Bulletin of Entomological Research (2018) **108**, 1–4
doi:10.1017/S0007485317000359

Polymorphism in male genitalia of *Aedes* (*Ochlerotatus*) *scapularis* Rondani, 1848

V. Petersen[1,2]*†, F. Virginio[1,2]† and L. Suesdek[1,3]

[1]Laboratório de Parasitologia, Instituto Butantan, Av. Vital Brazil – 1500, São Paulo, SP 05503-000, Brazil: [2]Programa de Pós-Graduação Biologia da Relação Patógeno-Hospedeiro, Instituto de Ciências Biomédicas, Universidade de São Paulo, Av. Prof. Lineu Prestes – 2415, São Paulo, SP 05508-900, Brasil: [3]Programa de Pós-Graduação em Medicina Tropical, Instituto de Medicina Tropical, Universidade de São Paulo, Av. Dr. Enéas de Carvalho Aguiar – 470, São Paulo, SP 05403-000, Brasil

Abstract

Morphology of male genitalia of culicids is generally species-specific and often used as a taxonomic marker. However, some characters of the male genitalia vary intraspecifically and are not taxonomically diagnostic. This might be the case of *Aedes scapularis*, a Neotropical culicid with vector competence for arboviruses and filarial worms. Males of this species may or not present a retrorse process (RP) in the genitalic claspette filaments, which led authors to suspect that this variance might be indicative of population divergence or incipient speciation process. This suspicion has not been investigated hitherto and it is not known if there are variable patterns of RPs. We hypothesized that the presence of the RP varies intraspecifically in *Ae. scapularis* and then we statistically evaluated the variability of this character in a single population. To this study the genitalia of 73 males of *Ae. scapularis* were prepared, and their RPs were meristically quantified and categorized according to the phenotypes observed. We noted that the presence or RPs is a polymorphic character because it varied inter and intra-individually. The presence of a single RP on each claspette filament was the predominant pattern (77%), but absent or multiple RPs in each filament were also found either in bilateral symmetry or asymmetry. Thus, we conclude that the presence of RPs owing to its high variability is not indicative of populational divergence or diagnostic of species complex within *Ae. scapularis*.

Keywords: morphology, taxonomy, retrorse process, claspette filament

(Accepted 21 March 2017; First published online 18 April 2017)

Introduction

Male genitalia have many morphological characters that are generally species-specific and therefore useful for species identification of mosquitoes (Eberhard, 1985; Huber, 1995, 2004; Song & Wenzel, 2008). Male genitalia characters have also been used to diagnose species, such as *Anopheles* (*Nyssorhynchus*) *albertoi* Unti, 1941 and *Anopheles* (*Nyssorhynchus*) *arthuri* Unti, 1941, and in the *Anopheles strodei* complex Faran 1980 (Sallum *et al.*, 2010). However, some authors have reported intraspecific variation of male genitalia characters in culicids, which leads us to believe that not all features of the genitalia are taxonomically diagnostic. Examples of this are showed in *Anopheles* (Hribar, 1994; Motoki *et al.*, 2009) and *Aedes* (*Ochlerotatus*) *scapularis* Rondani, 1848 (Petersen, 2012).

Aedes scapularis is a species with vector competence for arboviruses and filarial worms (Lourenço-de-Oliveira & Deane, 1995; Rachou *et al.*, 1995; Vasconcelos *et al.*, 2001; Pauvolid-Corrêa *et al.*, 2013), which belongs to the 'Scapularis group', a set of morphologically-related species such as *Aedes rhyacophilus* Costa Lima 1933 and *Aedes serratus* Theobald 1901 (Arnell, 1976; Sallum *et al.*, 1988). Arnell (1976)

*Author for correspondence
Phone/Fax: +55 11 2627-9785
E-mail: vivianpetersen@usp.br
†These authors contributed equally to this work.

