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Prevalence and susceptibility of infection to *Myxobolus cerebralis*, and genetic differences among populations of *Tubifex tubifex*

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ABSTRACT: The prevalence of infection and susceptibility of the aquatic oligochaete *Tubifex tubifex* to *Myxobolus cerebralis*, was examined in 2 studies on the upper Colorado River, Colorado, USA, where whirling disease occurs in wild trout populations. In the first study, the prevalence of infection ranged from 0.4 to 1.5%, as determined by counting the number of *T. tubifex* releasing triactinomyxons of *M. cerebralis* directly following their collection from the field. The susceptibility of those *T. tubifex* not releasing triactinomyxons was assessed by the number of these oligochaetes releasing triactinomyxons 3 mo following experimental exposures to spores of *M. cerebralis*. The prevalence of infection following experimental exposures of these *T. tubifex* ranged from 4.2 to 14.1%. In a second study, all *T. tubifex* collected at 2 different times directly from the 2 field sites in Colorado were exposed to spores of *M. cerebralis*. Individual oligochaetes representing those groups of *T. tubifex* releasing and those groups not releasing triactinomyxons at 3 mo were screened with molecular genetic markers. *T. tubifex* populations found at the 2 study sites consisted of 4 genetically distinct lineages that varied with respect to their susceptibility to experimental exposure to *M. cerebralis*. Lineages I and III contained the most oligochaetes susceptible to *M. cerebralis* and were the most prominent lineages at Windy Gap Reservoir, a site of high infectivity for wild rainbow trout on the upper Colorado River. In contrast, at the Breeze Bridge site which is below Windy Gap Reservoir and where *M. cerebralis* infections are less severe in wild trout, oligochaetes in lineages V and VI that are resistant to *M. cerebralis* were more prominent. These results suggest that certain habitats, such as Windy Gap Reservoir, are conducive to large and more homogenous populations of susceptible *T. tubifex* lineages that may serve as point sources of infection for *M. cerebralis.* Although not a direct objective of this study, there was no evidence of *M. cerebralis* infections among any oligochaetes other than those that would be classified as *T. tubifex* by standard morphological characteristics.

KEY WORDS: Whirling disease · *Myxobolus cerebralis* · *Tubifex tubifex*

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INTRODUCTION

The effects of parasites on wild and free-ranging fish populations are poorly understood, in part due to the complexity of the interrelating factors that influence host and pathogen relationships when the environment cannot be tightly controlled, as in aquaculture (Hedrick 1998). Parasites such as *Myxobolus cerebralis*, the causative agent of salmonid whirling disease, have recently been recognized as causes of catastrophic de-

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clines among wild rainbow trout populations in the states of Idaho, Montana, Colorado and Utah, USA (Nehring & Walker 1996, Vincent 1996, Hedrick et al. 1998). *M. cerebralis* is the best known of 1300 parasites grouped in the phylum Myxozoa and the first shown to possess a 2 host life cycle including salmonid fish and an aquatic oligochaete, *Tubifex tubifex* (Markiw & Wolf 1983, Wolf & Markiw 1984, Wolf et al. 1986). The parasite has been observed among wild fish in 23 states in the USA, but without the same disastrous effects observed in the intermountain west (Nickum 1999). The differences in the impacts on wild fish between geographic regions is not easily traced to different 'strains' of the parasite, since 18S and ITS-1 rDNA sequences were identical for isolates of the parasite from 4 North American sites and 1 German site (Andree et al. 1999). In addition, there was no apparent difference in the virulence for experimentally infected rainbow trout with parasites obtained from high- and low-impact enzootic locations of whirling disease (McDowell et al. unpubl. data). Salmonid species exhibit a range of susceptibility to *M. cerebralis* ranging from resistant to highly susceptible (O'Grodnick 1978, 1979, El-Matbouli et al. 1999a, Hedrick et al. 1999a,b). The mechanisms by which fish resist *M. cerebralis* infections are currently under investigation (Hedrick et al. unpubl. data). While our understanding of the parasite during development in the fish has progressed, much less is known about the parasite in tubificid oligochaetes, the hosts that are now viewed as potentially central to the severity and eventual management of whirling disease in certain wild trout populations.

