Biodiversity Information Facility (GBIF) includes nearly 200,000 records of myxomycetes and 1,600 records of *D. difforme* with georeferencing and collector data. The limitations of these data, however, include the absence of ecological information about the referenced specimens. The largest available data-base of myxomycetes that does include ecological data was developed at the University of Arkansas. This database–included in GBIF–contains more than 840 records for *D. difforme*. The database at the University of Arkansas (hereafter referred to as UARKM) represents a previously unavailable dataset for making broad conclusions about the ecology of particular species of myxomycetes. Ecological conclusions are extrapolated from the world-wide UARKM database and the historical literature.

The UARKM database contains 844 records of *Didymium difforme* with data on the ecological conditions under which this species has been found. Of these records, 623 specimens were obtained with the use of the moist chamber culture technique. Three collections are from moist chamber cultures for which no pH data were recorded, and the remaining 218 records are assumed to be specimens that fruited in the field under natural conditions, since the standard procedure used by all of the collectors listed is to determine the pH for all moist chamber culture dishes. For the records obtained in the field, the data collected represents the snapshot in time during which it is assumed that the environmental conditions favored growth. It is well known that myxomycetes show a seasonal preference for fruiting, although beyond recognizing that the particular season presents ideal conditions for fruiting, there is no known understanding of the specific factors, abiotic and biotic, that cause the seasonality. Collections from material that developed in moist chamber may not represent the expected seasonality of the species because the species may be found as a spore, amoeboflagellate, microcyst, macrocyst and/or microscopic plasmodium, which in the case of the spore, microcyst, or macrocyst, the individual organism is not functioning in that environment at that time. The moist chamber culture would stimulate development because favorable conditions are created.

The literature describes the usual habit of *D. difforme* as herbaceous litter, including dead

leaves and decaying herbaceous stalks, and dung from herbivorous animals (Lister & Lister 1925, Martin & Alexopoulos 1969). Although not specifically stated, we interpret decaying herbaceous stalks as being what is regularly termed aerial litter–dead plant material still attached to the plant above the ground. For 843 records in the worldwide database, substrate material is listed. Aerial litter is listed for 551 records, ground litter for 269 records, dung for 17 records, and bark for 6 records. While no assumptions can be made for the sampling scheme by which all of the substrate materials were sampled, a conclusion can be drawn that *D. difforme* shows a marked preference for decaying herbaceous litter, with over 97% of all collections in this database having been collected from herbaceous substrates. For the collections on herbaceous litter (820), 67% were found on aerial litter and the remaining (33%) on ground litter. Again, without the benefit of an appropriate understanding of the sampling scheme, it is not possible to analyze these data in a statistical fashion. However, the number of collections found on aerial substrates is just over twice the number from ground litter, suggesting a tendency for this species to fruit on aerial substrates. The percentages are similar whether the specimen was collected in the field or harvested from moist chamber cultures. This preference for aerial litter is noted in the literature, although for much smaller datasets in specific geographical areas (e.g., Schnittler & Stephenson 2002). As previously stated, specimens from moist chamber cultures develop from microscopic stages of the life cycle that may or may not be actually functioning on the substrate when it is collected but rather exist as one of the three resting stages–spore, microcyst or macrocyst. It is not possible to determine how this species occurred on the substrate prior to forming fruiting bodies in the moist chamber culture. In this study, fifty-six collections were sequenced for a DNA marker in the mitochondrial small subunit. Twenty-four of these collections are from Kenya, four collections from Costa Rica, sixteen from Mexico, seven from North Dakota in the United States, and one each from Wyoming, Kansas and Oklahoma in the United States (Table 1).

DNA extraction, amplification and sequencing

DNA was extracted from fruiting bodies using the Chelex method as described previously (Winsett & Stephenson 2008). Briefly, spores from four to five fruiting bodies and 150 µL of a 5% Chelex suspension were placed in a thermocycler held at 56°C for 4 hours and then 98°C for 30 minutes to release DNA from the other cellular material that is part of the spore. Extracted DNA was frozen in microcentrifuge tubes at -20°C until use.