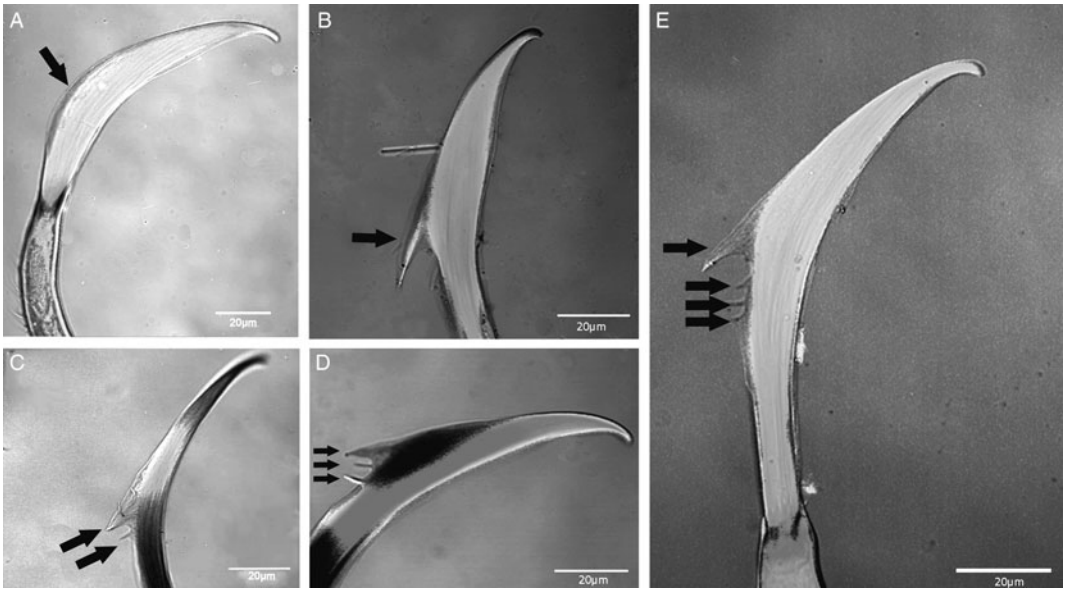

Fig. 1. Polymorphism in the number of RPs on the claspette filaments of the male genitalia of *Aedes scapularis*. (A) Absence of RP; (B) presence of one RP; (C) presence of two RPs; (D) presence of three RPs; (E) presence of four RPs.

reported that the claspette filament of male genitalia of *Ae. scapularis* might or not bear a retrorse process (RP), and put in doubt the diagnostic power of genitalic characters for the group. Conversely, Forattini (2002) considered that this morphological variation could be one of the indicatives of the existence of a complex of species and suggested that this possibility should be investigated.

The possible meaning of the variability of genitalia has not been further investigated until now, but our recently published morphogenetic findings support the idea that *Ae. scapularis* is a highly polymorphic species (Petersen *et al.*, 2015). In light of this, we hypothesized that the presence of RPs varies intraspecifically in *Ae. scapularis*. We then statistically investigated the variability of the presence of RPs in a single population of this species.

Material and methods

Collection of specimens

Adult mosquitoes were collected using an aspirator (Consoli & Oliveira, 1994) between 2013 and 2014 in the Parque Ecológico do Tietê (PET), located in the metropolitan area of Sao Paulo, Brazil (23 29′15″S, 46 31′90″W). This sampling site was selected because the first author had observed polymorphism of the male genitalia in some specimens of *Ae. scapularis*. The park has reforested areas and native species of the Atlantic Forest, and receives about 70,000 visitors monthly.

Sample preparation

Seventy-three males were identified at the species (Arnell, 1976; Consoli & Oliveira, 1994; Forattini, 2002), stored in silica gel and then dissected. The genitalia were detached from the abdominal segment VII and stained according to Lorenz & Suesdek (2013). This structure is rich in chitin, which is auto-fluorescent, thus each of them was evaluated using a laser-scanning microscope with differential interference contrast (Zeiss LSM 510 meta confocal system) and three-dimensional (3D) projection from a Z-section to assist in the analysis of RPs. A 488-nm laser was used for excitation and a LP 505-nm filter for emission. The images were photographed at 40× magnification and stored in the mosquito morphology database 'WingBank' (http://www.wingbank.com.br).

Analysis

The right and left (R–L) claspette filaments of the genitalia were analysed, and the number of RPs on each was scored separately. The RPs were scored by two of the authors (VP and FV, independently) and the scores compared; any discrepancies were resolved by re-examining the specimens involved.