A group of related aquatic annelids (Oligochaetae, Tubificidae) have been the focus of a number of ecological and systematic studies because of their importance as indicators of aquatic pollution and as alternate hosts to fish parasites (Kent et al. 1994, Day et al. 1995, Brinkhurst 1996). Three families of oligochaetes are known to host myxosporean parasites, but *Tubifex tubifex* is reported to be the only host for *Myxobolus cerebralis* (Markiw & Wolf 1983, Wolf et al. 1986, El-Matbouli & Hoffman 1989, 1995). Currently, the taxonomy and systematics of oligochaetes depends on morphological characteristics of sexually mature adults, and this has proven insufficient to describe differences among geographic isolates of tubificid oligochaetes serving as hosts for *M. cerebralis* (K. Beauchamp unpubl. obs.). Both field and laboratory exposures have demonstrated that certain populations classified as *T. tubifex* from the USA, Canada and Europe range from highly susceptible to completely resistant to infection by *M. cerebralis* (El-Matbouli et al. 1999b). Two recent studies on experimentally exposed *T. tubifex* have reported variable results on the release of the actinosporean stages of *M. cerebralis*, suggesting both a temporal and dose-response to parasite infection among different oligochaete populations (Gilbert & Granath 2001, Stevens et al. 2001). Our previous phylogenetic studies using mitochondrial 16S rDNA (mt16S rDNA) markers identified 4 genetically distinct lineages of morphologically indistinguishable *T. tubifex* from North America; 3 of the lineages are also found in Europe (Beauchamp et al. 2001). In addition, we found that individuals from 1 mitochondrial lineage are resistant to infection from *M. cerebralis*. Therefore, understanding the genetic diversity and distribution of *T. tubifex* species that are susceptible or resistant to *M. cerebralis* is of immediate concern for the potential control of whirling disease.

The upper Colorado River represents a well-known and highly prized wild rainbow trout fisheries in the state of Colorado, USA Serious declines in this sport fishery have been thoroughly documented (Nehring & Walker 1996, Nehring 1998, 1999, Thompson et al. 1999). The role of the Windy Gap Reservoir on the Colorado River is believed to be a major environmental factor contributing to the whirling disease that occurs among trout in the river below (Thompson & Nehring 2000). A recent study by Zendt & Bergerson (2000) examined the abundance and distribution of oligochaetes above, within and below the reservoir. However, what was not known, nor were the tools available for the assessment at that time, was the relative composition and genetics of susceptible and resistant *Tubifex tubifex* present in their samples. In this study we examined populations of aquatic oligochaetes for the prevalence of infection with *Myxobolus cerebralis* from the upper Colorado River, including Windy Gap Reservoir. We examined individual oligochaetes collected from each site to determine the prevalence of those releasing triactinomyxon stages of *M. cerebralis*. We then exposed those *T. tubifex* not found to be producing triactinomyxon stages, to spores of *M. cerebralis* obtained from infected trout to test their susceptibility as assessed 3 mo later by releases of triactinomyxons. We also applied recently developed molecular approaches to identify specific genotypes of *T. tubifex* that might be present in field samples and might correlate with the susceptibility or resistance of these oligochaetes to *M. cerebralis* infections.

MATERIALS AND METHODS

Sample collection and sorting. Two studies were carried out in 1998 and 1999. In the first study (1998), sediment samples were collected from the Windy Gap Reservoir (WG) and from several sites on the upper Colorado River (UCR, below Windy Gap Reservoir); samples from the UCR sites were combined for analysis. In the second study (1999), samples were collected

from only 2 sites, WG and Breeze Bridge (BB, below Windy Gap Reservoir). In both studies, samples contained aquatic insects, mollusks, crustaceans and annelids. Using a dissecting microscope, oligochaetes were first grossly sorted based on the presence or absence of hair chaetae. At each collection site we observed 3 families of oligochaetes: Lumbriculidae, Naididae and 2 groups of Tubificidae (one species with and the other without hair chaetae).

In the first study, oligochaetes were collected on 21 September and 28 October 1998, and individual oligochaetes were distributed into multi-well plates with dechlorinated water and maintained for approximately 1 wk at 15°C to determine if they were releasing actinosporean stages. Oligochaetes that did not release any actinosporeans were then placed in bulk culture according to the 4 groups mentioned above. The cultures were maintained in plastic containers (2 l) with 400 g sterilized sand and 1 l dechlorinated tap water at 15°C. The water was changed once per week and the oligochaetes were fed with Algamac-2000. The water from each container was examined weekly for the presence of actinosporeans. Approximately two-thirds of the water volume was passed through a 10 µm Nitex screen. Actinosporeans trapped on the screen were concentrated into a volume of 50 ml and viewed by phase-contrast microscopy as previously described (Markiw 1989, Andree et al. 1997). After 1 mo, individual oligochaetes were re-distributed into multi-well plates and screened for the presence of actinosporeans. In the second study, oligochaetes were collected on 13 September 1998 and sorted by the presence of hair chaetae (*Tubifex tubifex*). Only *T. tubifex* oligochaetes were transferred to bulk culture in the same manner as the first study.

