

SCIENTIFIC NOTE

Genetic diversity of field populations of the cat flea, *Ctenocephalides felis*, and the human flea, *Pulex irritans*, in the South Central United States¹

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ABSTRACT The cat flea, *Ctenocephalides felis*, and the human flea, *Pulex irritans*, are species of significant human and veterinary health concerns. Genetic analysis of these species may provide insight into the dispersal of these insects and the spread of insecticide resistance. For this study, a total of 58 fleas were collected from Texas and Arkansas from both dwellings and pet animals. A total of 52 fleas collected were identified as *C. felis*, and 6 were identified as *P. irritans*. Samples were subjected to PCR and DNA sequencing using two markers, a portion of the mitochondrial DNA 16S rRNA gene and the nuclear rRNA first internal transcribed spacer region. For both species, no genetic variation was observed in either the nuclear or mitochondrial markers. Based on this observed lack of genetic variation, *C. felis* and *P. irritans* possibly underwent a genetic bottleneck in the United States.

KEY WORDS cat flea, human flea, DNA sequence, genetic variation

The most common domestic flea in the United States is the cat flea, *Ctenocephalides felis* (Bouché). It can reproduce on both dogs and cats and is the most important ectoparasite of these species worldwide (Rust & Dryden 1997). The human flea, *Pulex irritans* L., is associated with humans as well as with wild mammals such as foxes, skunks and coyotes. This species is thought to have originated in South America, where its original host may have been the guinea pig or peccary that were living in close proximity to human dwellings (Buckland & Sadler 1989). It is one of six species in the genus *Pulex*; the other five are confined to the Nearctic and Neotropical regions (Whiting et al. 2008). Often, fleas can become established in dwellings where pet animals are infested, and then they will bite humans. In order to effectively control an infestation, fleas must be removed from the pet, the home, and the surrounding area. Over one billion dollars are spent annually by pet owners in the USA for flea control

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Table 1. Sample collection data and haplotype for *Ctenocephalides felis* and *Pulex irritans* found in this study.

Species	State	County	Haplotype (n)
<i>Ctenocephalides felis</i>	AR	Benton	F1(1)
		Jasper	F1(1)
		Johnson	F1(1)
		Phillips	F1(2)
		Saline	F1(1)
	TX	Washington	F1(5)
		Bexar	F1(1)
		Brazos	F1(29)
		Cherokee	F1(1)
		Gregg	F1(2)
		Harris	F1(2)
		Kaufman	F1(1)
		Montgomery	F1(1)
		Nueces	F1(1)
		Travis	F1(1)
		Waller	F1(1)
		Wharton	F1(1)
<i>Pulex irritans</i>	TX	Brazos	F2(4)
		Cherokee	F2(1)
		Wichita	F2(1)

(MacAllister 1993). The cat flea has developed insecticide resistance to at least five different classes of insecticides (WHO 1992).

Knowledge of genetic variation within medically important insect species is an essential element required for understanding vector transmission, disease epidemiology, and disease control (Tabachnick & Black 1995). Because nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) are inherited by independent evolutionary means, their combined application to elucidate gene flow of fleas can reveal both their sexually and matrilineally derived ancestry. A previous study by Vobis et al. (2004) was conducted on *C. felis* populations from the United States and Europe using DNA sequencing analysis of the nDNA ribosomal first and second internal transcribed spacer regions (ITS1 and ITS2), and the mtDNA rRNA 16S gene. The study by Vobis et al. (2004), which focused on lab colonies from the United States, did not find any genetic variation for either marker. Therefore, the objective of this study was to determine the extent of genetic variation within and among field populations of *C. felis* and *P. irritans* from the south-central United States using both mitochondrial and nuclear DNA markers.

Materials and Methods

Flea specimens were collected from Arkansas and Texas in 2007 and 2008 (Table 1). Samples were morphologically identified to species using keys by Furman & Catts (1986). Voucher specimens preserved in 100% ethanol are

maintained at the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, AR.