A 400 base pair hypervariable region of the mitochondrial small subunit (mtSSU) was amplified using the primers mtCore1 (5′–TAG TGT TAT TCG TGA TGA CT–3′) and mtCore2 (5′–CTC GAA TTA AAC CAC AT–3′). The DNA templates were amplified using a 25 µL reaction (12.5 µL Promega GoTaq® Green Master Mix, 8.5 µL dH2O, 1 µL each primer and 2 µL template DNA) using a PCR protocol, which consisted of an initial activation step at 94°C for 2 minutes (per instructions for Master Mix) and 35 repetitions of 94°C for 30 seconds, 40°C for one minute, and 72°C for one minute. The PCR amplicon was extracted from the PCR product by a modified gel electrophoresis and gel extraction method–modified from Dentinger et al. (2010). A 20 µL portion of each PCR product was run in a 1% agarose gel prepared with 1X TA buffer (20X: 193.6 g Trizma base, 45.7 mL glacial acetic acid, dH_2O up to 2L, dilute to 1X) with ethidium bromide included at 80 V for approximately 30 minutes. The resulting band was cut from the gel and placed in a filtered micropipette tip cut to fit into a 1.5 mL microcentrifuge tube. The tube with the piece of gel sitting on the filter was frozen to break up the agarose matrix then spun in a centrifuge at 13,000 rpm for 10 minutes. The filtered pipette tip was removed from the tube, leaving the extracted DNA within the flow-through. DNA samples were sequenced on a Perkin-Elmer ABI 3700 sequencer using amplification primers. Resulting chromatograms were checked and edited using Sequencher v4.3. All sequences were submitted to GenBank (Accession numbers HQ450397–HQ450452).

Table 1 List of specimens of *Didymium difforme* used in this study. All vouchers are herbarium specimens deposited in the University of Arkansas (UARK) myxomycete collection with data available online at http://slimemold.uark.edu.

Sequence Number	Country	Collector Number	Accession Number	GenBank Number	Haplotype
1	Kenya	GN116	22223	HQ450426	8
2	Kenya	GN117	22224	HQ450438	6
3	Kenya	GN118	22225	HQ450418	6
4	Kenya	GN122	22228	HQ450416	6
5	Kenya	GN157	23705	HQ450430	6
9	Kenya	GN313	27316	HQ450450	13
10	Kenya	GN401	27404	HQ450397	1
12	Kenya	GN484	27487	HQ450404	4
15	Kenya	GN690	27693	HQ450452	13
16	Kenya	GN1694	36451	HQ450401	3
19	Kenya	GN1986	36743	HQ450433	11
24	Kenya	GN2630	38970	HQ450402	3
25	Kenya	GN2695	39035	HQ450417	6
26	Kenya	GN2722	39062	HQ450429	10
28	Kenya	GN2849	39216	HQ450419	6
31	Kenya	GN2922	39289	HQ450422	6
34	Kenya	GN3066	39433	HQ450420	6
35	Kenya	GN3097	39464	HQ450449	3
37	Kenya	GN3135	39502	HQ450428	9
41	Kenya	GN3337	39704	HQ450399	3
42	Kenya	GN3369	39742	HQ450448	3
47	Kenya	GN3496	39869	HQ450405	4
49	Kenya	GN3534	39907	HQ450403	4
50	Kenya	GN3568	39941	HQ450442	6
51	Kenya	GN3598	39971	HQ450400	3
53	Costa Rica	CR1767	34694	HQ450423	6
54	Costa Rica	CR1791	34718	HQ450431	6
55	Costa Rica	CR1800	34727	HQ450406	5
57	Costa Rica	CR1943	35146	HQ450421	6
58	Mexico	CR2047	36259	HQ450409	7
59	Mexico	CR2062	36274	HQ450446	6
60	Mexico	CR1889	35098	HQ450447	12
61	Mexico	CR2085	36297	HQ450427	8
62	Mexico	CR2080	36292	HQ450443	6
63	Mexico	CR2038	36250	HQ450414	6
64	Mexico	CR1992	36204	HQ450424	6
65	Mexico	CR2066	36278	HQ450439	6
67	Mexico	CR2055	36267	HQ450444	6
69	Mexico	CR1886	35095	HQ450435	5
70	Mexico	CR1882	35091	HQ450432	6
71	Mexico	CR1881	35090	HQ450407	5
72	Mexico	CR1877	35086	HQ450440	6
74	Mexico	CR1895	35084	HQ450441	6
75	Mexico	CR1861	35070	HQ450445	6
76	Mexico	CR1221	30625	HQ450425	6
80	Mexico	CR1126	30569	HQ450398	2
82	North Dakota	AWR1191	26718	HQ450410	6
84	North Dakota	AWR535	26082	HQ450451	13
87	North Dakota	AWR995	26536	HQ450413	6
88	North Dakota	AWR1088	26620	HQ450437	6
91	North Dakota	AWR690	26237	HQ450415	6
93	North Dakota	AWR1199	26724	HQ450436	6
94	North Dakota	AWR1187	26715	HQ450408	6
96	Wyoming	AWR1271	27224	HQ450412	6
98	Kansas	AWR1063	26596	HQ450411	6
100	Oklahoma	AWR1151	26681	HQ450434	6