Experimental exposures to *Myxobolus cerebralis* spores. For the first study, oligochaetes that did not release actinosporeans after 1 mo observation were exposed to freshly harvested *M. cerebralis* spores from infected rainbow trout (5×10^2 spores per oligochaete) from the Mount Whitney hatchery, Independence, California, as previously described by El-Matbouli et al. (1999b). In the second study, only *Tubifex tubifex* were selected and separated for 2 experiments as follows: In Expt 1, oligochaetes were exposed on 12 October 1999 to *M. cerebralis* spores freshly harvested from rainbow trout (Mount Whitney, California) so that each oligochaete was exposed to 1.1×10^4 spores. In Expt 2, oligochaetes were exposed on 3 November 1999 to *M. cerebralis* spores obtained from brown trout (South Platte River, Colorado) and each oligochaete was exposed to 6×10^3 spores. Spores used in both studies were collected by the plankton centrifuge method (O'Grodnick 1975). After 3 mo, individual oligochaetes were plated and screened to determine which oligochaetes were releasing triactinomyxons (El-Matbouli et al. 1999b). At the end of the experiments (3 mo post-exposure) oligochaetes releasing or not releasing triactinomyxons of *M. cerebralis* were fixed for genetic screening and morphological and molecular identification.

Genotype screening of *Tubifex tubifex.* Due to previous findings that natural populations of *T. tubifex* contain a mixture of morphologically indistinguishable genotypes, a PCR-based genetic screening method was developed. For each of the 3 major lineages found in *T. tubifex* from Colorado, a specific primer was designed for 1 strand and used in combination with a previously known reverse primer of one mt16S rDNA to yield a size-specific PCR product (present Fig. 1 and Beauchamp et al. 2001). The PCR cocktail contained 3 lineage-specific primers and the *Tubifex*-specific reverse primer (1 µM of each). For the genetic screening, genomic DNA was extracted using the QIAmp Tissue Kit (Qiagen) from up to 50 individual oligochaetes for each of the groups releasing or not releasing triactinomyxons of *Myxobolus cerebralis*. When there were less than 50 oligochaetes in a group, all oligochaetes were analyzed. In those cases in which none of the lineage-specific primers yielded a band or in which multiple bands were observed, the product of the PCR using the universal primers for mt16S rDNA was sequenced to determine the genotype (see next section).

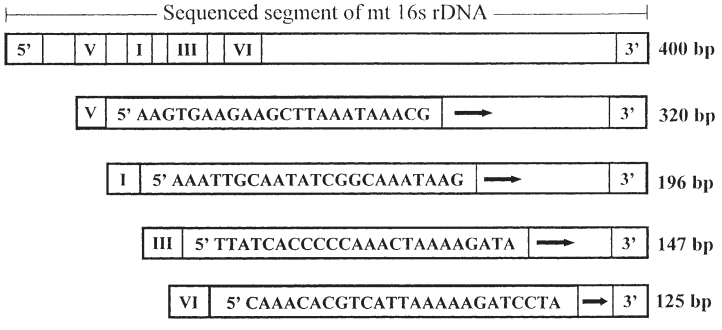

Fig. 1. Sequences and priming locations of 4 lineage specific primers within the 550 bp segment of the mt16S rDNA yielding 1 lineage-specific amplification product with the *Tubifex*-specific primer on the complementary strand (Tub16R: 5′ TAA RCC AAC ATY GAG GTG CCA 3′; Beauchamp et al. 2001). Top bar represents the entire segment of the mt16S rDNA amplified by the *Tubifex* spp.-specific primers and the priming sites of the 4 Lineage-Specific Primers I, III, V and VI (Sturmbauer et al. 1999, Beauchamp et al. 2001); lower bars represent lineage-specific amplification products and the orientation and sequence of each diagnostic primer. Only Lineage-Specific Primers I, III, and VI were used in this study. Size of each PCR-amplified product for each of the primers is shown on the right

Morphological and molecular identification. The examined oligochaetes were cut in half. The anterior portion was fixed in 10% neutral buffered formalin and mounted in CMCP-10 high-viscosity mountant for morphological identification according to Kathman & Brinkhurst (1998). The posterior of the oligochaete was used to extract genomic DNA by the same method described in the last section. For those oligochaetes that did not PCR-amplify with the lineage-specific primers, a 550 bp region of the mitochondrial 16S gene (mt16S rDNA) was PCR-amplified using the universal primers 16sar and 16sbr (Palumbi et al. 1991). PCR products were directly sequenced from double-stranded DNA using the Sequenase Version 2 and an automated sequencer (ABI 377). Both strands were sequenced. Additional sequences of *Tubifex tubifex* used in the analysis were from Sturmbauer et al. (1999) and Beauchamp et al. (2001).

