

# Genetic Differences Between *Culex pipiens* f. *molestus* and *Culex pipiens pipiens* (Diptera: Culicidae) in New York

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**ABSTRACT** The definition and phylogenetic placement of the autogenous *molestus* form of *Culex pipiens* has puzzled entomologists for decades. We identified genetic differences between *Cx. p. pipiens* (L.) and *Cx. pipiens* f. *molestus* Forskål in the SH60 fragment described previously. Single-strand conformation polymorphism analysis, cloning, and sequencing of this fragment demonstrated high polymorphism within and among individual *Cx. p. pipiens*, with common SH60 variants shared among individuals from distant locations. In contrast, *Cx. pipiens* f. *molestus* from New York City each contained a single SH60 variant, which was not identified in any other *Cx. p. pipiens* specimens analyzed. Supporting microsatellite analysis demonstrated significant but reduced gene flow between *Cx. p. pipiens* and *Cx. pipiens* f. *molestus* in New York relative to *Cx. p. pipiens* populations in New York and California. Results are discussed in the context of two contrasting hypotheses regarding the origin of *Cx. pipiens* f. *molestus* populations.

**KEY WORDS** *Culex pipiens pipiens*, *Culex pipiens* f. *molestus*, genetics, microsatellites, West Nile virus

*Culex pipiens* f. *molestus* Forskål is a morphologically identical ecological biotype of *Culex pipiens pipiens* (L.) defined by a host of behavioral and physiological characteristics. In contrast to *Cx. p. pipiens*, *Culex pipiens* f. *molestus* has the ability to produce eggs without a vertebrate bloodmeal (autogeny); can mate in confined spaces (stenogamy); foregoes winter diapause; occupies subterranean environments with limited surface access; and feeds readily on mammals, including humans (Mattingly 1952, Vinogradova 2000). The potential public health importance of the mammal-feeding *molestus* biotype of *Cx. p. pipiens* as a human disease vector has led to a search for markers to readily identify these two forms.

Despite the numerous biological differences that distinguish *Cx. pipiens* f. *molestus* from *Cx. p. pipiens*, reliable identification of *Cx. pipiens* f. *molestus* by using morphology and/or molecular tools and phylogenetic placement of this form within the *Cx. pipiens* complex remains unresolved. Morphological and biochemical studies using larval chaetotaxy, variation in length of dorsal and ventral arms of the phallus in adult males (DV/D ratios), and chromatography have all failed to reliably separate these two forms (Jobling 1938, Mattingly 1952, Micks 1954, Harbach et al. 1984). Micks and Scrollini (1954) identified biochemical differences among *Cx. p. pipiens*, *Cx. p. quinquefasciatus*

Say, and *Cx. pipiens* f. *molestus* by using infrared spectrometry. Recently Fonseca et al. (2004) reported unique microsatellite signatures among worldwide populations of *Cx. p. pipiens* and *Cx. pipiens* f. *molestus*. This report was followed by Bahnck and Fonseca (2006) who developed a rapid molecular assay to differentiate between *Cx. p. pipiens*, *Cx. pipiens* f. *molestus*, and putative hybrid populations based on sequence differences in the genomic regions flanking the CQ11 microsatellite locus. These advances provide evidence of a molecular and genetic basis for the observable phenotypes that distinguish *Cx. p. pipiens* and *Cx. pipiens* f. *molestus*, but they also demonstrate that progress in identifying genetic differences between them has been challenging despite decades of work. Identification of additional markers and a more thorough characterization of genetic differences between *Cx. p. pipiens* and *Cx. pipiens* f. *molestus* are needed as well as further evidence supporting the evolutionary origin of *Cx. pipiens* f. *molestus*.

Two hypotheses predominate regarding the origin of *Cx. pipiens* f. *molestus* populations. Byrne and Nichols (1999) concluded with allozyme analysis that underground, autogenous *Cx. pipiens* f. *molestus* in London were most likely founded from a single colonization event from local aboveground *Cx. p. pipiens* populations. This hypothesis was also proposed by Dobrotworsky (1967), who through comparative studies of DV/D ratios of *Cx. p. pipiens*, *Cx. pipiens* f. *molestus*, and *Cx. p. fatigans* Weidemann in the South Pacific postulated that *Cx. pipiens* f. *molestus* originated from *Cx. p. pipiens*. Alternatively, microsatellite data generated by Fonseca et al. (2004) supports a

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