Sequence analysis

Sequence alignments were performed using Clustal X and then manually edited in Seaview (Larkin et al. 2007, Gouy et al. 2010). Summary statistics for the sequences including the number of haplotypes, haplotype diversity, nucleotide diversity, and number of pairwise differences were calculated using DnaSP v. 5 (Librado & Rozas 2009). This software was also used to test for neutrality of mutation through D statistic Tajima (1989) and the D* and F* statistics of Fu & Li (1993). A gene tree was produced using Bayesian Markov Chain Monte Carlo methods using the software package BEAST v.1.5.4 (Drummond & Rambaut 2007). The alignment in nexus format was imported into BEAUTi, to format the appropriate XML file to be used by BEAST. The alignment was analyzed using estimated base frequencies and a chain length of 1,000,000. Performance suggestions for operators that were included in the output from BEAST were used to make operator modifications in BEAUTi to optimize the data set. The updated XML file produced by BEAUTi was imported into BEAST and reanalyzed. This step was repeated until the operator values were optimized according to the performance suggestions in the BEAST output file. The resulting ".trees" output was formatted using TreeAnnotator v.1.5.4 and saved as a ".tre" file that was viewed and edited in Fig Tree v. 1.3.1. A gene geneology (haplotype) network was constructed using TCS (Clement et al. 2000).

Results

Fifty-six sequences were obtained from isolates of *D. difforme* from different geographic locations. The amplified region was a nearly 400 base pair (bp) hypervariable region of the mitochondrial small subunit. The region of the small subunit amplified in this study is located within the small subunit gene 740 bases from the beginning of the available sequence (GenBank Accession: X75591.1), which is 1814 bp in length. The sequence length from the amplified region ranged from 370 bp to 483 bp. However, much of the variation in length was attributed to incomplete sequencing of the PCR product as the missing sections resulting in length variation were at the ends of the sequences so these sections were removed.

A total of 368 sites were analyzed for 56 sequences of the hypervariable region of the mtSSU for isolates of *Didymium difforme* from three different regions of the world. These were Central America (Costa Rica and Mexico), the central United States grasslands, and Kenya. Of the 368 sites, 73 were polymorphic with 66 sites parsimony informative. Summary statistics are found in Table 2

Based on these sequences, 13 haplotypes emerged, seven of which included only one sequence (singleton haplotypes). Haplotype designations for each sequence are found in Table 1. Haplotype designations are an arbitrary number between one and thirteen that correspond to the output from DnaSP, but have no specific meaning except to separate the sequences into the 13 categories. The largest haplotype included 32 sequences of isolates found in all three regions. Nucleotide diversity (π) was 0.03065, and the average nucleotide difference (k) among the sequences was 8.79545. Two of the three test statistics for neutral mutations Fu and Li's D* and F* were not significant suggesting that all mutations are neutral. Tajima's D was significant with a P value less than 0.05.

Summary statistics and test statistics for neutrality were also calculated for each region (Central America, central United States grasslands, and Kenya) independently (Table 2). The sequences from isolates collected in Kenya represented the highest number of haplotypes (9). Central American isolates included six haplotypes, and the grasslands represented two haplotypes. The test statistics for neutrality were not significant for Kenya, but in the sequences from both Central America and the grasslands, these statistics were significant.

