

Introduction

Myrmarachne formicaria (Salticidae) is an invasive ant-mimicking spider native to Eurasia which was first identified in North America in 2001 (Bradley et al. 2006). It has since been found across western New York, Pennsylvania, Ohio, and Ontario, but it seems to be expanding its range. Little is known about its introduction to North America and dispersal patterns since, except that most early sightings were in buildings or near residences and other human-disturbed areas. However, its distinctive appearance supports the idea that this species had a recent introduction as it would be unlikely to remain unnoticed for long (Bradley et al. 2006).



Fig. 2 Myrmarachne formicaria male.

Fig. 1 Myrmarachne formicaria female.



Invasive species often have less genetic diversity in their invaded range than their source populations. This is especially true when resulting from a single introduction event (Frantz et. al 2013). Characterizing the mitochondrial genetic diversity of *M*. formicaria can help to determine the history of its introduction to North America and its dispersal patterns.

Methods

M. formicaria samples were collected across different locations in New York, Pennsylvania, and Ohio (Fig. 3) in summer and fall 2019. Molecular analysis included extraction of DNA from the legs of the spiders using a Qiagen DNeasy Blood and Tissue Kit. Polymerase chain reaction (PCR) was performed to amplify part of the mitochondrial (mtDNA) cytochrome c oxidase 1 gene (CO1). The CO1 gene was found to be invariant (Fleischman 2020). Consequently, we selected another mtDNA locus that might be more likely to be variable, a 600-bp gene region spanning the 16s rRNA, leucine tRNA, and part of the ND1 gene. Primers were chosen based on prior studies with related spiders (Hedin 1997). PCR

products were purified using the QiaQuick PCR Purification kit. Sequencing of purified PCR products was performed by GeneWiz. Sequences were trimmed and aligned using the program MEGA X (Kumar et al. 2018). Several samples were sequenced more than once to confirm sequence variation was not due to sequencing or PCR artifacts.



Fig. 3 Sampling localities for populations of *M*. formicaria. Map prepared by Daniel Fleischman.

Describing Genetic Diversity in a Non-Native Ant-Mimicking Spider **Cassidy Mills and Jennifer L. Apple Department of Biology, SUNY Geneseo**

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Results

Sequencing of the ND1 gene region only yielded one insertion polymorphism in the leucine tRNA region as well as 1 replacement polymorphism out of 615 base pairs. Three salticid species, Myrmarachne erythrocephala, Myrmarachne luctuosa, and Habronattus ustulatus, with sequences in GenBank in the same mitochondrial gene region, exhibit much more variation than *Myrmarachne formicaria* (Figs. 4, 5, 6; Table 1).

Fig. 4 Example of variation in ND1 in M. erythrocephala. (Pekár et al. 2015)

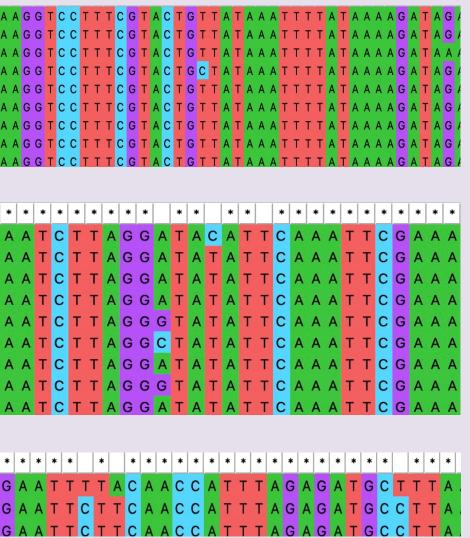
Fig. 5 Example of variation in ND1 in H. ustulatus. (Rosenblum et al. 2015)

Fig. 6 Example of variation in ND1 in M. luctuosa. (Pekár et al. 2015)