DNA sequences and phylogenetic reconstructions. Sequences were aligned with the aid of the computer program MacDNASIS Version 3.7 (Hitachi Software Engineering America) and Clustal V (Higgins & Sharp 1989). Phylogenetic relationships were assessed by maximum-parsimony (MP), using the heuristic search and the neighbor-joining (NJ) methods as implemented by the software package PAUP (Version 4.0; Swofford 1998) with 1000 bootstrap replicates (Felsenstein 1985, Hedges 1992).

Statistical analyses. All statistical analyses were performed using the software program SAS Version 8.1 (SAS Institute). Tests of proportions of the prevalence and susceptibility of oligochaetes and the genetic screening comparisons between all sites, dates or lineages employed Fisher's exact test of independence (Christensen 1990). We used Fisher's exact test throughout our study rather than the more common chi-square comparison because, at times, sample sizes were too small for the chi-square analysis (see SAS Version 8.1).

RESULTS

Prevalence of infection

In the first study, initial screening of the 4 groups of oligochaetes from both sites and at both collection times demonstrated that only Tubificidae with hair chaetae (*Tubifex tubifex*) were found releasing triactinomyxon stages of *Myxobolus cerebralis*. Throughout the studies, no *T. tubifex* were found releasing any actinosporeans other than those characteristic of *M. cerebralis*. Combining data from both collection times, the number of infected *T. tubifex* was 38 from a total of 4349 oligochaetes from the WG site and 22 from a total of 3376 from the UCR site (Table 1). The prevalence of *T. tubifex* releasing triactinomyxons from naturally acquired infections ranged from 0.4 to 1.4% (0.7% combined) at WG and 0.4 to 0.8% at UCR (1.0% combined).

Susceptibility of the *Tubifex tubifex* not initially releasing triactinomyxons stages was assessed in the laboratory following exposure to spores of *Myxobolus cerebralis*. There were 230 *T. tubifex* from a total of 3532 from the WG site and 181 from a total of 1789 from the UCR site found releasing triactinomyxons at 3 mo post exposure (Table 1). The prevalence of infection therefore ranged from 5.3 to 8.5% (6.5% combined) at WG and 4.2 to 14.1% (10.1% combined) at UCR in 1998. There was no significant difference in susceptibility of oligochaetes between the WG and UCR sites ($p = 0.8107$) or between the collection dates 13 September ($p = 0.3168$) and 12 October ($p = 0.1617$) in 1999. However, there were more susceptible oligochaetes on the first collection date (13 September) within each site, (WG: $p = 0.0012$; UCR: $p = 0.0020$) compared to the other collection dates.

In the second study, all *Tubifex tubifex* collected were exposed to *Myxobolus cerebralis* spores from 2 sources, brown trout from the South Platter River and rainbow trout from Mount Whitney, immediately upon return to the laboratory. Therefore, no information on the initial prevalence of infection in the oligochaetes was obtained. Also in contrast to the first study, oligochaetes were examined with newly developed genetic markers at the termination of the study. The number of oligochaetes releasing triactinomyxons

Table 1. *Tubifex tubifex*. Prevalence of *Myxobolus cerebralis* infection among Colorado River oligochaetes in Study 1 (1998). The prevalence of infection among oligochaetes from the field was determined as the percentage releasing triactinomyxons out of the total collected. Susceptibility of the oligochaetes from the field not found releasing triactinomyxons was assessed 3 mo after exposure to *M. cerebralis* spores in the laboratory. Sites of oligochaete collections were Windy Gap Reservoir (WG) and Upper Colorado River (UCR)

Experiment	Collection site			
	WG		UCR	
	21Sep	28Oct	21Sep	28Oct
Field experiment				
Total oligochaetes collected	2075	3312	2699	2078
Number of *T. tubifex*	1929	2420	2522	854
Number initially infected	29	9	19	3
Prevalence (%)	1.5	0.4	0.8	0.4
Laboratory experiment				
Number exposed	1362	2170	1069	720
Laboratory infected	116	114	151	30
Susceptibility (%)	10.4	5.6	15.6	4.6
Prevalence (%)	8.5	5.3	14.1	4.2

