

A First Assessment of Mitochondrial DNA Variation and Geographic Distribution of Haplotypes in Hessian fly (Diptera: Cecidomyiidae)

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ABSTRACT Domain III of the mitochondrial 12S rRNA gene from Hessian fly, *Mayetiola destructor* (Say), was sequenced in 21 populations from the United States, two populations from Canada, five populations from the Mediterranean basin, one population from Southwest Asia, and one population from New Zealand. From the total alignment, seven unique mitochondrial DNA (mtDNA) sequences (haplotypes) were identified. Of the seven 12S haplotypes only three (haplotypes 1, 2, and 3) occurred in populations from North America, indicating these were most likely the introduced haplotypes. Haplotypes were not restricted to any given biotype of *M. destructor* as defined by virulence to resistance genes in wheat. Thus, neutral markers did not show structure based on biotype. Populations of *M. destructor* showed a predominance of one haplotype over the others in specific geographic regions. However, *Wolbachia* DNA was not detected in any population, indicating that inheritance patterns of mtDNA in *M. destructor* were most likely due to repeated bottlenecks leading to the expansion of one lineage over another. The complete complement of 12S sequences in the *M. destructor* populations was subjected to a phylogenetic reconstruction by using haplotypes 1 and 3 of the gall midge *Orseolia oryzae* (Wood-Mason), as outgroups. Results from this initial study indicate a more robust phylogenetic reconstruction and analysis of population history will test the hypothesis of a single introduction of *M. destructor* into North America.

KEY WORDS *Mayetiola destructor*, mtDNA, intraspecific variation, dispersal, *Wolbachia*

THE HESSIAN FLY, *Mayetiola destructor* (Say), poses a significant economic threat to crop production in all wheat-growing areas of the United States and is the most important insect pest of wheat in the eastern soft-winter-wheat region. It is thought to be endemic to the southern Caucasus and Southwest Asia, the presumed center of origin of the genus *Triticum* L. (Harlan and Zohary 1966), and to have dispersed to North Africa and Europe (Ratcliffe and Hatchett 1997). Historically, the first report of *M. destructor* infesting wheat in North America was on Long Island, NY, in 1779, and it was believed Hessian mercenaries serving with Lord Howe's army during the Revolutionary War had brought the pest 3 yr earlier from Europe in bedding straw (Ratcliffe and Hatchett 1997). However, the possibility of additional introductions of the pest during the colonization of North America by Europeans has been suggested, specifically introduction of *M. destructor* into California by the Spanish (Packard 1928). The possibility of multiple introductions into North America has implications concerning genetic variation and the ability of the pest to respond to resistance genes in wheat.

The primary method of control of the insect is through genetically resistant wheat (Gallun 1977). However, the development of biotypes capable of surviving on formerly resistant wheat is a threat to the durability of resistance (Ratcliffe et al. 1994). Resistance in wheat is expressed as larval antibiosis and is controlled by dominant alleles at one or two loci. Virulence in the insect (ability of larvae to survive on and stunt plants) for some resistance genes in wheat is controlled by recessive alleles at single loci and operates in a gene-for-gene relationship with resistance (Hatchett and Gallun 1970, Gallun 1978, Formusoh et al. 1996, Zantoko and Shukle 1997). Populations of *M. destructor* generally contain a mixture of virulence alleles (Ratcliffe et al. 2000), suggesting that gene flow occurs across biotypes. Previous analysis of allozyme variation among *M. destructor* populations suggested considerable genetic differentiation among local populations (Black et al. 1990). The most likely explanation of this local differentiation was considered to be genetic drift among natural populations. Several aspects of the biology of *M. destructor* were considered likely to reduce gene flow among local populations in the field. Dispersal by adults is limited and probably does not exceed a few hundred meters at most (Harris et al. 2003). Males and females are short lived (generally 1 d for males; 2 to 3 d for

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females), and behaviors have evolved for rapid mate location within proximity (Harris and Foster 1991). Oviposition by females begins 1 to 3 h after mating and limits the time a female can spend in dispersal (Harris and Rose 1991). Gene flow or rate of migration among populations is fundamental to the rate at which virulence can evolve and spread in populations. In *M. destructor*, immigration of dominant alleles for avirulence would reduce virulent, recessive homozygotes and decrease the rate at which new virulent phenotypes arise. Restricted local gene flow would increase the probability of new recessive mutations becoming homozygous.