The results of the molecular analysis of the mtDNA marker indicated that several groups were apparent (Fig. 1). In *D. difforme* three major, apparently significant, groups (clades) were resolved. Two of the clades (I and III) are made up of two and three isolates, respectively. The remaining clade (II) is made up of the majority of the specimens included in the analysis and is further resolved in Fig. 2.

Table 2 Summary statistics for mtDNA polymoprhisms in sequences of *Didymium difforme*. n_s is the number of sequences; h_n is number of haplotypes; H_d (±SD) is haplotype diversity ± standard deviation; π (k) is nucleotide diversity and mean number of nucleotide differences.

Location	n_s	h_n	H_d (±SD)	π (k)	Fu and Li's D*(P)	Fu and Li's F*(P)	Tajima's D (P)
Kenya	25	9	0.817 (0.055)	0.04122 (11.83000)	-0.79084 (ns)	-1.29175 (ns)	-1.75712 (ns)
Central America	21	6	0.552 (0.122)	0.01691 (4.85238)	-3.92687 (P<0.02)	-4.06715 (P<0.02)	-2.46275 (P<0.001)
North American grasslands	10	2	0.200 (0.154)	0.03275 (9.40000)	-2.51341 (P<0.02)	-2.72698 (P<0.02)	-2.11847 (P<0.001)
Total	56	13	0.662 (0.069)	0.03065 (8.79545)	0.90327 (ns)	-0.16997 (ns)	-1.84919 (P<0.05)

Major clades were numbered with Roman numerals for ease of communication.

Clade I was made up of two isolates, one from Kenya and the other from Mexico, with a significant posterior probability (PP) of 99%. Clade III groups two Mexican isolates with one from North Dakota in the United States, with a significant PP score of 97%. The largest significant clade of *D. difforme* contained 51 sequences that were significantly grouped with a 97% PP. Fig. 2 is a subset of the full tree shown in Clade II.

Clade II is further divided into three significant groups (IV, V and VI). Clade VI, with a PP of 94%, was the only significant group having sequences from a single geographic region, with nine sequences from specimens collected in Kenya. Clades IV and V made up one large group that was separated from the Kenyan group (VI). Clade IV included four sequences, all from Central America, with three from Mexico and one from Costa Rica. The three Mexican sequences, however, did not group together. Two of the Mexican sequences are grouped with the Costa Rican sequence, with a PP of 89%. Clade V included 38 sequences. The group of 38 sequences was supported as a single group with a significant PP of 92%. Only three of the interior nodes, however, had significant level (greater than 70%) of PP. Two Kenyan isolates were grouped with a Costa Rican isolate with 84% PP, a single Mexican and one Kenyan isolate were grouped with a PP of 94%, and two Kenyan isolates were grouped with 99% PP. The remaining sequences were resolved into clades within the larger group V. However, the PP values were not significant, averaging only 18%. Group V was composed of sequences from a wide geographic range that encompassed Kenya, Costa Rica and Mexico, and the central United States (Kansas, North Dakota, Wyoming and Oklahoma).

Haplotype designations were congruent to the gene tree constructed for these sequences. The significant (>75% PP) tip clades on the gene tree (Figs 1, 2) were composed of a single haplotype (e.g., III, IV, and VI) or included all the sequences for a certain haplotype along with singleton haplotypes (e.g., Ken26-Ken19-Ken05-CR54 and clade V).

Discussion

The overall observation from this analysis of sequence data for the isolates of *D. difforme* considered in the present study is that no true geographical pattern emerges. The distribution of haplotypes and the topology of the gene tree point to the conclusion that a readily apparent biogeographical pattern in which sequences from collections originating from the same locality simply does not exist.

The significance of the tests for neutrality in each region is called into question by sample size. These statistics were significant for the sequences from both Central America and the North American grasslands, thus suggesting that the mutations are not neutral. However, the sample sizes for the subsets are 25 individuals or less, which is considered too low for appropriate statistical power. Therefore, rejecting the null hypothesis that the polymorphisms are neutral may not be the appropriate course of action (Simonsen et al. 1995).

