Ibis (2005), doi: 10.1111/j.1474-919x.2005.00468.x

Short Communication

Establishment of the avian disease vector *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae) on the Galápagos Islands, Ecuador

NOAH KERNESS WHITEMAN,[1*] SIMON J. GOODMAN,[2] BRADLEY J. SINCLAIR,[3] TIM WALSH,[4,5†] ANDREW A. CUNNINGHAM,[2] LAURA D. KRAMER[6] & PATRICIA G. PARKER[1]

[1] *Department of Biology & International Center for Tropical Ecology, University of Missouri-Saint Louis, Research Building 223, 8001 Natural Bridge Road, Saint Louis, MO 63121, USA*

[2] *Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK*

[3] *Zoologisches Forschungsinstitut und Museum Alexander Koenig, Adenauerallee 160, D-53113 Bonn, Germany*

[4] *Charles Darwin Research Station, Puerto Ayora, Isla Santa Cruz, Galápagos, Ecuador*

[5] *Saint Louis Zoo, One Government Drive, St. Louis, MO 63110, USA*

[6] *Wadsworth Center, New York State Department of Health, Slingerlands, NY 12159, USA*

Avian disease has been implicated as a major factor in the decline of the endemic Hawaiian avifauna (Warner 1968, Van Riper *et al.* 1986, 2002, Atkinson *et al.* 2000, Yorinks & Atkinson 2000). The introduction into Hawaii of avian pox *Avipoxvirus* spp., avian malaria *Plasmodium relictum* and a suitable vector, the Southern House Mosquito *Culex quinquefasciatus* Say, 1823 (Hardy 1960), are thought to be the mechanisms driving this decline (Van Riper & Scott 2001, Van Riper *et al.* 2002). *C. quinquefasciatus* is a cyclopropagative vector (in which the pathogen undergoes further development and multiplication) for avian malaria, and a mechanical vector (in which the pathogen is carried on or in mouthparts, legs, etc., but does not undergo further development or multiplication) for avian pox in Hawaii. The endemic birds of Hawaii are more susceptible to both of these pathogens than are introduced birds (Atkinson *et al.* 2000, Yorinks & Atkinson 2000, Van Riper *et al.* 2002).

By contrast, the avifauna of the Galápagos Islands is largely intact (due to relatively recent human colonization; Snell *et al.* 2002), yet is highly endemic (84% of land birds are unique; Tye *et al.* 2002). Several endemic bird populations are in decline (Snell *et al.* 2002), although none is extinct archipelago-wide. For example, the Galápagos Hawk *Buteo galapagoensis* Gould, 1837 has been extirpated on three human-inhabited islands (de Vries 1975), while breeding populations still reside on eight islands. Invasive organisms and disease agents, including viruses such as West Nile Virus (WNV), now pose the greatest threat to the continued persistence of Galápagos's unique birds (Wikelski *et al.* 2004, Thiel *et al.* 2005). We report here the establishment in the Galápagos Islands of the avian disease vector *C. quinquefasciatus*, first reported from the archipelago in 1989 (Peck *et al.* 1998), and documented now as part of a larger survey of avian disease and their vectors in the archipelago begun in 2001. We also report the date '1985' as the first collection of this mosquito in the archipelago, earlier than was published previously (1989). The implications of the establishment of this insect in the Galápagos Islands, specifically the threat it poses to avian health, are discussed.

METHODS

Adult mosquitoes were sampled during a total of nine trapping attempts using US Centers for Disease Control & Prevention miniature ultraviolet light traps on Isla Santa Cruz in the Galápagos Islands (Archipelago de Colón), Ecuador, in July and August 2003 (purchased from BioQuip Products, Rancho Dominguez, CA, USA). Light traps were turned on approximately 1 h before dusk (~17:00 h local time) and turned off from 1 to 5 h after dawn (~07:00–11:00 h). Culicids were then separated from other insect taxa and stored in 95% ethanol for identification. Label information from specimens collected prior to this study was obtained from vouchers housed at the Canadian National Collection of Insects in Ottawa, Canada. All 2003 collections were made in and around the coastal town of Puerto Ayora, Isla Santa Cruz, which lies within the Arid Zone (with focused sampling at the Charles Darwin Research Station; 0°44′20″S, 90°18′25″W; 6 m asl) and within the town of Bellavista, which lies within the upper Transition Zone (0°42′S, 90°22′W; 194 m). Bellavista, Isla Santa Cruz, annually receives more rainfall and is cooler than Puerto Ayora, Isla Santa Cruz (Snell & Rea 1999).