We evaluated the polymorphism of two characters: the number of RPs per claspette filament and the bilateral asymmetry of genitalia according to the number of RPs. Asymmetry scores were calculated as the differences between the number of RPs on R–L sides, and asymmetry was expressed by the modules, $|R–L|$, of the scores for each trait (Palmer & Strobeck, 2003; Souza *et al.*, 2007). Individuals with equal numbers of RPs on R–L sides of genitalia were considered symmetrical. Individuals with unequal numbers of RPs on R/L sides were considered asymmetrical. The Shapiro & Wilk's (1965) normality test was employed to evaluate the type of asymmetry. The distribution of the sample was evaluated according to the kurtosis and skewness values.

Results and discussion

We observed both inter- and intra-individual polymorphism of RPs among the specimens analysed. Moreover, we found both the absence and presence of RPs in symmetric and asymmetric conditions. Considering that RP presence varied in such a fashion in a single population, we conclude that

https://doi.org/10.1017/S0007485317000359 Published online by Cambridge University Press

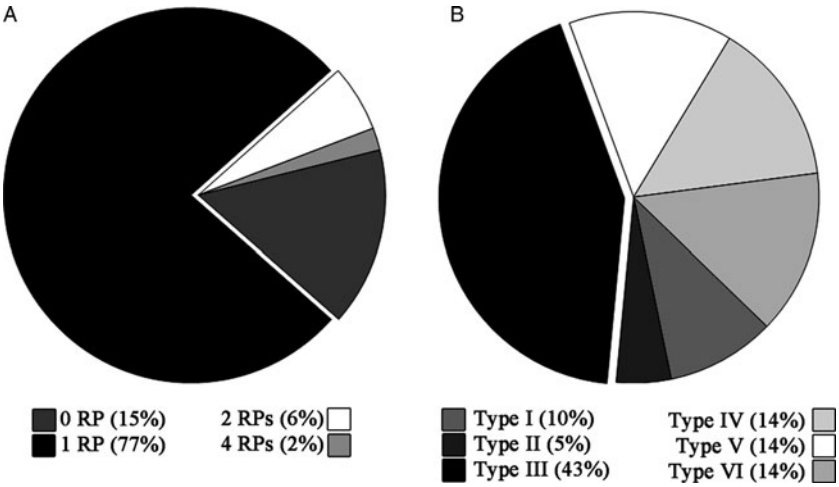

Fig. 2. Quantitative and qualitative graphical representation of percentage of males with the same number of RPs on both claspettes. (A) and those with different numbers of RPs on the left and right claspettes (B). Type I: 0 and one RP. Type II: 0 and two RPs. Type III: one and two RPs. Type IV: one and three RPs. Type V: two and three RPs. Type VI: two and four RPs.

this trait is not taxonomically informative. Based on our results and the literature (Arnell, 1976; Forattini, 2002), we consider that this character cannot be used as diagnostic for species.

The amount of RPs of most individuals was symmetrical (71%), and among them we found the following phenotypes (fig. 1): 0 (absence of RP), 1, 2 and 4 RPs. The presence of a single RP on each claspette (77%) was the predominant condition. The absence of a RP was the second most common condition, representing 15% of the individuals, followed by two RPs (6%) and four RPs (2%). Among the asymmetric genitalia (29%), there were six different phenotypes, as follows: I (0 and one RP), II (0 and two RPs), III (one and two RPs), IV (one and three RPs), V (two and three RPs) and VI (two and four RPs). This classification does not consider whether the RPs were found on the R/L claspette filament.

The most common phenotype was type III (43%) and the least common was type II (5%) (fig. 2). The presence of more than one RP an individual, as well as the different number of RPs on the R–L claspettes of genitalia suggests that the variability of this structure is high.

In addition, the Shapiro–Wilk test ($W = 0.73$, $P = 0.00001$) rejected the null hypothesis, which considers that the data comes from a population with a normal distribution. The kurtosis observed was the leptokurtic type, and the distribution was negatively skewed ($S = -0.37$). The fluctuating asymmetry (FA) was characterized by a combination of different averages and variances of the distribution between the characteristics present on R–L claspettes (VanValen, 1962).