For the full dataset, Tajima's D statistic for neutrality was significant with a P value

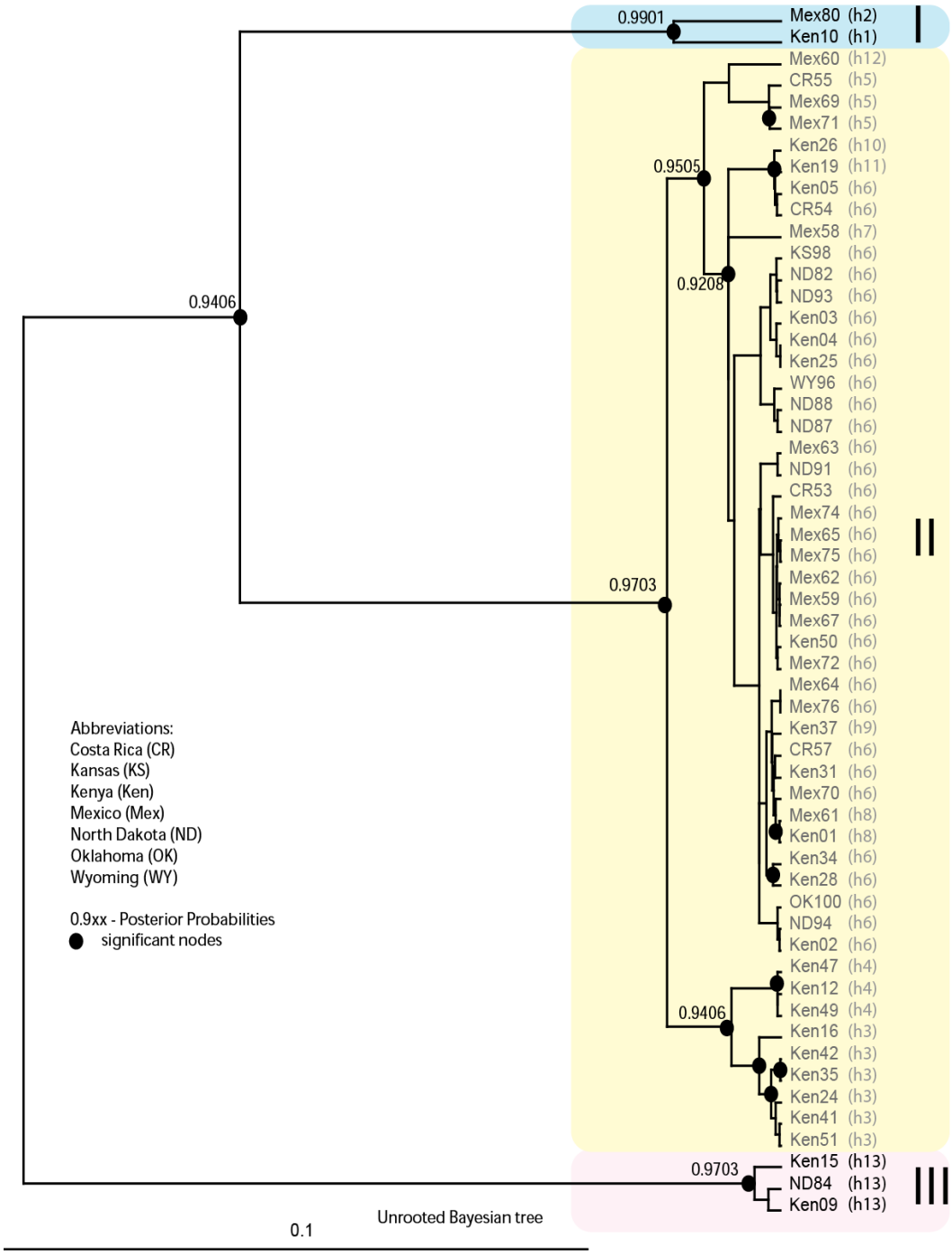

Fig. 1 – An unrooted Bayesian tree showing relationships among the 56 isolates used in this study. Node labels are posterior probabilities. As the number approaches one, the significance increases.

less than 0.05 suggesting the polymorphisms were not neutral. However, it has been suggested that Tajima's test is more powerful than Fu and Li's statistics in cases of population growth and genetic hitchhiking (Fu 1997) thus these results may indicate an alternative hypothesis that the population is increasing or the locus sequenced is linked to another that is the object of selection as the mechanism responsible for these polymorphisms.