Oviposition traps were made from 5-L 'pitcher'-style plastic water containers by cutting away the neck and front walls of the vessel to half height. The containers were filled with ~1.5 L of fresh, potable water and a handful of dry straw and placed in partially shaded locations around the Galápagos National Park Service Headquarters in Puerto

*Corresponding author.
Email: nkwf38@studentmail.umsl.edu

†Present address: Washington State University, Washington Animal Disease Diagnostic Laboratory, PO Box 2037, Pullman, WA 99165-2037, USA.

Ayora. Two traps were set on consecutive days from 28 April to 14 May 2004. Traps were checked daily and the number of eggs counted. Egg rafts were removed to separate hatching containers and allowed to complete the development cycle, after which a selection of adults was collected for identification. Identifications of culicid specimens were made using a species-diagnostic molecular analysis of the internal transcribed spacers (ITS1 and ITS2) of the nuclear ribosomal gene array (Crabtree *et al.* 1995), conducted at the Arbovirus Laboratories, Wadsworth Center, NY, USA.

RESULTS

Eleven adult individuals of the Southern House Mosquito were collected from two traps placed at two locations (one trap within the Arid Zone and one trap within the upper Transition Zone) on Isla Santa Cruz in August 2003 (Table 1). One of the traps (placed in Bellavista) that produced two Southern House Mosquitoes also produced 11 individuals of the Black Salt Marsh Mosquito *Ochlerotatus taeniorhynchus* (Wiedemann 1821). Seven traps placed in other areas, including near the Charles Darwin Research Station, produced 155 *O. taeniorhynchus* individuals and no *Culex* individuals. Thus, 11 Southern House Mosquitoes and 166 Black Salt Marsh Mosquitoes were collected from the nine trapping attempts. Voucher specimens of both species have been placed at the Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany. Re-examination of museum label data from *C. quinquefasciatus* collected in the Galápagos Islands prior to this study indicate that the date of first record of occurrence in the Galápagos was not 1989 as reported by Peck *et al.* (1998), but rather 1985.

In total, 27 egg rafts were laid in oviposition traps between 28 April and 14 May 2004. Adults reared from these eggs rafts were subsequently confirmed as *C. quinquefasciatus* using the molecular analysis described above.

DISCUSSION

The establishment of *C. quinquefasciatus* on the Galápagos Islands after its first detection two decades ago, in 1985, is troubling from an avian conservation perspective. This species is capable of biting humans or migrating birds and transmitting exotic disease agents, such as WNV (Turell *et al.* 2001). WNV is present within other island systems in the New World tropics and it may be simply a matter of time before it enters the Galápagos ecosystem (Dupuis *et al.* 2003). This mosquito is also a mechanical vector for *Avipoxvirus*, now present in both domesticated and wild birds in the Galápagos (Thiel *et al.* 2005), and thus its presence may exacerbate the spread of pox within and between islands. If *Plasmodium relictum* or another avian malaria species ever enters the Galápagos, *C. quinquefasciatus* can serve as a competent vector. This combination of events would probably be devastating to the local bird community.

Interestingly, the first 2003 *C. quinquefasciatus* collection locality on Isla Santa Cruz was in a small town (Bellavista), and only 5 km from the first collection locality (in 1985) on Isla Santa Cruz, at the Media Luna.

Table 1. Collection records of the Southern House Mosquito (Diptera: Culicidae) *Culex quinquefasciatus* and the Black Salt Marsh Mosquito (*Ochlerotatus taeniorhynchus*) from the Galápagos Islands, Ecuador.