Although *M. destructor* populations have been sampled to monitor biotype composition, no molecular analyses have been conducted to study introduced populations of the pest in North America in reference to ancestral populations in the Old World. This can restrict interpretation of genetic variation among populations in North America. Additionally, despite the economic importance of the pest, dispersal patterns and intraspecific variability have been studied infrequently. Sequence variation within mitochondrial DNA (mtDNA) has been used extensively to study insect populations (Caterino et al. 2000), including intraspecific phylogeography of phlebotomine sandflies (Essegir et al. 1997, Ready et al. 1997) and the blowfly *Lucilia cuprina* (Weidemann), (Gleeson and Sarre 1997) and can reveal historical and phylogeographical patterns that reflect dispersal of the organism and fragmentation of its environment (Avise et al. 1987, Avise 1994).

The objective of the current study with *M. destructor* was to make an initial assessment of mitochondrial DNA variation and the geographic distribution of haplotypes, to determine the feasibility of addressing ancestry and historical biogeography, and to test the prevailing hypothesis that there was a single introduction of *M. destructor* into North America.

Materials and Methods

Experimental Insect. Samples of populations from different geographic origins within the United States, Canada, the Mediterranean basin, Southwest Asia, and New Zealand were obtained between 2000 and 2003. Population samples from within the United States and from Canada, New Zealand, and Israel were composed of puparia from which adults were allowed to emerge. Population samples from Spain, Morocco, Syria, and Kazakhstan were preserved in 100% ethanol. Samples of *M. destructor* populations from Manitoba and Ontario, Canada, were provided by Dr. Jay Whistlecraft (Agriculture Canada, London, Ontario, Canada). The Horticultural Research Institute (Mt. Albert, New Zealand) provided the sample of *M. destructor* from New Zealand (Harris et al. 2001). The population sample from Spain was provided by Dr. Jose Del Moral (SIDT, Department de Fitopatologia, Barajoz, Spain). Population samples from Israel were provided by Dr. Phyllis G. Weintraub (Gilat Research Center, Negev, Israel), and population samples from Syria,

Kazakhstan, and Morocco were kindly provided by Dr. Mustapha El Bouhssini (Germplasm Program, ICARDA, Aleppo, Syria). Samples of the wheat midge, *Sitodiplosis mosellana* (Géhin), were provided by Dr. Robert Lamb (Agriculture Canada, Winnipeg, Canada).

Biotype composition of population samples as defined by virulence to resistance genes in wheat was determined in the present work as described by Ratcliffe et al. (2000) for populations from North America, New Zealand, and Israel. Virulence to resistance genes within the population from Spain was determined by Dr. Jose Del Moral. Virulence to resistance genes is present at varying frequencies in the populations from Morocco, Syria, and Kazakhstan (Naber et al. 2000, 2003). Populations from Israel, New Zealand, and Canada were established in culture for future reference as described by Black et al. 1990.

Amplification and Sequencing. For polymerase chain reaction (PCR) amplifications, DNA was extracted from individual adults or pupae by using the Omega Mollusc DNA EZ-kit (Life Science Products, Frederick, CO). Domain III of the mitochondrial 12S rRNA gene was amplified using total DNA extracted from *M. destructor* and *S. mosellana*, respectively, and primers 12S-F, 5'-AATTA AAAACGACGGGCAATATGT-3' and 12S-R, 5'-AAACTAGGATTAGATACCCTTTTAT-3' based on the sequence of 12S rRNA primers SRJ-14233 and SR-N-14588 (Simon et al. 1994) for *Drosophila* and other insects, with modification based on sequence data obtained for the 12S rRNA gene from *M. destructor*.

Infection with *Wolbachia* was assayed with primers based on the 16S rRNA gene (16S-F, 5'-TTGTAGCTGCTATGGTATAACT-3' and 16S-R, 5'-GAATAGTATGATTTTCATGT-3') of *Wolbachia pipientis* (O'Neill et al. 1992, Behura et al. 2001) and primers designed to the *Wolbachia* *ftsZ* gene (FtsZ-F, 5'-TACTGACTGTTGGAGTTGTAAC TAAGCCGT-3' and FtsZ-R, 5'-TGCCAGTTGCAAGAACAGAACTCTAACTC-3') and *wsp* gene (WspF-, 5'-TGGTCAATAAGTGATGAAGAACTAGCTA-3' and WspR, 5'-AAAAATTA AACGCTACTCCAGCTTCTGCAC-3') (Jeyaprakash and Hoy 2000).