Table 2. *Tubifex tubifex* from Expts 1 and 2 of Study 2 (1999) found releasing (positive) or not releasing (negative) triactinomyxons 3 mo following experimental exposure to spores of *Myxobolus cerebralis*. The number of dead oligochaetes represents the difference between the number of oligochaetes initially exposed and the number remaining 3 mo post-exposure. Oligochaetes were collected from Windy Gap Reservoir (WG) and Breeze Bridge (BB), Colorado, and exposed to *M. cerebralis* spores harvested from rainbow trout at Mount Whitney, California (Expt 1), or from brown trout at South Platte River,Colorado (Expt 2)

	Expt 1		Expt 2	
Date of collection	13 Sep		13 Sep	
Date of exposure	12 Oct		3 Nov	
Oligochaete collection site	WG	BB	WG	BB
Number exposed	295[a]	288[a]	245[b]	264[b]
Number positive	21	9	24	12
Number negative	24	208	199	166
Number dead	250	71	22	86

[a] Each oligochaete exposed to 1.1×10^4 spores
[b] Each oligochaete exposed to 6×10^3 spores

(susceptible) ranged from 9 to 21 in Expt 1 with spores originating from Mount Whitney and 12 to 24 in Expt 2 with spores from the South Platte River origin (Table 2). The number of oligochaetes not releasing triactinomyxons (resistant) ranged from 24 to 208 in both experiments. There was no significant difference in the proportion of susceptible or resistant oligochaetes between Expts 1 and 2 at either site ($p = 0.3531$). The greater mortality of oligochaetes from WG in Expt 1 was unexplained. To reduce stress on the oligochaetes, they were only counted at the onset and termination (3 mo) of the experiments, and this precluded determining the susceptibility or genotype of oligochaetes dying during the experimental period. There was a difference in the susceptibility of oligochaetes between the 2 sites in Expt 1 and for the 2 experiments combined (both $p < 0.0001$), but not between the WG and BB sites in Expt 2 ($p = 0.2180$). The mortality in Expt 2 was 0.09% (22 of 245) among WG oligochaetes and 0.326% (86 of 264) among those from BB, i.e. they were significantly different from each other ($p < 0.0001$).

Genetic screening

The genetic screening assay based on the mt16S lineage-specific PCR separated each mitochondrial lineage by the size of the resulting amplified fragment. Individual oligochaetes representing the 3 lineages (I, III, VI) yield a single PCR product of defined length (Fig. 2). Those oligochaetes from which no amplified fragment was observed using the lineage-specific primers, and whose DNA sequence was determined

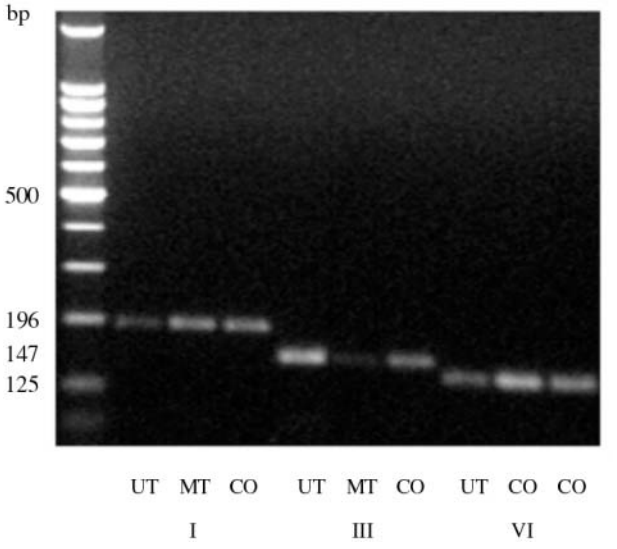

Fig. 2. *Tubifex tubifex*. Agarose gel electrophoresis of PCR-amplified genomic DNA using mt 16S rDNA Lineage-Specific Primers I (196 bp), III (147 bp) and VI (125 bp) of oligochaetes from Logan River, Utah (UT), Bozeman Fish Tech Center, Montana (MT) and Windy Gap Reservoir, Colorado (CO). A DNA size standard ladder (100 bp) is shown on the left side of the gel (2%)

using the 16sar and 16sbr primer, all belonged to Lineage V (GenBank Accession No. AF426845–AF426860), with the exception of 1 WG oligochaete from Lineage III (AF426844: Fig. 3). The analysis of samples from WG revealed that susceptible and resistant oligochaetes were found in Lineages I and III in different proportions (Table 3). At WG, Lineage III was more likely to have resistant oligochaetes than lineage I ($p = 0.0360$). In contrast to WG reservoir, BB was more likely to have resistant oligochaetes in Lineage V than III ($p = 0.0063$). At BB, the only lineage with susceptible oligochaetes was III; no susceptible oligochaetes were observed in Lineages I ($n = 12$), V ($n = 21$) and VI ($n = 6$). Interestingly, oligochaetes in lineage V were resistant and only found at BB. The lowest number of oligochaetes was found in lineage VI at both sites, and they were all resistant. There was no evidence of Lineages II and IV (Sturmbauer et al. 1999) in our study.