The discussion of a geographical pattern relies upon the analysis of haplotype data and the topology of the gene tree, which offer no major clade that includes all of the sequences originating from a single locality. However, there are several small groups that contain isolates from the same location. One example is clade VI, which is composed only of Kenyan isolates. The other isolates from Kenya, though, are significantly grouped with isolates from

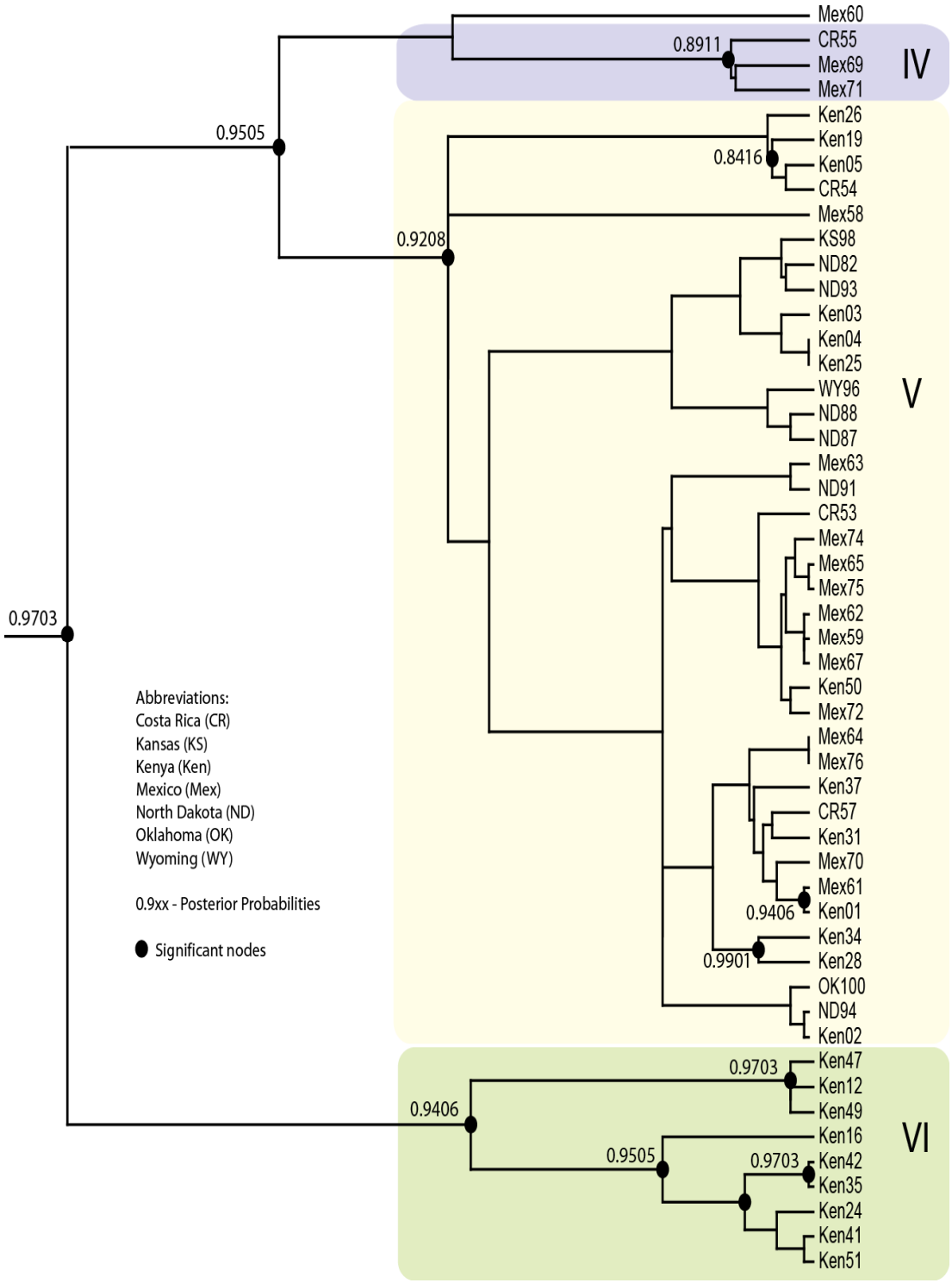

Fig. 2 – A subset (Clade II) of the tree in shown in Fig. 1

distant geographic locations such as Mexico, Costa Rica and North Dakota. As such, it can be assumed that these sequences are more similar to those sequences from Central American isolates than those originating from material collected at a much closer geographic location. All three localities included multiple haplotypes (Table 3). The sequences representing collections from Central America had six haplotypes, the grasslands had two and Kenyan collections had nine haplotypes. No locality was made up of a single haplotype and multiple haplotypes were found in more than one locality, further supporting the assertion that no geographic affinity separated sequences from any one location from sequences representing the other locations.