PCR amplifications for the 12S rRNA sequences were carried out in 25- μ l volumes of 1 \times PCR buffer (Promega, Madison, WI), 2 mM MgCl₂, 400 μ M dNTPs, 2 μ l of template DNA (vide supra), 0.5 μ M each primer, and 2.5 U of *Taq* DNA polymerase (Promega). PCR amplifications for *Wolbachia* DNA were by Long PCR by using the linked thermal cycler profiles described by Jeyaprakash and Hoy (2000). Amplifications were performed in an MJ Research DNA Engine Dyad thermal cycler (MJ Research, Watertown, MA). Amplification of 12S rRNA sequences was with the following program steps: 1) 94°C for 1 min, 2) 50°C for 30 s, 3) 72°C for 1 min, 4) cycle to step 1 thirty-four times, 5) 72°C for 5 min, and 6) 4°C hold. A positive control for amplification of the *Wolbachia* DNA was with total DNA extracted from both individuals and bulks of the weevil *Callosobruchus chinensis* L., which is known to be infected with *Wolbachia*

(Ijichi et al. 2002). DNA negative controls were included in all PCR amplifications.

PCR products were separated in 1% agarose gels and visualized by ethidium bromide staining. PCR amplicons were extracted from gel slices using the QIAquick gel extraction kit (QIAGEN, Valencia, CA) and sequenced using dye-termination cycle sequencing (Sanger et al. 1977) by the Purdue Genomics Facility over both strands.

Sequence Analysis. Sequences were aligned using ClustalX (1.81) software (Thompson et al. 1997). The distance/neighbor-joining (NJ; Saitou and Nei 1987) method was used for phylogeny reconstruction. *Anopheles gambiae* (Giles) was used as the outgroup for interspecific analysis of 12S sequences from *M. destructor* and other cecidomyiid species. 12S rRNA sequences for the following species, accession numbers in parentheses, were downloaded from GenBank: *An. gambiae* (L20934), *O. oryzae* haplotype 1 (AF306553), *O. oryzae* haplotype 2 (AF306554), *O. oryzae* haplotype 3 (AF306555), gall midges in the subtribe Baldratiina (Diptera: Cecidomyiidae) *Stefaniella trinacriae* Stefani (AJ495756), *Stefaniola brevis* Mohn (AJ495743), *Stefaniola siliqua* Dorchin (AJ495749), *Izeniola obesula* Dorchin (AJ495742), *Baldratia salicorniae* Kieffer (AJ495744), *Baldratia suaedae* Mohn (AJ495745), and *Baldratia occulta* Dorchin (AJ495754). For intraspecific analysis of 12S sequences in the *M. destructor* populations studied, *Orseolia oryzae* (Wood-Mason) haplotypes 1 and 3 were used as outgroups. We used PAUP 4.0b10 for Windows (Swofford 1998) to conduct the analyses [NJ: minimum evolution criterion, Felsenstein's distance estimator (Felsenstein 1984) for unequal base frequencies, unequal transitions (Ti):transversions (Tv)]. Confidence values for groupings in the trees were assessed by bootstrap resampling (Felsenstein 1985) with 10,000 repetitions for distance/NJ. Gaps were excluded from the analyses.

Data Deposition. The 12S rRNA nucleotide sequence data for *M. destructor* and *S. mosellana* used in phylogenetic reconstructions were submitted to GenBank and have the following accession numbers: *M. destructor* haplotypes 1–7, AY460205–AY460211; and *S. mosellana* haplotype 1, AY461598.

Results and Discussion

Using primers for amplification of domain III of the mitochondrial 12S rRNA gene, a fragment of ≈ 400 bp from 30 populations of *M. destructor* was amplified. Primers used for *M. destructor* also amplified a fragment of the expected size from *S. mosellana*. Analysis of PCR products in agarose gels revealed a single amplicon and no indication of heteroplasmy or possible pseudogenes. Blastn searches of the sequences showed high homology to 12S rRNA genes from other insect species, confirming the PCR amplicons were from the mitochondrial 12S rRNA gene. From the total alignment, there were seven unique mitochondrial DNA sequences (haplotypes) identified among the *M. destructor* populations. Gaps were ignored, and

each haplotype was numbered in the order it was identified. Five transitions were revealed, including two C→T and three A→G. Only two transversions were identified, and both were A→T.

Three 12S haplotypes occurred in the North American populations (Fig. 1). Haplotype 1 was predominant in populations from the southeastern United States and the Northwest, whereas haplotype 2 showed prevalence in populations from Illinois, Indiana, North Carolina, and Winnipeg (Manitoba, Canada). Haplotype 3 was present at low frequency in populations from Illinois, Indiana, and North Carolina but was present at nearly equal frequency with haplotype 2 in the population from Ontario, Canada.

Within the populations from the Mediterranean basin, Southwest Asia, and Australasia, all seven 12S haplotypes were found (Fig. 2). Haplotype 1 was predominant in the populations from Spain, Morocco, and New Zealand. Haplotype 2 was the predominant haplotype in the population from Kazakhstan. Haplotype 3 was identified at low frequencies in the populations from Spain and Morocco. Haplotype 4 was found in Kazakhstani populations at a low frequency. Haplotypes 5 and 6, two previously unseen types, were identified in the population from Syria. The Israeli populations showed only haplotype 7.