DNA was extracted from individual fleas using the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN). Extracted DNA was resuspended in 50 μ l of Tris:EDTA and stored at -20°C . Polymerase chain reaction was conducted using the primers LR-J-13007 (5'-TTACGCTGTTATCCCTAA-3') (Kambhampati & Smith 1995) and LR-N-13398 (5'-CGCCTGTTTATCAAAAA CAT-3') (Simon et al. 1994). These PCR primers amplify an approximately 428 bp region of the mtDNA 16S rRNA gene. The PCR reactions were conducted with 1 μ l of the extracted DNA (Szalanski et al. 2000), having a profile consisting of 35 cycles of 94°C for 45 s, 46°C for 45 s, and 72°C for 60 s for the 16S marker. PCR was also conducted using the primers rDNA1.58s and rDNA2 to amplify the small subunit RNA ITS marker. For the ITS1 marker, a 3' portion of 18S rDNA, all of ITS1, and the 5' portion of 5.8S was first amplified using the primers rDNA2 (5'-TTGATTACGTCCCTGCCCTTT-3') (Vrain et al. 1992) and rDNA 1.58S (5'-GCCACCTAGTGAGCCGAGCA-3') (Cherry et al. 1997) with a thermalcycler profile consisting of 40 cycles of 94°C for 45 s, 53°C for 1 min, and 72°C for 1 min as described by Szalanski and Owens (2003). Amplified DNA from individual fleas was purified and concentrated with minicolumns (Wizard PCRpreps, Promega, Madison, WI) according to the manufacturer's instructions. Samples were sent to the University of Arkansas Medical Center DNA Sequencing Facility (Little Rock, AR) for direct sequencing in both directions. GenBank accession numbers were GQ387496 to GQ387498 for the new sequences found in this study. Consensus sequences for each sample were obtained using Bioedit 5.09 (Hall 1999). Sequences were compared with ones available on GenBank using BLAST search (www.ncbi.nlm.nih.gov/blast/, which is an algorithm for comparing the similarity of DNA sequences).

Results and Discussion

Of the 58 fleas that were subjected to DNA sequencing analysis, 52 were *C. felis* and the remainder were *P. irritans* (Table 1). The ITS1 marker was 948 bp in size for *P. irritans* and 830 bp in size for *C. felis*. No intraspecific variation was observed for either species for the ITS1 marker. Using a BLAST search, the *P. irritans* ITS1 sequence was 89% similar to *P. irritans* from Cameroon (GenBank accession number EU169198), and the *C. felis* ITS1 sequence was identical to *C. felis* from Cameroon (GenBank accession number EU170156).

The mtDNA 16S marker was 419 bp in size for both *C. felis* and *P. irritans*. As with the nuclear rRNA ITS1 marker, no intraspecific variation was observed for either species. No DNA sequences for this marker in *C. felis* or *P. irritans* exist on GenBank, so a BLAST search was not preformed.

Vobis et al. (2004) found little variation in cat flea DNA collected from Europe and from laboratory colonies from the United States using both the mtDNA 16S marker and the nuclear ITS1 and ITS2 markers. Vobis et al. (2004) also studied a population of *P. irritans* from Austria and found no intraspecific genetic variation. We also found a lack of genetic variation from our field collections of *C. felis* and *P. irritans* from Arkansas and Texas. Unfortunately, the DNA sequences from Vobis et al. (2004) were not deposited to GenBank, so comparisons of our data with theirs cannot be made. Molecular genetic variation

studies of other Siphonaptera are limited. The only other species that has been subjected to DNA sequencing analysis is *Tunga penetrans* (L.) from South America and Africa populations using the mtDNA cytochrome oxidase II and ITS2 markers (Luchetti et al. 2007). As with *C. felis* and *P. irritans*, low levels of mtDNA genetic variation were observed, while the ITS2 marker revealed a distinction between populations from Ecuador with those from Brazil or Africa. This difference in mitochondrial and nuclear genetic markers has also been observed for another blood-feeding insect, *Cimex lectularius* L. (Szalanski et al. 2008); however, with this bed bug, there was a large amount of mitochondrial genetic variation and a lack of ITS1 genetic variation, indirectly supporting suppositions about the chronology of reemerging populations of this pest.

Based on our results, field populations of *C. felis* and *P. irritans* in Arkansas and Texas have either undergone a genetic bottleneck due to insecticide pressure or they are a recent introduction. Some populations of *C. felis* that might constitute possible assemblages of resistance to insecticides such as imidacloprid, (Rust et al. 2002), carbaryl, chlorpyrifos, malathion, and PBO-synergized pyrethrin (Bass et al. 2004a,b, Bossard et al. 2002), have been reported. However, if insecticides are driving this genetic isolation, insect growth regulators (IGRs) may be the most likely candidates to affect this genetic shift due to their broad use by veterinarians and the easy access to consumers from the over-the-counter market segment of animal health products. This being stated, reports of insecticide resistance are few (Rust 2005). In the future, a more in-depth study involving microsatellite markers and a broader geographical sample may provide more insight into the genetic diversity of these two economically important flea species. For now, this is the first attempt in Texas or Arkansas to observe these public and animal nuisance pests from a genetic perspective. Future evaluation of IGR contribution to genetic isolation and resistance of these species is highly recommended.

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