Date	Island	Location	Abundance
Culex quinquefasciatus			
14.v.–13.7.1985	Santa Cruz*	4 km N Bellavista, Media Luna, 620 m	4
10.2.1989*	San Cristóbal*	Puerto Baquerizo, hotel light, swarming	9
01.8.2003	Santa Cruz	Town of Bellavista	2
03.8.2003	Santa Cruz	Near laundry room of private residence in Puerto Ayora	9
			2003 total: 11
Ochlerotatus taeniorhynchus			
16–17.7.2003	Santa Cruz	Charles Darwin Research Station, ~1 km E Puerto Ayora, 6 m (CDRS, near scientists' dormitories)	2
17.7.2003	Santa Cruz	Same data	1
20.7.2003	Santa Cruz	CDRS (near Iguana rearing pens)	4
20.7.2003	Santa Cruz	CDRS (near scientists' dormitories)	10
23.7.2003	Santa Cruz	CDRS (near Iguana rearing pens)	36
28.7.2003	Santa Cruz	CDRS (near scientists' dormitories)	91
01.8.2003	Santa Cruz	Town of Bellavista, 194 m (collected in same light trap as *C. quinquefasciatus* collected on this date)	11
03.8.2003	Santa Cruz	CDRS (outside Ornithology Laboratory)	11
			2003 total: 166

*Same collection data reported previously (Peck *et al.* 1998).

However, these two sites, although geographically proximate, are separated by *c.* 400 m in elevation. Bellavista is an agricultural settlement located *c.* 8 km inland, situated in the more mesic highlands of the upper Transition Zone. The 1985 sampling locality (the Media Luna) remains uninhabited and is in the mesic Miconia Zone. The second 2003 collection location on Santa Cruz was located within the Arid Zone but a trap was intentionally placed near a laundry room of a private residence, where mosquitoes had been observed previously. *C. quinquefasciatus* also oviposited readily in freshwater traps on Santa Cruz. Thus, *C. quinquefasciatus* has now been reported from three altitudinal zones within Isla Santa Cruz and from the Arid Zone within Isla San Cristóbal. As breeding by *C. quinquefasciatus* could be limited by the presence of freshwater (it is a freshwater obligate; Patrick & Bradley 2000) its distribution in the Galápagos is probably most common near human habitations where freshwater can be found. However, *C. quinquefasciatus* is likely to increase its range within the Arid Zone during the wet season. Furthermore, the absence of *C. quinquefasciatus* from most light traps may be due to the fact that we sampled during the dry season and not the wet season. Nonetheless, this species was present within both the Arid and the Transition zones during the dry season, which emphasizes the potential for *C. quinquefasciatus* to invade coastal areas of other islands, particularly during the wet season and during El Niño Southern Oscillation events. Simple control measures, such as reducing the availability of artificial oviposition sites (e.g. used tyres, open containers), may reduce the local abundance and the eventual spread of these obligate freshwater breeding mosquitoes in the archipelago. Other control measures, such as the use of the biological control agent *Bacillus sphaericus*, which is toxic to *C. quinquefasciatus* (Regis *et al.* 2000), could be implemented. However, resistance to the 'Bin toxin' has been observed (Oliveira *et al.* 2004). The toxin produced by *Bacillus thuringiensis israelensis* (Bti), the effects of which are also relatively specific to larval dipterans, would be preferable because mosquitoes do not develop resistance to it. However, non-target taxa, particularly other insects within the dipteran suborder Nematocera, such as chironomid midges, may be negatively affected by its application (Hershey *et al.* 1998).

Peck *et al.* (1998) speculated that *C. quinquefasciatus* arrived in the archipelago as larvae in water. However, local air travel now occurs among three islands within the archipelago (Islas Isabela, Santa Cruz, San Cristóbal) and between two islands and the mainland, including the city of Guayaquil, Ecuador, situated in the humid tropical lowlands. As Peck *et al.* (1998) noted, 11 448 insect specimens were collected from aircraft in Hawaii (Dethier 1945, see also Lounibos 2002). This route of dispersal is likely to ensure the presence of such invasive pests in Galápagos, and new mosquito-borne diseases are likely to be introduced unless control measures are implemented for aircraft flying into the archipelago (A.M. Kilpatrick *et al.* unpubl. data). Tour operators, tourists, residents and scientists on interisland boat trips should be vigilant in ensuring that they are not transporting these mosquitoes. An educational campaign should be instituted to alert communities on the Galápagos to eliminate standing water. Nonetheless, *C. quinquefasciatus* now appears to be established on Isla Santa Cruz and is quite likely still to be present on Isla San Cristóbal, where it was collected in 1989. It seems probable that this species is also present on Islas Isabela and Floreana, the only other islands inhabited by humans in the archipelago, but further sampling is needed to confirm this.