Of the 12S haplotypes identified in the present work, only three of the seven were found within North America. Having been identified in other parts of the world, the presence of haplotypes 1, 2, and 3 indicate that these mutations are not specific to North American *M. destructor* populations and therefore these haplotypes have not evolved here. Furthermore, the presence of only haplotypes 1, 2, and 3 are most likely representative of the initial introduction/s into North America.

A phylogenetic analysis was conducted based on the seven *M. destructor* 12S sequences, the *S. mosellana* 12S sequence amplified in the current work, and the complete complement of 12S sequences in GenBank for named cecidomyiid species. This matrix provided 463 total characters of which 144 were parsimony-informative and 88 were uninformative. The distance/neighbor-joining phylogenetic reconstruction (Fig. 3) positioned *O. oryzae* haplotypes 1 and 3 distant from the other cecidomyiid species. *S. mosellana* was positioned basally among the remaining cecidomyiid species. A clade, whose topology was congruent with previous studies (Dorchin et al. 2004), contained gall midges within the subtribe Baldratiina (Diptera: Cecidomyiidae). A second clade contained the seven *M. destructor* haplotypes. Within this clade, haplotype 7 from the Israeli populations was positioned basally, suggesting that haplotype 7 is the most ancestral mitotype sequenced to date. Mild statistical support is given for the separation of *M. destructor* haplotypes; however, there is resolution of the seven haplotypes via restriction enzyme analysis. Each of the haplotypes can be detected through a precise series of restrictions. Haplotypes 1, 4, and 5 share several restriction fragment length polymorphisms in common, which reduces the amount of variation between the three