DISCUSSION

In this study over 10 164 oligochaetes were examined and only *Tubifex tubifex* was found to be susceptible to *Myxobolus cerebralis* infections, as demonstrated by the release of the triactinomyxons stages following either natural or experimental exposure to the parasite. These findings are in agreement with previous studies that report *T. tubifex* as the sole host for

M. cerebralis (Markiw & Wolf 1983, Wolf & Markiw 1984, Markiw 1986, Wolf et al. 1986, El-Matbouli & Hoffman 1989, 1995, 1998). Our results do not support the proposal by Brinkhurst (1996) that there may be other alternate oligochaete hosts for *M. cerebralis* in the families Lumbriculidae, Naididae and other Tubificidae. Our daily screening of individual oligochaetes never revealed releases of more than 1 morphological type of actinosporean. This suggests that in our study sites *T. tubifex* is supporting few other myxosporean infections. In contrast, Yokoyama et al. (1991) found several types of actinosporeans released from a single *T. tubifex* obtained from a fish pond in Japan.

The susceptibility of *Tubifex tubifex* at our study sites in the upper Colorado River ranged from 0.4 to 1.5%; This is very similar to the 1 to 2.0% previously

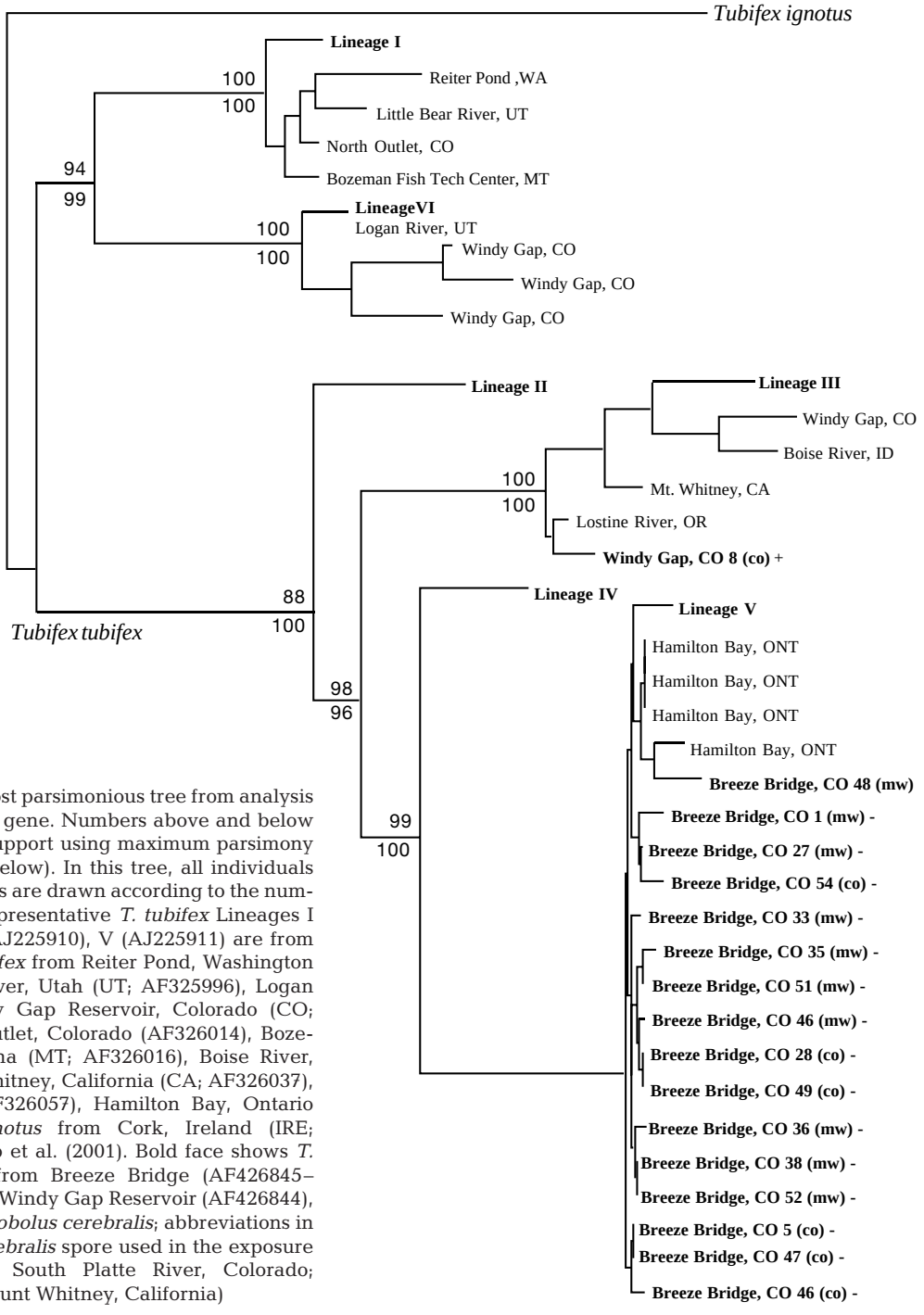

Fig. 3. *Tubifex tubifex*. Single most parsimonious tree from analysis of a portion of the mt 16S rDNA gene. Numbers above and below the nodes represent bootstrap support using maximum parsimony (above) and neighbor joining (below). In this tree, all individuals are represented and the branches are drawn according to the number of inferred substitutions. Representative *T. tubifex* Lineages I (AJ225906), II (AJ225907), IV (AJ225910), V (AJ225911) are