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1. KT364863.1_Myrmarachne_erythrocephala_	Α	Т	A	A	G	A	Т	A	ГТ	А	Α	T	A	Т	Α	ТΤ	Т	ΤA	T	A
2. KT364854.1_Myrmarachne_erythrocephala_																				
3. KT364860.1_Myrmarachne_erythrocephala_																				
 KT364857.1_Myrmarachne_erythrocephala_ 	Α	Т	A	A	G	A	Т	A	ГТ	А	Α	T /	A	Т	А	ТΤ	Т	ТА	. Т	A
KT364862.1_Myrmarachne_erythrocephala_	Α	т	A	A	G	A	Т	A	ГΤ	А	Α	ТА	A	Т	А	ТΤ	Т	ΤA	. Т	A
KT364855.1_Myrmarachne_erythrocephala_																				
7. KT364856.1_Myrmarachne_erythrocephala_																				
KT364858.1_Myrmarachne_erythrocephala_																				
9. KT364853.1_Myrmarachne_erythrocephala_	Α	Т	A	Α	G	A	Т	A	ГТ	A	A	T /	A	T	Α	ТТ	Т	TA	Т	A
Species/Abbrv					*	1	*	*	*	*	•	*	*	*	*	*	*	*	*	,
1. KT751600.1_Habronattus_ust	ula	at	us	<u>_</u>	Т	•	г	т	Т	A	10	3	Ą	Α	С	G	Α	Α	Α	1
2. KT751603.1_Habronattus_ust	tul	at	u	s_	Т	•	г	т	Т	A	10	3	Ą	A	С	G	Α	Α	A	1
3. KT751617.1_Habronattus_ust	ula	atu	us	_i	Т		г	т	Т	A	10	3	Ą	Α	С	G	Α	Α	A	1
4. KT751611.1_Habronattus_ust	ula	atı	us	_i	Т		г	т	Т	A	1	3	4	Α	С	G	Α	Α	A	1
5. KT751596.1_Habronattus_ust	tul	at	u	s_	Т	•	г	т	Т	A	1	3	Ą	A	С	G	А	Α	A	1
6. KT751602.1_Habronattus_ust	tul	at	u	s_	Т	• •	г	т	Т	A	10	3	Ą	A	С	G	Α	Α	A	1
7 KT7E160E 1 Habrapattua uat	ul				I _		-	т	-		0				-		A	A	A	1
7. KT751605.1_Habronattus_ust	ui	at	us	S_			L .		- 1	~	۱ U	> /	4	Α	С	G				
																				1
7. KT751605.1_Habronattus_ust 8. KT751598.1_Habronattus_ust 9. KT751595.1 Habronattus_ust	tul	at	u	s_	т		Г	т	Т	A	•	3	4	A	с	G	A	A	A	
8. KT751598.1_Habronattus_ust	tul	at	u	s_	т		Г	т	Т	A	•	3	4	A	с	G	A	A	A	
8. KT751598.1_Habronattus_ust 9. KT751595.1_Habronattus_ust	tul	at	u	s_	T		Г	т	Т	A		3	4 4	A A	C C	G G	A A	A A	A A	
8. KT751598.1_Habronattus_ust	tul	at	u	s_	T		Г	т	Т	A		3	4 4	A A	C C	G G	A A	A	A A	
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8. KT751598.1_Habronattus_ust 9. KT751595.1_Habronattus_ust Species/Abbrv	tul	at	:u: :u: a_	<u>s_</u> s_	T T	*	T T ¥	T T *	т т * А	A A * G	* G	> / > /	а 4 *	A A *	C C	G G *	A A *	A A * A	A A * G	*

Discussion

We were able to sequence 27 spiders from 14 locations. Sequencing of the ND1 mtDNA gene region revealed two polymorphisms (Figs. 7, 8). These insertions of A nucleotides only occurred in samples from the Geneseo, NY, region and a single sample from Ohio (Fig. 8); other individuals from the same locations had the most common sequence variant. The comparison to other salticid species illustrated the degree of intraspecific variation in this gene region we may expect (Table 1). However, the variation in M. formicaria is very low in comparison. Our data are consistent with a single introduction of M. formicaria into North America. This result is also consistent with the idea of a recent introduction, as there has been little time for mutations to • 2012-2017 accumulate as this spider expands its range in North America. • 2018 • 2019 Some possible routes for further research include testing for • 2020 variation in a different gene region. Also, since our sampling efforts, • 2021 the spider has been reported in Chicago and Burlington, VT, two sites beyond the current range (Fig. 9). Ontario also has a high frequency of reports and potentially could harbor more genetic variation. Additional samples from newly colonized locations could Fig. 9 Map of range of M. formicaria over time provide us with more information about the genetic history of this Map created with R packages 'ggmap' and 'rinat.' ant-mimicking spider.