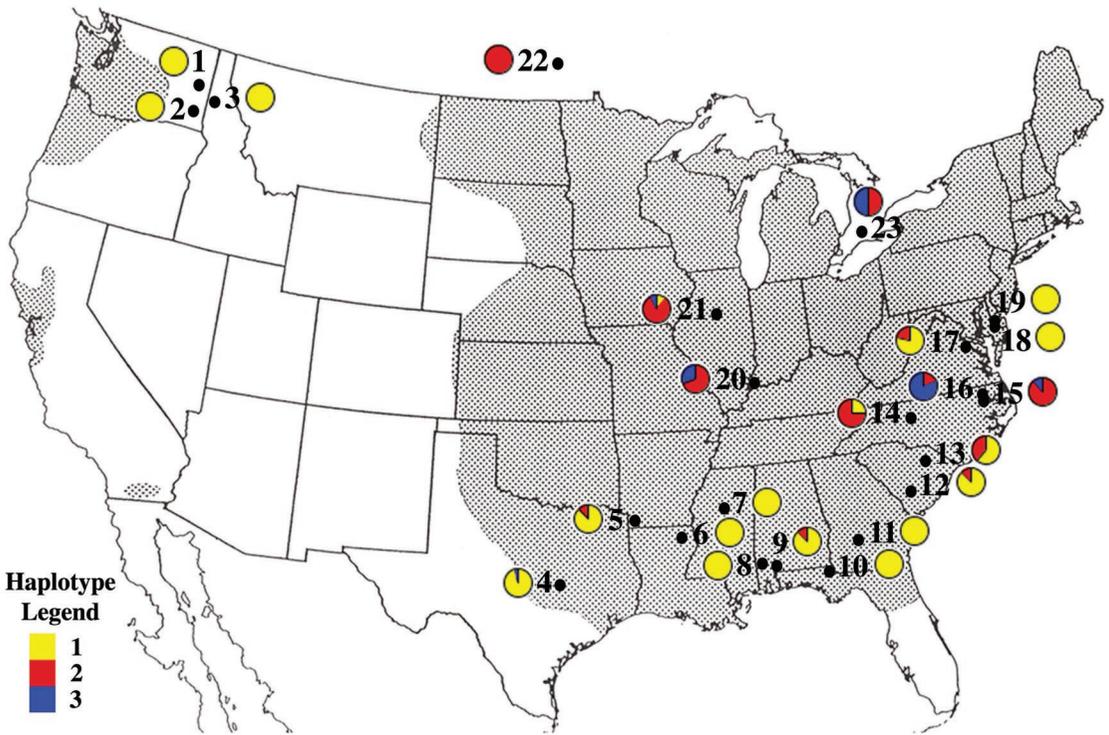


Fig. 1. Distribution range of *M. destructor* within the United States sampling sites used. Shaded areas represent reported distribution (Foster et al. 1986). An increase in the distribution range has occurred in the Northwest since 1986 as *M. destructor* has moved eastward. Numbers on the map indicate collection site. Frequency distribution of haplotypes is indicated by the pie charts as follows where the number before the parenthesis indicates haplotype number and the number within indicates the number of individuals: 1. Spokane County (Co.), WA, 1 (32); 2. Garfield Co., WA, 1 (40); 3. Lewis Co., ID, 1 (24); 4. Milan Co., TX, 1 (29), 3 (1); 5. Miller Co., AR, 1 (28), 2 (4); 6. Richland Co., LA, 1 (48); 7. Washington Co., MS, 1 (20); 8. Hale Co., AL, 1 (40); 9. Baldwin Co., AL, 1 (42), 2 (6); 10. Jackson Co., FL, 1 (20); 11. Sumter Co., GA, 1 (32); 12. Barnwell Co., SC, 1 (35), 2 (5); 13. Florence Co., SC, 1 (23), 2 (15); 14. Rowan Co., NC, 1 (10), 2 (30); 15. Beaufort Co., NC, 2 (25), 3 (3); 16. Washington Co., NC, 2 (6), (34); 17. New Kent Co., VA, 1 (30), 2 (8); 18. Wicomico Co., MD, 1 (48); 19. Sussex Co., DE, 1 (30); 20. Posey Co., IN, 2 (22), 3 (10); 21. Peoria Co., IL, 1 (30, 2 (22), 3 (2); 22. Winnipeg, Manitoba, Canada, 2 (25); and 23. London, Ontario, Canada, 2 (27), 3 (28).

types to only five parsimonious informative characters supporting the close association in the phylogenetic reconstruction.

Genetic distances (Table 1) among the cecidomyiid 12S sequences were estimated by the method of Felsenstein (1984). The *A. gambiae* 12S sequence, as

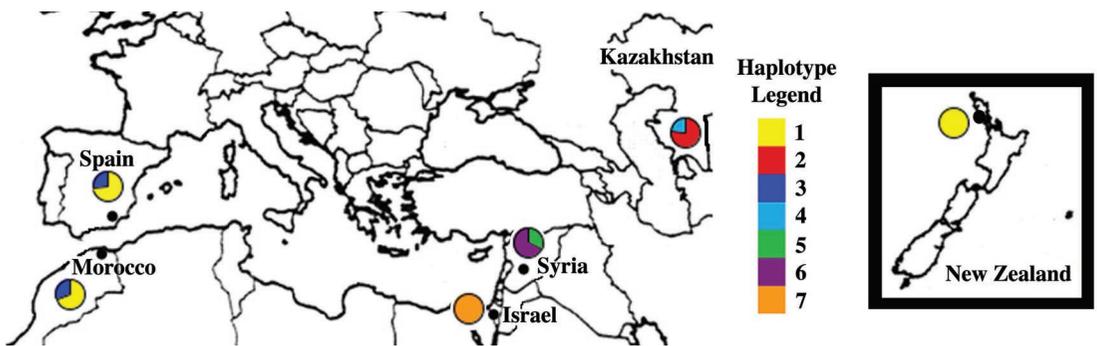


Fig. 2. Geographic locations of *M. destructor* in the Mediterranean basin, Southwest Asia, and New Zealand. Dots on map indicate collection site. Frequency distribution of haplotypes is indicated by the pie charts as follows: Spain, 1 (26), 3 (10); Morocco, 1 (20), 3 (9); Rahama, Israel, 7 (20); Gilat, Israel, 7 (50); Syria, 5 (23), 6 (46); Kazakhstan, 2 (53), 4 (16); and New Zealand, 1 (48).