from Sturmbauer et al. (1999). *T. tubifex* from Reiter Pond, Washington (WA; AF325998), Little Bear River, Utah (UT; AF325996), Logan River, Utah (AF325991), Windy Gap Reservoir, Colorado (CO; AF326006-AF326009), North Outlet, Colorado (AF326014), Bozeman Fish Tech Center, Montana (MT; AF326016), Boise River, Idaho (ID; AF326004), Mount Whitney, California (CA; AF326037), Lostine River, Oregon (OR; AF326057), Hamilton Bay, Ontario (ONT; AF326025) and *T. ignotus* from Cork, Ireland (IRE; AF325987) are from Beauchamp et al. (2001). Bold face shows *T. tubifex* that are negative (–) from Breeze Bridge (AF426845–AF426860) and positive (+) from Windy Gap Reservoir (AF426844), Colorado, after exposure to *Myxobolus cerebralis*; abbreviations in parentheses: locations of *M. cerebralis* spore used in the exposure experiment (co: brown trout, South Platte River, Colorado; mw: rainbow trout, Mount Whitney, California)

Table 3. *Tubifex tubifex*. Distribution into four 16S mtDNA lineages of oligochaetes releasing (+) and not releasing (–) triactinomyxons of *Myxobolus cerebralis* in Study 2 from genetically heterogeneous populations collected from Windy Gap Reservoir (WG) and Breeze Bridge (BB), Colorado (see Table 2), and numbers in geographic populations for Lineages I, III, V and VI (see Fig. 2). *T. tubifex* were exposed to spores from rainbow trout (Mount Whitney, California) and brown trout (South Platte River, Colorado) in Expts 1 and 2, respectively

Lineage	Expt 1				Expt 2				Combined results			
	WG		BB		WG		BB		WG		BB	
	(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)
I	1	17	0	7	23	7	0	5	24	24	0	12
III	20	5	9	35	1	43	12	28	21	48	21	63
V	0	0	0	10	0	0	0	11	0	0	0	21
VI	0	1	0	1	0	0	0	5	0	1	0	6

