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SCIENTIFIC NOTE

GYNANDROMORPHIC SPECIMENS OF *CULEX ERYTHROTHORAX*, *CULEX PIPIENS* COMPLEX, *CULEX TARSALIS*, AND *CULISETA INCIDENS* COLLECTED IN NORTHERN SAN JOAQUIN VALLEY, CALIFORNIA

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ABSTRACT. Gynandromorphic insects bear both male and female characteristics. In mosquitoes, gynandromorphy has been documented in multiple species and it can include many different combinations of sex-specific traits such as plumose or filiform antennae, short or elongated palpi, and male or female terminalia. Here we report 4 gynandromorphic specimens of *Culex erythrothorax*, *Cx. pipiens* complex, *Cx. tarsalis*, and *Culiseta incidens* collected in the Northern San Joaquin Valley of California. Each of the specimens exhibited heads with female characteristics of short palpi and filiform antennae while displaying fully developed external male terminalia including basistyles and dististyles. The potential implication of gynandromorphism in genetic control of mosquito vectors is discussed.

KEY WORDS *Culex erythrothorax, Culex pipiens* complex, *Culex tarsalis*, *Culiseta incidens*, gynandromorphy, Northern San Joaquin Valley, California

Gynandromorphy is the occurrence of both male and female characteristics in an individual animal, a phenomenon that has been documented for decades in numerous species (Chue and Smith 2011, Kronefeld et al. 2013). Several types of gynandromorphy exist, including polar, bilateral, and oblique gynandromorphy: polar gynandromorphs display one sex phenotype on the anterior half and the opposite sex phenotype on the posterior half, bilateral gynandromorphs display phenotypic traits of one sex on the left lateral side and traits of the opposite sex on the right lateral side, and oblique gynandromorphs display one phenotype on the head and one lateral half of the thorax while the other half of the thorax and abdomen display the other sex phenotype (Barreto et al. 2008). Gynandromorphy in insects has been well documented in the literature, and many gynandromorph mosquito findings have been reported in 20th-century literature compared with the present day (Roth 1948, Brust 1966, Hall 1987, Kronefeld et al. 2013). Here we chronologically report 4 cases of gynandromorphy observed in mosquitoes collected in the Northern San Joaquin Valley of California.

The 1st gynandromorphic specimen is a *Culex erythrothorax* Dyar collected at the City of Stockton wastewater treatment facility (37°56′22.8″N, 121°20′42.5″W). Aquatic flora such as tules (*Schoenoplectus acutus* (Muhl. ex Bigelow) Á. Löve & D. Löve) and the large amount of organic matter present in the wastewater ponds make the facility a prolific habitat for *Culex* species. The specimen was collected on September 29, 2014, in a carbon dioxide–baited encephalitis vector survey (EVS) trap (Bioquip Products, Inc., Rancho Dominguez, CA) for routine West Nile virus surveillance. The trap collected 9,022 mosquitoes containing 8,867 *Cx. erythrothorax*. This specimen can be considered gynandromorphic in that it has a clear division of male and female parts (Fig. 1A), as opposed to an intersex individual that may display different gender traits in an ambiguous manner (Narita et al. 2010). The anterior of the body presents as a female, with filiform antennae and short palpi. However, the posterior of the body contains typical male structural anatomy with a characteristically thin abdomen and complete external male terminalia. All major structures are apparent, including the basistyles and dististyles. The foretarsi of this specimen also present large single-toothed tarsal claws, which are typical of males. To date, this specimen is the only field-collected gynandromorphic *Cx. erythrothorax* that has been documented. The only previous record of a gynandromorph of this species was from a laboratory colony (Blakeslee and Rigby 1965). Both specimens are similar, displaying a female anterior and a male posterior body type.

The 2nd gynandromorphic specimen is a *Cx. pipiens* complex mosquito that displayed a female head with short palpi and nonplumose antennae while also exhibiting male terminalia. This specimen was collected on October 7, 2014, in a weekly EVS trap at a dairy in southeast rural Manteca, CA (37°42′35.1″N, 121°12′22.5″W). The trap collected 71 total mosquitoes, which consisted of 27 *Cx.*