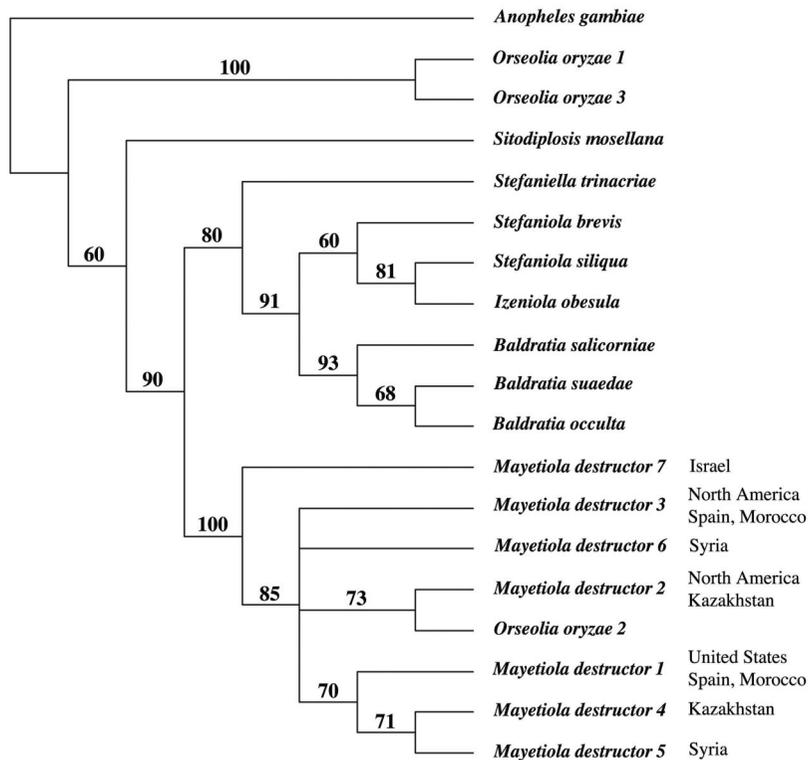


Fig. 3. Phylogenetic reconstruction based on domain III of the mitochondrial 12S rRNA gene from *M. destructor* (haplotypes 1–7), *S. mosellana*, *O. oryzae* (haplotypes 1–3), and gall midge species within the subtribe Baldratiina. *An. gambiae* was included as the outgroup. The topology and branch length of the cladogram is produced by distance/neighbor-joining. Numbers above branches correspond to bootstrap support above 50% by using the distance estimator of Felsenstein (1984). *M. destructor* haplotypes were numbered according to the order of their finding, and the place of incidence is listed for each haplotype.

expected from a distant relative, differed in nucleotide sequence divergence from the cecidomyiid 12S sequences by 45.6% (*O. oryzae* haplotype 1) to 53.5% (*O. oryzae* haplotype 2). Divergence among the *M. destructor* 12S haplotypes ranged from 0.76 to 3.44%. The greatest degree of divergence among the *M. destructor* haplotypes occurred with respect to haplotype 7 (Israeli populations), which differed in nucleotide sequence from the remaining haplotypes by 2.61% (haplotype 1) to 3.44% (haplotype 5). This high percentage is indicative of between-species comparisons; therefore, further analysis is needed to determine whether haplotype 7 is ancestral or an incipient species. The range of pairwise sequence divergence rates for mitochondrial DNA of higher eukaryotes is generally quoted at 1.0–2.3% per million years (Brown 1983, Hasegawa et al. 1985, Brower 1994, Hewitt 1996). Whereas the present analysis of sequence divergence in the mitochondrial genome of *M. destructor* was defined by variation in domain III of the 12S rRNA gene (vide supra), results suggest the 12S haplotypes revealed in this study shared a common ancestral mitochondrial genome \approx 1.5–3.4 million years ago.

Surprisingly, the phylogenetic analysis supported positioning haplotype 2 of *O. oryzae* with haplotype 2

of *M. destructor*. Divergence among the *M. destructor* haplotypes and *O. oryzae* haplotype 2 ranged from 2.39% (*M. destructor* haplotype 2) to 5.47% (*M. destructor* haplotype 7). In contrast, *O. oryzae* within species divergence was greater between both haplotype 1 and haplotype 3 in comparison with haplotype 2 (34.7 and 36.8%, respectively). This strange lack of divergence between *O. oryzae* haplotype 2 and *M. destructor* haplotype 2 could be reflective of the common ancestral mitotype once shared by the cecidomyiid relatives several million years; however, it is beyond the scope of this research to further elucidate the significance of this find. Yet, if present results are supported by later study with additional genes, the presence of an incipient species within *O. oryzae* may be supported.

The complete complement of 12S sequences in the *M. destructor* populations studied also was subjected to an intraspecific phylogenetic reconstruction by using the distance/neighbor-joining method with *O. oryzae* 12S haplotypes 1 and 3 serving as outgroups (Fig. 4). This analysis supported the previous reconstruction by positioning haplotype 7 in the Israeli populations basally among the *M. destructor* haplotypes, further supporting the possibility that haplotype 7 could be an