reported for wild populations of *T. tubifex* and other oligochaetes infected with myxosporeans (Markiw 1986, Burtle et al. 1991, Yokoyama et al. 1991, 1993, Styer et al. 1991, Kent et al. 1993, El-Matbouli & Hoffman 1995, Xiao & Desser 1998). Rather than assessing prevalence by releases of actinosporeans, Rognlie & Knapp (1998) and Zendt & Bergerson (2000) obtained similar prevalence estimates (0.8 to 2.6%) using PCR to detect *M. cerebralis* infections among wild *T. tubifex* collected from enzootic areas of whirling disease in the states of Montana and Colorado. Our experimental exposures of *T. tubifex*, either after holding them in laboratory or immediately after collection, clearly showed that there is a much larger proportion of the population (up to 14%) that is susceptible to *M. cerebralis* infections, and this may differ between study sites (Tables 1 and 2). Several factors potentially influence the susceptibility of *T. tubifex* to *M. cerebralis* infections. They may include a number of environmental factors such as water quality and temperature (El-Matbouli et al. 1999b), season, day-length, etc. (Yokoyama et al. 1991, Rognlie & Knapp 1998, Xiao & Desser 1998). The dose and perhaps age of spores that the oligochaetes ingest are also critical (Stevens et al. 2001). In contrast, spore origin appeared not to greatly influence the susceptibility of *T. tubifex* to spores originating from 2 host fish species (rainbow and brown trout) and geographic sites (California and Colorado) in our study. Studies characterizing the genotype and virulence of strains of *M. cerebralis* suggest they comprise a fairly homogeneous group, regardless of geographic origin (Andree et al. 1997, T. McDowell et al. unpubl. data). Host factors, such as size, age and genotype of the oligochaete, are also presumed to greatly influence the susceptibility to *M. cerebralis* infections.

Our initial molecular screening of *Tubifex tubifex* in the upper Colorado River suggest that it is not a homogeneous taxon but consists instead of several genetically distinct lineages. Of the 4 lineages of *T. tubifex* found in our study, 2 (V, VI) appear to be resistant to infection with *Myxobolus cerebralis*, while the other 2 (I, III) appear to contain both susceptible and resistant oligochaetes. There was no evidence at our study sites for the 2 lineages of *T. tubifex* described by Sturmbauer et al. (1999) that are known from freshwater habitats in Europe.

One lineage of *Tubifex tubifex* (V) found in our study at the Breeze Bridge site is related to oligochaetes from Hamilton Bay, Ontario, Canada, that have been shown to be resistant to *Myxobolus cerebralis* infections in the laboratory. The Hamilton Bay oligochaetes have been shown to be capable of ingesting and then inactivating the spores of *M. cerebralis* (M. El-Matbouli unpubl. data). The spores ingested by these oligochaetes hatch in the intestine but cannot complete the infection cycle; thus the oligochaetes effectively remove the spores they consume from the environment. These oligochaetes essentially act as biological filters, preventing susceptible oligochaetes from coming into contact with the parasite, a phenomenon demonstrated by laboratory trials examining experimental infections of mixed populations of susceptible and resistant *Tubifex* spp. (M. El-Matbouli unpubl. data). The relative abundance of *Tubifex* species that are completely or partially resistant to *M. cerebralis* infections could therefore exert considerable influence on the infectivity and therefore the impact of whirling disease on wild or captive populations of trout. Other important features of infections once they become established in the oligochaete host, including duration and patterns of release of triactinomyxons, may influence the severity of whirling disease in a given watershed (Gilbert & Granath 2001, Stevens et al. 2001).

The ecology of the habitat throughout a species range may also influence host-parasite interactions among populations of *Tubifex* spp. Certain waters in the state of Colorado have been characterized as highly impacted by whirling disease, based upon the severity of infections in wild and sentinel trout and high numbers of triactinomyxons of *Myxobolus cerebralis* found in the water by periodic filtration (Nehring 1998, 1999, Thompson et al. 1999, Thompson & Nehring 2000, Nehring & Thompson 2001). The upper Colorado River, downstream from Windy Gap Reservoir, is one such highly impacted site. The dam creating the reservoir is presumed to have established a new habitat conducive to the greater abundance of oligochaetes susceptible to *M. cerebralis* (Zendt & Bergersen 2000). Water flow, temperature and dissolved gases have also

been studied in relation to the severity of whirling disease at this site (Schisler et al. 2000). Together, these data suggest that Windy Gap Reservoir represents a significant point source of infection for wild trout in the upper Colorado River. Our studies of the oligochaete populations in this area suggest that particular lineages (e.g. I and III) that contain oligochaetes susceptible to *M. cerebralis* are abundant and may in part be responsible for the severity of whirling disease at this site. Of interest however, is the presence at BB of oligochaetes from lineages that may represent more resistant oligochaetes (e.g. V and VI), whose abundance, if increased, might dampen the overall impact of whirling disease in the upper Colorado River.

Our data supports the hypothesis that the abundance and genotype of *Tubifex tubifex* are important in defining high- and low-impact areas in the upper Colorado River. More in-depth genetic characterizations of *T. tubifex* acting as hosts for *Myxobolus cerebralis* are underway with the ultimate goal of determining how the abundance and distribution of these naturally occurring oligochaete populations influence the severity of whirling disease in wild trout populations.

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