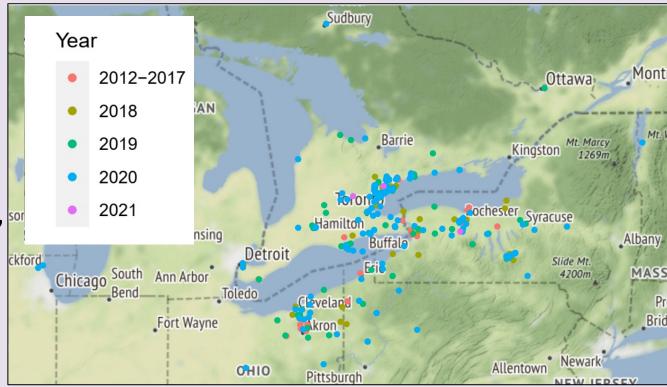


6. MM2-F	СТААТТАААССААААТТТААААА	A G A A A G T C T G T T	6. MM2-F	C A T T A G A A T T A G A A T C T	-
7. JL1-f-new	СТААТТААА <mark>GС</mark> ААААТТТААААА	А <mark>Б А А А Б Т С Т Б Т Т</mark>	7. JL1-f-new	САТТАБААТТАБААТСТ	ааааааааттаааттт
8. ITH6-f	с т а а т т а а а д с а а а а т т т а а а а а	. А <mark>Б А А А Б Т С Т Б Т Т</mark>	8. ITH6-f	САТТАБААТТАБААТСТ	- А А А А А А А <mark>Т Т</mark> А А А <mark>Т Т Т</mark>
9. ITH-9-Con	с т а а т т а а а д с а а а а т т т а а а а а	A G A A A G <mark>T C</mark> T G T T	9. ITH-9-Con	C A T T A G A A T T A G A A T C T	- A A A A A A A <mark>T T</mark> A A A <mark>T T T</mark>
10. ITH9-f-new	стааттааадсаааатттааааа	. A <mark>G A A A G T C</mark> T G T T	10. ITH9-f-new	C A T T A G A A T T A G A A T C T	- A A A A A A A <mark>T T</mark> A A A <mark>T T T</mark>
11. ER-5-Con	С Т А А Т Т А А А G C А А А А Т Т Т А А А А А	. A <mark>G A A A G T C T G T T</mark>	11. ER-5-Con	C A T T A G A A T T A G A A T C T	- A A A A A A A <mark>T T</mark> A A A <mark>T T T</mark>
12. ER-2-Con	С Т А А Т Т А А А G С А А А А Т Т Т А А А А А	A G A A A G <mark>T C T G</mark> T T	12. ER-2-Con	C A T T A G A A T T A G A A T C T	- A A A A A A A <mark>T T</mark> A A A <mark>T T T</mark>
13. ER2-f-new	С Т А А Т Т А А А G С А А А А Т Т Т А А А А А	A G A A A G <mark>T C T G T T</mark>	13. ER2-f-new	C A T T A G A A T T A G A A T C T	- A A A A A A A <mark>T T</mark> A A A <mark>T T T</mark>
14. HC3-F	СТ АА <mark>ТТ</mark> ААА <mark>GС</mark> АААА <mark>ТТТ</mark> ААААА	A G A A A G <mark>T C T G T T</mark>	14. HC3-F	C A T T A G A A T T A G A A T C T	- A A A A A A A <mark>T T</mark> A A A <mark>T T T</mark>
15. HC-4-Con	СТ АА <mark>ТТ</mark> ААА <mark>GС</mark> АААА <mark>ТТТ</mark> ААААА	A G A A A G <mark>T C T G T T</mark>	15. HC-4-Con	C A T T A G A A T T A G A A T C T	- A A A A A A A <mark>T T</mark> A A A <mark>T T T</mark>
16. FMA-5-F	C T A A T T A A A G C A A A A T T T A A A A A	A G A A A G T C T G T T	16. FMA-5-F	C A T T A G A A T T A G A A T C T	- A A A A A A A <mark>T T</mark> A A A <mark>T T T</mark>
17. Buf6-f-new	СТААТТАААССААААТТТААААА	A G A A A G <mark>T C T G T T</mark>	17. Buf6-f-new	C A T T A G A A T T A G A A T C T	- A A A A A A A <mark>T T</mark> A A A <mark>T T T</mark>
18. Buf-2-Con	C T A A T T A A A G C A A A T T T T A A A A A	A G A A A G <mark>T C T G T T</mark>	18. Buf-2-Con	C A T T A G A A T T A G A A T C T	- A A A A A A A <mark>T T</mark> A A A <mark>T T T</mark>