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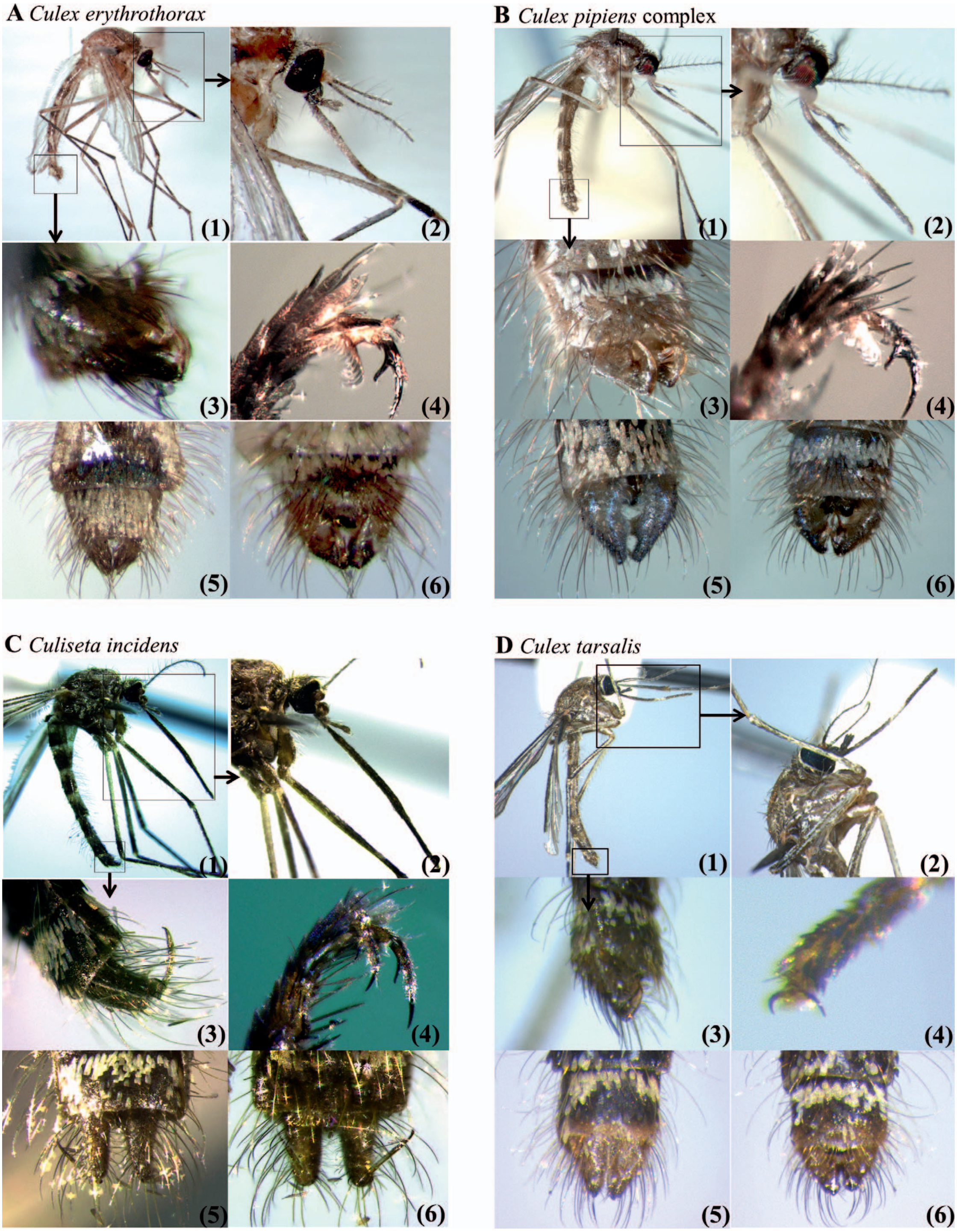

Fig. 1. Physical manifestation of gynandromorphism in (A) *Culex erythrothorax*, (B) *Cx. pipiens* complex, (C) *Culiseta incidens*, and (D) *Cx. tarsalis*. (1) General habitus, lateral view. (2) Head with proboscis, lateral view. (3) Lateral view of male terminalia. (4) Foretarsus. (5) Dorsal view of male terminalia. (6) Ventral view of male terminalia.

pipiens complex mosquitoes and 1 *Cx. pipiens* complex mosquito exhibiting both male and female anatomical structures (Fig. 1B). This gynandromorphic mosquito displayed a female head with filiform antennae and a single short palpus with several hairs on the distal end; the opposing palpus was not present. Several tarsi were missing the terminal segments, but a single intact foretarsus remained on which a single-toothed tarsal claw was present. The posterior of the mosquito exhibited male terminalia with all major structures present. As *Cx. pipiens* complex mosquitoes are the most common mosquitoes worldwide distributed in a variety of habitats, it is not surprising that gynandromorphy in mosquitoes has been frequently documented in the *Cx. pipiens* complex (Ali and Rasheed 2008, Kronefeld et al. 2013). The 1st record of gynandromorphic *Cx. pipiens* complex was described in 1938 in Great Britain and Germany (Marshall 1938, Weyer 1938), and the most recent documented occurrence was in Spain in 2016 (Ortiz et al. 2019).

The 3rd specimen displaying gynandromorphy is a *Culiseta incidens* (Thomson) collected from a routine EVS trap located by the Stanislaus River in Ripon, CA, and surrounded by a walnut (*Juglans regia* L.) orchard and golf course (37°44′32.6″N, 121°06′13.0″W). The trap was placed on April 10, 2017, and collected a total of 65 mosquitoes that consisted of 26 *Cs. incidens*. The gynandromorphic *Cs. incidens* presented with a female head with short maxillary palpi and a single filiform antenna (Fig. 1C). The foretarsus had 2 single-toothed tarsal claws. The abdomen displayed male characteristics with a slender shape as well as male terminalia with all structures present. This is the 1st documented occurrence of gynandromorphy in this species.