This kind of asymmetry in insects may be a result of environmental disturbances, such as pollution and/or climatic conditions, or genetic stress due to inbreeding, which may increase the phenotypic and genotypic variations of a population (Float & Fox, 2000; Mpho *et al.*, 2002). This effect has been seen in the number of sternopleural and outer orbital bristles of *Drosophila melanogaster* Meigen, 1830 (Woods *et al.*, 1999), and in the number of frontal bristles and postocular setae (R–L sides) of *Anastrepha fraterculus* complex Wiedemann, 1830 (Souza *et al.*, 2007). According to the results obtained by our team (Moratore, 2009; Peruzin, 2009) in a *Culex quinquefasciatus* Say, 1823 population also collected in PET, in which wing shape asymmetry was observed (bilateral asymmetry), it is possible that artificial environmental factors probably contributed to FA expression. This asymmetry may also be endogenous due an 'epigenetic control' in gene expression of RP development, interfering in gene effectiveness. Although we detected RP asymmetry, an explanation for the observed patterns is yet to be elucidated.

Conclusion

We concluded that the variable presence of the RPs on the genitalic claspette filament is not indicative of populational divergence or diagnostic of cryptic species within *Ae. scapularis*. Moreover, we found both inter- and intra-individual polymorphism and bilateral fluctuant asymmetry of RPs confirming that this is a labile character.

Acknowledgements

We would like to thank Aristides Fernandes for taxonomic identification of the specimens, Fernanda Silva Almeida for mounting genitalia slides and Henrique Krambeck Rofatto for operating the CLS microscope (*Fundação de Amparo a Pesquisa do Estado de São Paulo – FAPESP* grant # 00/11624-5). Financial support was provided by *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (*CNPq* Grants # 140032/2013-4 and #311805/2014-0), and *Coordenação de Aperfeiçoamento Pessoal de Nível Superior* (*CAPES* Grant # 23038.005274/2011-24 and 1275/2011).

References

Arnell, J.H. (1976) Mosquito studies (Diptera, Culicidae). XXXIII – a revision of the Scapularis group of *Aedes* (*Ochlerotatus*). *Contribution of American Entomological Institute* **13**, 1–144.

Consoli, R.A.G.B. & Oliveira, R.L. (1994) *Principais mosquitos de importância sanitária no Brasil*. Rio de Janeiro, Fiocruz, 228 pp.

Eberhard, W.G. (1985) *Sexual Selection and Animal Genitalia*. Cambridge, MA, Harvard University Press, 244 pp.

https://doi.org/10.1017/S0007485317000359 Published online by Cambridge University Press

Float, K.D. & Fox, A.S. (2000) Flies under stress: a test of fluctuating asymmetry as a biomonitor of environmental quality. *Ecological Applications* **10**, 1541–1550.

Forattini, O.P. (2002) *Culicidologia médica* 2. São Paulo, University of São Paulo, *Edusp*, 860 pp.

Hribar, L.J. (1994) Geographic variation of male genitalia of *Anopheles nuneztovari* (Diptera: Culicidae). *American Mosquito Control Association* **26**, 132–144.

Huber, B.A. (1995) Genital morphology and copulatory mechanics in *Anyphaena accentuate* (Anyphaenidae) and *Clubiona pallidula* (Clubionidae: Araneae). *Journal of Zoology* **235**, 689–702.

Huber, B.A. (2004) Evidence for functional segregation in the directionally asymmetric male genitalia of the spider *Metagonia mariguitarensis* (González-Sponga) (Pholcidae: Araneae). *Journal of Zoology* **262**, 317–326.

Lorenz, C. & Suesdek, L. (2013) Evaluation of chemical preparation on insect wing shape for geometric morphometrics. *American Journal of Tropical Medicine and Hygiene* **89**, 928–931.

Lourenço-de-Oliveira, R. & Deane, L.M. (1995) Presumed *Dirofilaria immitis* infections in wild-caught *Aedes taeniorhynchus* and *Aedes scapularis* in Rio de Janeiro, Brazil. *Memórias do Instituto Oswaldo Cruz* **90**, 387–388.

Moratore, C. (2009) Genetic and morphological patterns in populations of *Culex quinquefasciatus* (Diptera: Culicidae). Dissertation of Master degree in Science, University of Sao Paulo, Institute of Biomedical Sciences, São Paulo, Brazil.

Motoki, M.T., Santos, C.L.S.D. & Sallum, M.A.M. (2009) intraspecific variation on the aedeagus of *Anopheles oswaldoi* (Peryassú) (Diptera: Culicidae). *Neotropical Entomology* **38**, 144–148.

Mpho, M., Callaghan, A. & Holloway, G.J. (2002) Temperature and genotypic effects on life history and fluctuating asymmetry in a field strain of *Culex pipiens*. *Heredity* **88**, 307–312.