The Black Salt Marsh Mosquito is present on all the main islands within the Galápagos and has been known since it was first recorded in the late 1890s (Linsley & Usinger 1966). This species breeds in brackish water and is regarded as less threatening as a vector of avian disease agents. However, it should not be ignored as a threat, because, although it may prefer feeding upon mammals, individuals also feed on birds (Edman 1971). *Ochlerotatus taeniorhynchus* individuals have been observed feeding on endemic birds within the Galápagos and locally high mosquito population densities have led to cases of nest desertion by endemic birds (Anderson & Fortner 1988). Moreover, individuals of *O. taeniorhynchus* have tested positive for WNV elsewhere (Hribar *et al.* 2003), and individuals are capable of transmitting WNV (Turell *et al.* 2001). This insect is also likely to serve as a mechanical vector of *Avipoxvirus* among birds in the Galápagos Islands (Thiel *et al.* 2005).

Data on host preferences (by genetically characterizing the identity of mosquito blood meals; Ngo & Kramer 2003), distribution, and intra- and interisland movement of these mosquitoes (e.g. population genetics), and how each of these interacts with seasonality, are needed to understand more fully the threat posed by these vectors to the unique Galápagos avifauna.

N.K.W. and P.G.P. were supported by an International Dissertation Enhancement Grant from the US National Science Foundation (INT-030759), a Trans World Airlines scholarship in environmental studies, grants from the Field Research for Conservation Program of the Saint Louis Zoo, International Center for Tropical Ecology, Sigma Xi, and funds from the E. Desmond Lee and Family Fund's Collaborative Vision in Zoological Studies from UM-St. Louis and the Saint Louis Zoo. S.J.G. and A.A.C. were supported by a UK government Darwin Initiative Grant (no. 162-12-017, 'Building capacity and determining disease threats to endemic Galápagos fauna'). We gratefully acknowledge the logistical support and research permits provided by the Servicio Parqué Nacional de Galápagos and the Estación Científica Charles Darwin, Isla Santa Cruz, Galápagos, Ecuador, with special thanks to Poly Robayo, Howard Snell, Hernan Vargas and David Wiedenfeld. TAME provided discounted roundtrip air-travel within Ecuador. Dr Stewart Peck provided helpful comments on a previous version of this manuscript.

REFERENCES

Anderson, D.A. & Fortner, S. 1988. Waved Albatross egg neglect and associated mosquito ectoparasitism. *Condor* **90**: 27–29.

Atkinson, C.T., Dusek, R.J., Woods, K.L. & Iko, W.M. 2000. Pathogenicity of avian malaria in experimentally-infected Hawaii Amakihi. *J. Wildl. Dis.* **36**: 197–204.

Crabtree, M.B., Savage, H.M. & Miller, B.R. 1995. Development of a species-diagnostic polymerase chain reaction assay for the identification of *Culex* vectors of St. Louis encephalitis virus based on interspecies sequence variation in ribosomal DNA spacers. *Am. J. Trop. Med. Hyg.* **53**: 105–109.

Dethier, V.G. 1945. The transport of insects in aircraft. *J. Econ. Entomol.* **38**: 528–531.

Dupuis, A.P., Marra, P.P.I.I. & Kramer, L.D. 2003. Serologic evidence of West Nile virus transmission, Jamaica, West Indies. (Dispatches). *Emerg. Infect. Dis.* **9**: 860–863.

Edman, J.D. 1971. Host-feeding patterns of Florida mosquitoes. I. *Aedes, Anopheles*, *Coquillettidia*, *Mansonia*, and *Psorophora*. *J. Med. Entomol.* **8**: 687–695.

Hardy, D.E. 1960. *Insects of Hawaii*, Vol. 10. Honolulu, Hawaii: University of Hawaii Press.

Hershey, A.E., Lima, A.R., Niemi, G.J. & Regal, R.R. 1998. Effects of *Bacillus thuringiensis israelensis* (Bti) and methoprene on nontarget macroinvertebrates in Minnesota wetlands. *Ecol. Appl.* **8**: 41–61.

Hribar, L.J., Vlach, J.J., Demay, D.J., Stark, L.M., Stoner, R.L., Godsey, M.S., Burkhalter, K.L., Spoto, M.C., James, S.S., Smith, J.M. & Fussell, E.M. 2003. Mosquitoes infected with West Nile Virus in the Florida Keys, Monroe County, Florida, USA. *J. Med. Entomol.* **40**: 361–363.

Linsley, E.G. & Usinger, R.L. 1966. Insects of the Galápagos Islands. *Proc. Cal. Acad. Sci.* **33**: 113–116.