19. FMA-2-Con	C T A A T T A A A G C A A A A T T T A A A A A	A G A A A G <mark>T C T G T T</mark>	19. FMA-2-Con	C A T T A G A A T T A G A A T C T	- A A A A A A A <mark>T T</mark> A A A <mark>T T T</mark>
20. 116-Con	C T A A T T A A A G C A A A A T T T A A A A A	A G A A A G T C T G T T	20. 116-Con	C A T T A G A A T T A G A A T C T	-
21. 136-Con	C T A A T T A A A G C A A A A T T T A A A A A	A G A A A G T C T G T T	21. 136-Con	C A T T A G A A T T A G A A T C T	- А А А А А А А <mark>Т Т</mark> А А А <mark>Т Т Т</mark>
22. 137-f-new	C T A A T T A A A G C A A A A T T T A A A A A	A G A A A G T C T G T T	22. 137-f-new	C A T T A G A A T T A G A A T C T	АААААААА ТТААА ТТТ
23. 144A-f	C T A A T T A A A G C A A A A T T T A A A A A	A G A A A G T C T G T T	23. 144A-f	C A T T A G A A T T A G A A T C T	АААААААА ТТААА ТТТ
24. 153-Con	СТ АА ТТ ААА <mark>GС</mark> ААААТТТ ААААА	A G A A A G T C T G T T	24. 153-Con	C A T T A G A A T T A G A A T C T	- А А А А А А А <mark>Т Т</mark> А А А <mark>Т Т Т</mark>
	26° C	A G A A A G T C T G T T	25. IF4-f-NI-J-1226	[•] С А Т Т А <mark>G</mark> А А <mark>Т Т</mark> А <mark>G</mark> А А Т С Т	A A A A A A A A A T T A A A T T T
26. RR2-f-new	C T A A T T A A A G C A A A A T T T A A A A A	A G A A A G T C T G T T	26. RR2-f-new	C A T T A G A A T T A G A A T C T	АААААААА ТТААА ТТТ
27. PYM-5-Con	C T A A T T A A A G C A A A A T T T A A A A A	A G A A A G T C T G T T	27. PYM-5-Con	C A T T A G A A T T A G A A T C T	- A A A A A A A A <mark>T T</mark> A A A <mark>T T T</mark>
		•			1 /1

Fig. 7 Comparison of ND1 sequences across samples (bp 8-42 of 615 base pairs). A polymorphism exists in the Buf-2 sample from Buffalo, NY.

M. luctuoso, and H. ustulatus than in M. formicaria.

Species	M. formicaria	M. erythrocephala	M. luctuoso	H. ustulatus
% Divergence	0.3%	1.9%	5.5%	4.4%
Sample Size	27	9	3	13



based on iNaturalist research-grade observations.

References

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Fig. 8 Comparison of ND1 sequences across samples (bp 177-210 of 615 base pairs). Some of the Geneseo, NY, samples and one sample from Ohio (JL-1) exhibit an insertion of an A nucleotide outside of the protein-coding region.

Table 1 Percent divergences observed over the 615-bp region for *M. formicaria* and three other salticid species. Comparisons were made from the same gene regions. There is much more intraspecific variation in *M. erythrocephala*,

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