Table 3 Haplotype frequency by locality.

Locality	Haplotype (frequency)
Central America	2(1), 5(3), 6(14), 7(1), 8(1), 12(1)
Central United States grasslands	9(6), 13(1)
Kenya	1(1), 3(6), 4(3), 6(9), 8(1), 9(1), 10(1), 11(1), 13(2)

Didymium difforme has a global distribution, and as an apparently cosmopolitan species, it is assumed that this can be achieved through mechanisms related to long-distance dispersal by wind (Stephenson et al. 2008). Because of the size of myxomycete spores–approximately 10 µm on average–it is theoretically possible for them to travel indefinitely in this way (Finlay 2002, Schnittler & Tesmer 2008). If this method of dispersal is indeed common, then cosmopolitan morphospecies are expected and the possibility of ubiquity–cosmopolitan genetic identity–emerges as the most likely situation rather than the more restricted biogeographical patterns found in higher animals and plants (Finlay 2002). The data presented above are, with a few exceptions, geographically incongruous with isolates of a cosmopolitan morphospecies from very distant locations with the same or similar sequence identity at this locus of the mitochondrial genome. Previous data from intraspecific DNA sequencing of the nuclear ribosomal internal transcribed spacers (ITS) in geographically separated collections of *Didymium squamulosum* offered similar conclusions wherein the groups of the most closely related sequences were not resolved by geography (Winsett & Stephenson 2008).

An initial observation would be that these data support the idea of long-distance dispersal wherein spores traveled long distances–between continents–and were deposited in another geographic location, giving rise to a new population of the species in question.

Theoretically, this long distance dispersal is possible because of the combination of small spore size and an immense reproductive potential from the huge numbers of spores produced by each fruiting body. The average myxomycete fruiting body produces on order of magnitude 10^5 to 10^6 spores (Schnittler & Tesmer 2008). From each plasmodium more than a single fruiting body, and often as many as ten or more fruiting bodies, will form in *D. difforme*, thus increasing the likelihood that random events, such as a passing wind current or water flow, will pick up spores and deposit them elsewhere.

With the similarities in sequences among isolates from very distant collecting locations, these data would support the long-distance dispersal concept. There are, however, some suggestions of geographical patterns. The group of Kenyan isolates collected together with a very high posterior probability to the exclusion of other isolates introduces the idea of local genetic patterns or populations. The idea of a population in myxomycetes is a difficult concept to circumscribe because of the variety of reproductive strategies–both sexual and asexual–possible within an individual morphospecies and the large numbers of amoebae that can result from mitosis, beginning with the protoplast that emerges from a single spore. In asexual isolates, each of these amoebae is capable of producing a plasmodium and, by extension, the millions of spores found in the fruiting bodies that are derived from one plasmodium. The amoebae and plasmodium are also both mobile over short (albeit still unknown) distances, so that all of the fruiting bodies in a single area can theoretically be the same individual, not a collection of individuals. In this way, it would be expected that a pattern of diversity would emerge such that those isolates collected in a single area would be more closely related, as shown by DNA sequence analysis of an appropriate molecule.