Lounibos, L.P. 2002. Invasions by insect vectors of human disease. *Ann. Rev. Entomol.* **47**: 233–266.

Ngo, K.A. & Kramer, L.D. 2003. Identification of mosquito bloodmeals using polymerase chain reaction (PCR) with order-specific primers. *J. Med. Entomol.* **40**: 215–222.

Oliveira, C.M.F., Siva-Filha, M.H., Nielsen-Leroux, C., Pei, G., Yuan, Z. & Regis, L. 2004. Inheritance and mechanism of resistance to *Bacillus sphaericus*. *Culex quinquefasciatus* (Diptera: Culicidae) from China and Brazil. *J. Med. Entomol.* **41**: 58–64.

Patrick, M.L. & Bradley, T.J. 2000. The physiology of salinity tolerance in two species of *Culex* mosquitoes: the role of compatible solutes. *J. Exp. Biol.* **203**: 821–830.

Peck, S.B., Heraty, J., Landry, B. & Sinclair, B.J. 1998. Introduced insect fauna of an oceanic archipelago: The Galápagos Islands, Ecuador. *Am. Entomol.* **44**: 218–237.

Regis, L., Oliveira, M.H., Silva-Filha, S.B., Maciel, A. & Furtado, A.F. 2000. Efficacy of *Bacillus sphaericus* in control of the filariasis vector *Culex quinquefasciatus* in an urban area of Olinda, Brazil. *Trans. Roy. Soc. Trop. Med. Hyg.* **94**: 488–492.

Snell, H.L. & Rea, S. 1999. The 1997–1998 El Niño in Galápagos: can 34 years of data estimate 120 years of pattern? *Noticias Galápagos* **60**: 11–20.

Snell, H.L., Tye, A., Causton, C.E. & Bensted-Smith, R. 2002. Current status of and threats to the terrestrial biodiversity of Galápagos. In Bensted-Smith, R. (ed.) *A Biodiversity Vision for the Galápagos Islands*: 30–47. Puerto Ayora, Galápagos: Charles Darwin Foundation & World Wildlife Fund.

Thiel, T., Whiteman, N.K., Tirapé, A., Baquero, M.I., Cedeño, V., Walsh, T., Uzcátegui, G.J. & Parker, P.G. 2005. Characterization of canarypox-like viruses infecting endemic birds in the Galápagos Islands. *J. Wild. Dis.* in press.

Turell, M.J., O'Guinn, M.L., Dohm, D.J. & Jones, J.W. 2001. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile Virus. *J. Med. Entomol.* **38**: 130–134.

Tye, A., Snell, H.L., Peck, S.B. & Adsersen, H. 2002. Outstanding terrestrial features of the Galápagos archipelago. In Bensted-Smith, R. (ed.) *A Biodiversity Vision for the Galápagos Islands*: 12–23. Puerto Ayora, Galápagos: Charles Darwin Foundation & World Wildlife Fund.

Van Riper, C. III & Scott, J.M. 2001. Limiting factors affecting Hawaiian native birds. *Stud. Avian Biol.* **22**: 221–233.

Van Riper, C. III, Van Riper, S.G., Goff, M.L. & Laird, M. 1986. The epizootiology and ecological significance of malaria in Hawaiian land birds. *Ecol. Monogr.* **56**: 327–344.

Van Riper, C. III, Van Riper, S.G. & Hansen, W.R. 2002. Epizootiology and effect of avian pox on Hawaiian forest birds. *Auk* **119**: 949–942.

de Vries, Tj. 1975. The breeding biology of the Galápagos Hawk, *Buteo galapagoensis*. *Le Gerfaut* **65**: 29–57.

Warner, R.E. 1968. The role of introduced diseases in the extinction of the endemic Hawaiian avifauna. *Condor* **70**: 101–120.

Wikelski, M., Foufopoulos, J., Vargas, H. & Snell, H. 2004. Galápagos birds and diseases: invasive pathogens as threats for island species. *Ecology and Society* **9**: article 5, http://www.ecologyandsociety.org/vol9/iss1/art5

Yorinks, N. & Atkinson, C.T. 2000. Effects of malaria on activity budgets of experimentally infected juvenile Apapane (*Himatione sanguinea*). *Auk* **117**: 731–738.

Received 4 October 2004; revision accepted 10 June 2005.