Palmer, A.R. & Strobeck, C. (2003) Fluctuating asymmetry revisited. 279–319 pp. *in* Polak, M. (*Ed.*) *Developmental Instability (DI): Causes and Consequences*. New York, Oxford Univ. Press.

Pauvolid-Corrêa, A., Kenney, J.L., Couto-Lima, D., Campos, Z. M., Schatzmayr, H.G., Nogueira, R.M., Brault, A.C. & Komar, N. (2013) Ilheus virus isolation in the Pantanal, west-central Brazil. *PLoS Neglected Tropical Diseases* **7**, 1–8.

Peruzin, M.C. (2009) Comparative populational analyses of *Culex quinquefasciatus* of two sites of Sao Paulo State. Dissertation of Master degree in Science, University of Sao Paulo, Institute of Biomedical Sciences, São Paulo, Brazil.

Petersen, V. (2012) Characterization of three populations of *Ochlerotatus scapularis* (Rondani, 1848) of the Rio de Janeiro-Sao Paulo, using morphological and genetic markers. Dissertation of Master degree in Science, University of Sao Paulo, Institute of Biomedical Sciences, São Paulo, Brazil.

Petersen, V., Devicari, M. & Suesdek, L. (2015) High morphological and genetic variabilities of *Aedes scapularis*, a potential vector of filarias and arboviruses. *Parasites & Vectors* **8**, 128.

Rachou, R.G., Lima, M.M., Neto, J.A.F. & Martins, C.M. (1995) Inquérito epidemiológico de filariose bancroftiana em uma localidade de Santa Catarina, como fase preliminar de uma prova profilática. Constatação de transmissão extradomiciliária por um novo vetor, *Aedes scapularis*. *Revista Brasileira de Malariologia e Doenças Tropicais* **7**, 51–70.

Sallum, M.A.M., Uramoto, K. & Forattini, O.P. (1988) Redescription, and resurrection from synonymy, of *Aedes* (Ochlerotatus) *rhyacophilus* Costa Lima, 1933. *Memórias do Instituto Oswaldo Cruz* **83**, 67–77.

Sallum, M.A.M., Foster, P.G., Santos, C.L.S.D., Flores, D.C., Motoki, M.T. & Bergo, E.S. (2010) Resurrection of two species from synonymy of *Anopheles* (*Nyssorhynchus*) *strodei* Root, and characterization of a distinct morphological form from the Strodei Complex (Diptera: Culicidae). *Journal of Medical Entomology* **47**, 504–526.

Shapiro, S.S. & Wilk, M.B. (1965) An analysis of variance test for normality (complete samples). *Biometrika Trust Stable* **52**, 591–611 pp. Available online at http://www.jstor.org/stable/2333709.

Song, H. & Wenzel, J.W. (2008) Mosaic pattern of genital divergence in three populations of *Schistocerca lineata* Scudder, 1899 (Orthoptera: Acrididae: Cyrtacanthacridinae). *Biology Journal Linnean Society* **94**, 289–301.

Souza, J.M.G.A., Gouveia, M., Perondini, A.L.P. & Selivon, D. (2007) Asymmetry frontal bristles and postocular setae in species and hybrids of the *Anastrepha fraterculus* complex (Diptera, Tephritidae). *Genetics and Molecular Biology* **30**, 145–151.

VanValen, L. (1962) A study of fluctuating asymmetry. *Evolution* **16**, 125–142.

Vasconcelos, P.F.C., Costa, Z.G., Travassos da Rosa, E.S., Luna, E., Rodrigues, S.G., Barros, V.L.R.S., Dias, J.P., Monteiro, H.A.O., Oliva, O.F.P., Vasconcelos, H.B., Oliveira, R.C., Sousa, M.R.S., Barbosa Da Silva, J., Cruz, A.C.R., Martins, E.C. & Travassos Da Rosa, J.F.S. (2001) Epidemic of Jungle Yellow Fever in Brazil, 2000: implications of climatic alterations in disease spread. *Journal of Medical Virology* **65**, 598–604.

Woods, R.E., Sgro, C.M., Hercus, M.J. & Hoffmann, A.A. (1999) The association between fluctuating asymmetry, trait variability, trait heritability, and stress: a multiply replicated experiment on combined stresses in *Drosophila melanogaster*. *Evolution* **53**, 493–505.