Table 1. Distance matrix from sequence analysis of domain III of the mitochondrial 12S rRNA gene for cecidomyiid species studied plus *An. gambiae*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
0																			
1 <i>O. oryzae-1</i>	0																		
2 <i>O. oryzae-3</i>	0.1035	0																	
3 <i>S. sitiqua</i>	0.2598	0.2954	0																
4 <i>I. obsvella</i>	0.2435	0.2456	0.0570	0															
5 <i>S. brevis</i>	0.2377	0.2863	0.0713	0.0729	0														
6 <i>B. staeade</i>	0.2260	0.2633	0.0735	0.0745	0.0867	0													
7 <i>B. occulta</i>	0.2520	0.2912	0.0864	0.0879	0.0834	0.0294	0												
8 <i>B. salicomyiae</i>	0.2384	0.2766	0.0899	0.0846	0.0767	0.0354	0.0294	0											
9 <i>S. trinaeriae</i>	0.2538	0.2933	0.1240	0.1292	0.1098	0.1208	0.1010	0.0967	0										
10 <i>S. mosellana</i>	0.2022	0.2562	0.2421	0.2546	0.2433	0.2473	0.2473	0.2335	0.2314	0									
11 <i>M. destructor-4</i>	0.3719	0.3945	0.2575	0.2427	0.2288	0.2414	0.2411	0.2320	0.2255	0.2706	0								
12 <i>M. destructor-5</i>	0.3637	0.3804	0.2490	0.2342	0.2207	0.2330	0.2327	0.2237	0.2174	0.2704	0.0102	0							
13 <i>M. destructor-1</i>	0.3589	0.3756	0.2487	0.2342	0.2254	0.2326	0.2324	0.2234	0.2130	0.2661	0.0076	0.0076	0						
14 <i>M. destructor-2</i>	0.3491	0.3749	0.2456	0.2310	0.2172	0.2288	0.2245	0.2196	0.2140	0.2733	0.0128	0.0129	0.0102	0					
15 <i>M. destructor-3</i>	0.3555	0.3682	0.2533	0.2388	0.2303	0.2418	0.2415	0.2324	0.2176	0.2673	0.0153	0.0154	0.0076	0.0128	0				
16 <i>M. destructor-6</i>	0.3530	0.3788	0.2405	0.2303	0.2130	0.2294	0.2293	0.2203	0.2094	0.2771	0.0260	0.0234	0.0233	0.0154	0.0128	0			
17 <i>M. destructor-7</i>	0.3568	0.3817	0.2371	0.2274	0.2199	0.2220	0.2265	0.2131	0.2069	0.2682	0.0341	0.0344	0.0261	0.0316	0.0287	0.0340	0		
18 <i>O. oryzae-2</i>	0.3466	0.3685	0.2594	0.2427	0.2460	0.2340	0.2507	0.2296	0.2380	0.3051	0.0426	0.0401	0.0345	0.0239	0.0345	0.0408	0.0547	0	
19 <i>An. gambiae</i>	0.4559	0.4640	0.5156	0.4679	0.4986	0.4833	0.4695	0.4772	0.4654	0.4840	0.5332	0.5217	0.5164	0.5083	0.5231	0.5203	0.5159	0.5348	0

Genetic distances were estimated by the method of Felsenstein (1984).

ancestral mitotype. Although the relationships among the *M. destructor* haplotypes were not strongly supported, the topology of the tree was generally the same as in the previous analysis (Fig. 3).

Unlike the association of greenbug, *Schizaphis graminum* (Rondani), resistance and mitochondrial haplotypes, no sequence difference in the 12S rRNA gene was observed between biotypes that vary in host-adapted alleles within the *M. destructor* populations studied (Shufran et al. 2000). Thus, neutral markers did not show structure based on biotype. This suggests the biotype composition of field populations reflects selection pressure from genes for resistance in hexaploid wheat and did not diverge through reproductive isolation predating the cultivation of wheat, *Triticum aestivum* L., ≈12,000 yr ago.

An assessment of phylogenetic relationships based entirely on mitochondrial genes, however, may not be appropriate (Nigro and Prout 1990). *Wolbachia* bacteria are maternally inherited cytoplasmic symbionts and occur in many insect species as well as many species of mites, isopods, and filarial worms (Werren 1997). The role of *Wolbachia* in insect population dynamics has been studied, and as infection progresses through a species, the frequency of mitochondrial genes from infected individuals increases in populations due to the maternal inheritance of *Wolbachia* and mitochondria (Turelli et al. 1992). In *O. oryzae*, all female flies of all biotypes are infected with *Wolbachia*, whereas infection in males varies in different biotypes, effectively producing mating barriers between haplotypes leading to proliferation of one type over another (Behura et al. 2001). Like *Aedes albopictus* (Skuse) Birungi and Munstermann 2002), *M. destructor* in North America shows a predominance of one haplotype over the others in specific geographic regions. Therefore, we tested for the presence of *Wolbachia* DNA in the 30 geographic populations of *M. destructor* included in the current study and six Purdue University laboratory biotypes (biotypes GP, B, C, D, E, and L) by Long PCR by using primers for *Wolbachia* DNA (see *Materials and Methods*).