The Kenyan clade would suggest the occurrence of a population of fruiting bodies from the same individual within the same area. However, these specimens were not collected in the same locality, but in localities as far as 40 km apart. Other specimens of *D. difforme* were collected in the same locality as those in group VI but are found in different parts of the tree. In this way, the groups of sequences are illogical. Those from the same collecting localities do not group together with similar sequences at the mitochondrial locus. The other

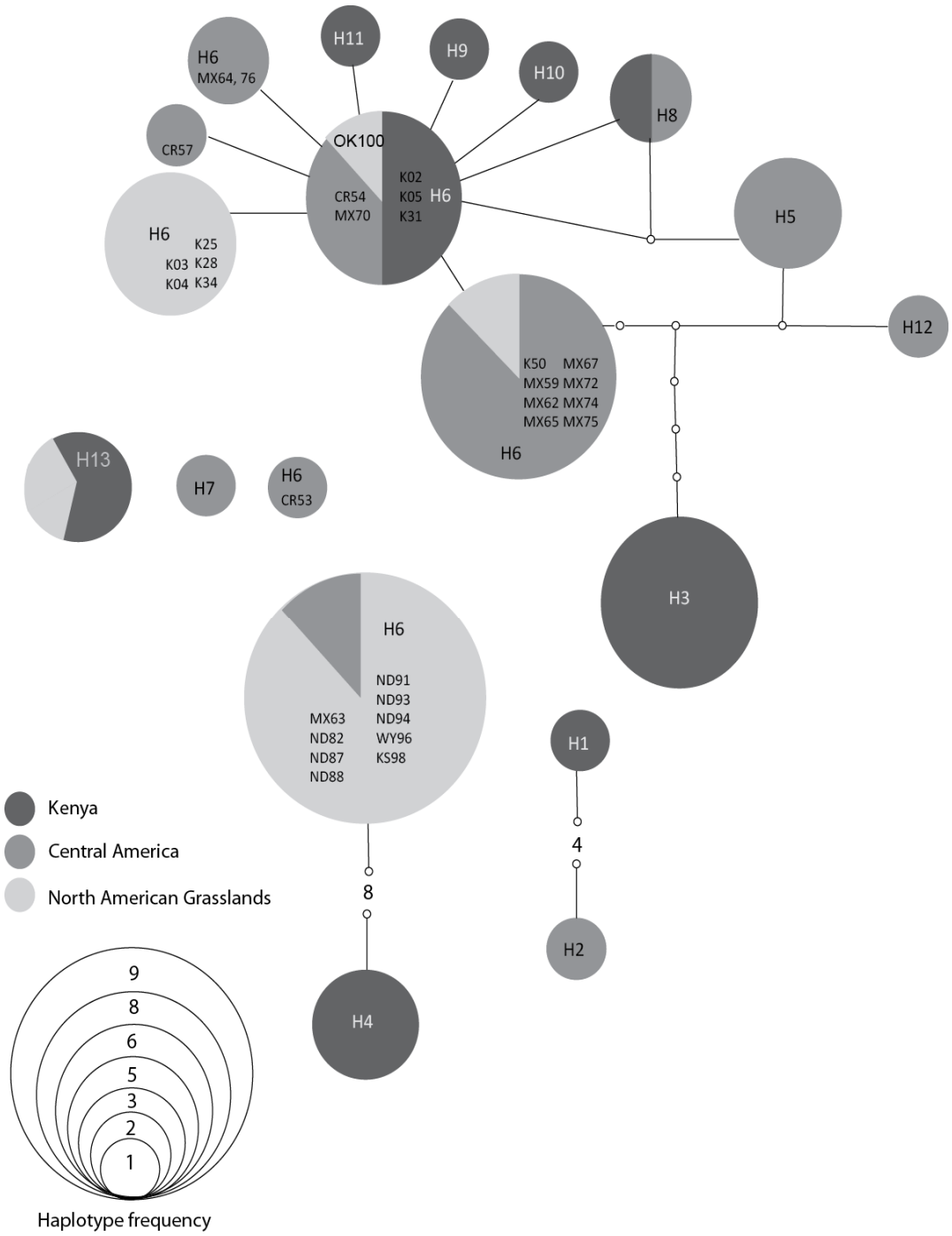

Fig. 3 – Gene geneology for the mitochondrial small subunit locus sequences estimated by TCS (Clement et al. 2000). Line segments indicate one mutation. Square and oval sizes are proportional to the sequences represented by the haplotype. Haplotype number corresponds to those given in Table 1.

groups in this analysis tell the same story. Sequences from isolates collected in the same location do not necessarily group together, but instead group with sequences from collections from distant localities, further suggesting the occurrence of long distance dispersal of spores by a mechanism, presumed most likely to be wind.

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