The 4th specimen is a *Culex tarsalis* Coq. collected on July 23, 2018, from a weekly EVS trap located in an agricultural field in the San Joaquin Delta, CA (37°56′05.8″N, 121°31′32.6″W). The trap collected a total of 40 mosquitoes, 29 of which were *Cx. tarsalis*. The anterior of the specimen presented as female and possessed filiform antennae and short maxillary palpi (Fig. 1D). There was a single foretarsus present with 2 nontoothed tarsal claws. The abdomen was slender in shape, and the posterior end displayed male terminalia with basistyles and dististyles present. There have been several documented occurrences of gynandromorphy in this species in the 20th century, with the 1st record from 1955 in California and the most recent from 1971 in Texas (Keh 1955, Harmston 1971).

The recent outbreaks of dengue, chikungunya, and Zika arboviral diseases in Latin America have driven the genetic control of mosquito vectors such as *Aedes aegypti* (L.) into a new era. Currently, there are multiple field trials in progress or recently completed where large numbers of males with self-limiting genes or *Wolbachia* bacteria infections are released with the goal of outcompeting wild males to reduce field populations (Benjamin et al. 2019, Crawford et al. 2020). While the exact behavior of gynandromorphic mosquitoes is unclear due to the lack of sufficient number of live and reproducible individuals, theoretically, several traits of gynandromorphic mosquitoes may provide alternatives to the few existing methods in genetic control of mosquitoes and vector-borne disease prevention.

First, gynandromorphs could serve as a barrier to mosquito reproduction. According to Roth (1948), male *Ae. aegypti* mosquitoes are attracted for mating by the frequency of sound produced by the female mosquito's wings. The chance of a male attempting to mate with the male-headed gynandromorph could be reduced. It was also indicated that insects do not behave based on the genitalia present but are influenced more heavily by the sex of their anterior portion (Bates 1941). This means in polar gynandromorphy, it is unlikely that reproduction would occur as a male-behaving gynandromorph that may seek and grasp a female would not have the correct terminalia for reproduction, and a female-behaving gynandromorph would not seek to mate despite having male genitalia. Second, gynandromorphs are expected to be poor and unlikely vectors for vector-borne diseases. Mosquito bloodfeeding behavior is driven by the need of nutrients to produce eggs. A gynandromorph with a female head and a male abdomen likely would not seek blood meals, whereas an oblique gynandromorph that is able to successfully derive host cues and obtain a blood meal may not have the necessary components to digest a blood meal. A gynandromorph with a male head and a female abdomen may not be able to obtain blood meals successfully because the male proboscis has a hypopharynx fused with the labium and greatly reduced maxillae and mandible structures that are required to pierce through dermal layers to obtain blood. In addition, a gynandromorph with a male head may not have the sensilla receptive to receiving host cues for locating a suitable host.

The molecular mechanisms of gynandromorphism in mosquitoes are not fully understood. Recent studies of the genera *Aedes* and *Anopheles* identified factors involved in sex determination and documented successful production of gynandromorphs in the laboratory (Alphey 2013, Adelman and Tu 2017, Macias et al. 2017). In *Ae. aegypti*, the *Nix* gene was shown to function as a male-determining factor and its induced expression can promote the development of male genitalia in female mosquitoes, creating a gynandromorph (Hall et al. 2015). In *Anopheles gambiae* Giles, *Yob1* was found to function as a *Y*-linked male determining factor (Krzywinska et al. 2016). Most recently, it was shown that in *An. gambiae*, the gene *doublesex* (*Agdsx*) is alternatively spliced to produce 2 mRNA transcripts, *dsx-female* (*AgdsxF*) and *dsx-male* (*AgdsxM*), which control sex determination (Kyrou et al. 2018). Disruption of *AgdsxF* by CRISPR-Cas9 editing did not affect male development but resulted in intersex characteristics and sterility in females homozygous for disrupted

AgdsxF. Mating between males homozygous for disrupted *AgdsxF* and females with normal *AgdsxF* can produce heterozygous females that can serve as vehicles to propagate an *AgdsxF* deficiency to the next generation. The authors showed that the disrupted *AgdsxF* alleles could rapidly spread in caged mosquitoes, resulting in complete population collapse in 7–11 generations.

These gene drive mechanisms for population collapse are of advantage over the mechanisms used by *Wolbachia*-infected or self-limiting gene-containing mosquitoes, which require frequent continued releases of large numbers of male mosquitoes to achieve satisfactory results. Therefore, if gynandromorphism can be controlled in the laboratory to produce gynandromorphs on a large scale, it could open up the possibility for the use of gynandromorphism as a genetic control method. More research on mechanisms for induced gynandromorphy in vector species could prove valuable to the future of mosquito control.

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