PCR with DNA extracted from bulks or individuals of *C. chinensis*, the positive control, resulted in amplicons of the expected size, the authenticity of which was confirmed by sequencing. However, results were negative with DNA extracted from individuals or bulks from the geographic populations and laboratory biotypes of *M. destructor*. To date, no reproductive barriers or anomalies have been observed in matings within or between different geographic populations or laboratory biotypes of *M. destructor*. Although it is possible *Wolbachia* may be later detected in some populations of *M. destructor*, it seems likely that *Wolbachia* was not present in any of the populations or laboratory biotypes of *M. destructor* tested in this study. In the absence of *Wolbachia*, small founding populations with few females could be the main factor influencing repeated bottlenecks leading to the expansion of one lineage over another.

We provide here a first assessment of mitochondrial DNA sequence divergence in *M. destructor* and the

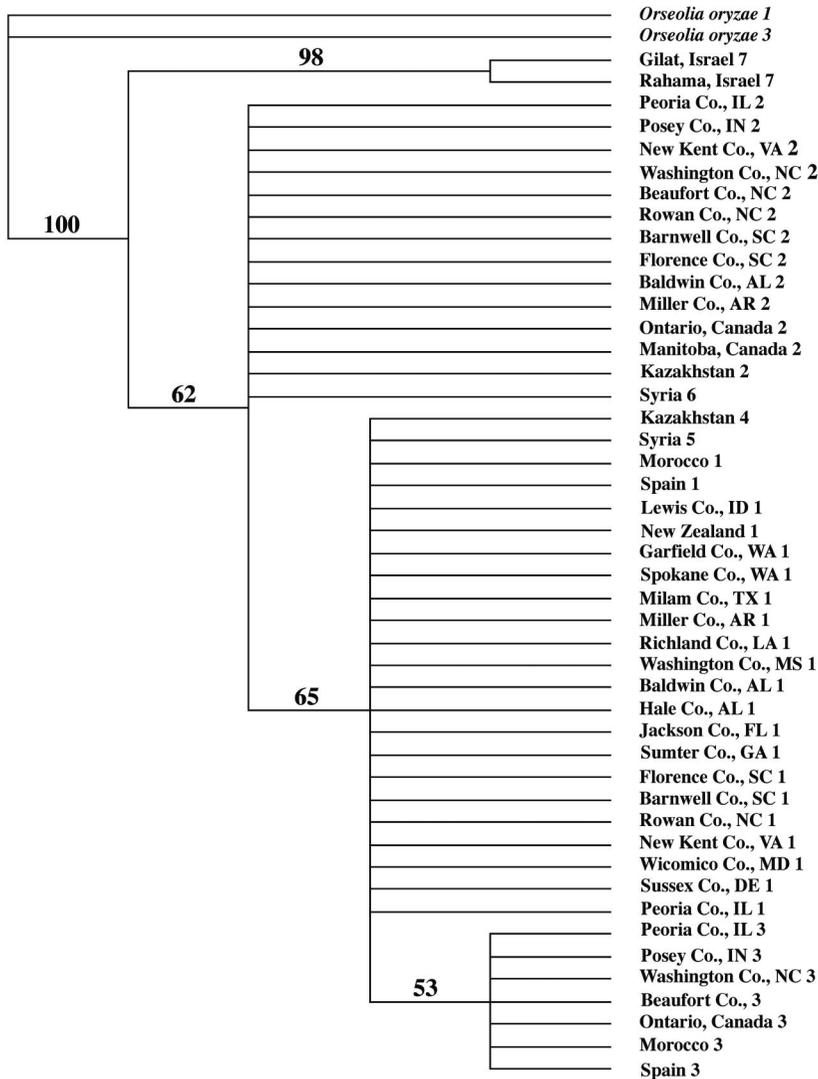


Fig. 4. Intraspecific phylogenetic reconstruction based on the mitochondrial 12S rRNA sequences in the complete complement of *M. destructor* populations studied using *O. oryzae* haplotypes 1 and 3 as the outgroups. Topology of the cladogram is produced by distance/neighbor-joining. The percentage of bootstrap replications above 50% supporting each branch is shown.

geographic distribution of mitochondrial haplotypes in North America, the Mediterranean basin, and Southwest Asia. Recent results from additional mitochondrial sequence information in conjunction with the 12S rRNA sequence have revealed additional resolution within Haplotype 1, which is predominant in most of the United States, as well as the other 12S haplotypes. This suggests that a more robust phylogenetic reconstruction with the inclusion of additional genes as well as populations from additional geographic sites both in North America (i.e., New York and Pennsylvania) and the Old World (northern Europe) can test the prevailing hypothesis concerning a single introduction of *M. destructor* into North Amer-

ica by the Hessians during the Revolutionary War. More than one introduction of this major pest of wheat into North America could have significance concerning geographic variation, genetic diversity, and the ability to respond to resistance genes in the host plant.

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