Infection of New- and Old-World *Aedes albopictus* (Diptera: Culicidae) by the Intracellular Parasite *Wolbachia*: Implications for Host Mitochondrial DNA Evolution

PETER ARMBRUSTER,[1] WILLIAM E. DAMSKY, JR., ROSANNA GIORDANO, JOSEPHINE BIRUNGI,[2] LEONARD E. MUNSTERMANN,[3] AND JAN E. CONN[4]

Department of Biology, University of Vermont, Burlington, VT 05405Ð0086

J. Med. Entomol. 40(3): 356Ð360 (2003)

ABSTRACT *Wolbachia* are cytoplasmically inherited, endosymbiotic bacteria known to infect a wide variety of arthropods. Polymerase chain reaction (PCR) ampliÞcation of the *Wolbachia* surface protein (*wsp*) gene was used to assay the infection of geographically disparate populations of *Aedes albopictus* (Skuse) by *Wolbachia*. Nine North American, four South American, one Hawaiian, and four Old World populations of *A. albopictus* were all doubly infected with both the *w*AlbA and *w*AlbB strains of *Wolbachia*. A 365-bp region of the *w*AlbA *wsp*gene was sequenced from seven geographically disparate host populations, and all sequences were identical. Similarly, a 474-bp region of the *w*AlbB *wsp* gene was sequenced from the same populations, and all sequences were identical. These results suggest a role for *Wolbachia* infection in causing the previously established pattern of low mitochon-drial DNA variability, but average nuclear gene diversity, within and among populations of *A. albopictus*.

KEY WORDS *Aedes albopictus*, *Wolbachia*, *wsp* gene, mitochondrial DNA

THE ASIAN TIGER MOSQUITO, *Aedes albopictus*, is an invasive mosquito of considerable medical concern. It is native to Asia but has recently invaded North America (Hawley et al. 1987), South America (Forattini 1986), Europe (Urbanelli et al. 2000), and Africa (Savage et al. 1992). It has been implicated as a vector of dengue fever in both its native (Shroyer 1986) and introduced ranges (Iban˜ez-Bernal et al. 1997), and Þeld-collected samples in North America have tested positive for arboviruses, including eastern equine encephalitis (Mitchell et al. 1992), LaCrosse encephalitis (Ger-hardt et al. 2001), and West Nile virus (Holick et al. 2002). Throughout its current range, *A. albopictus* in-habits tropical, subtropical, and temperate habitats and has shown a remarkable degree of evolutionary plasticityfortraitsrelatedtoclimaticadaptationacross wide environmental gradients (Lounibos 2002).

Analysis of biochemical variation (e.g., allozymes and DNA) has the potential to illuminate various aspects of invasion history, such as historical population demography, the geographic origin of invasive populations, and the number of invasion events. These insights may help biologists to understand the processes that facilitate the rapid spread and adaptation of invasive species (Tsutsui et al. 2000, Fonseca et al. 2001, Lee 2002), including *A. albopictus* (Birungi and Munstermann 2002). Several investigations have examined allozyme (Black et al. 1988a, 1988b, Kambhampati et al. 1990, 1991) and mitochondrial DNA (mtDNA) variation in *A. albopictus* (Kambhampati and Rai 1991, Birungi and Munstermann 2002). One pattern that emerges from these studies is that North American (Black et al. 1988a, Kambhampati et al. 1991), South American (Black et al. 1988a, Kambham-pati et al. 1991), and Asian (Black et al. 1988b) pop-ulations of *A. albopictus* display levels of allozyme variation typical for most insects. However, across this wide geographic range, *A. albopictus* displays ex-tremely low mtDNA variation both within and among populations (Kambhampati and Rai 1991, Birungi and Munstermann 2002).

Previous authors have invoked explanations involving genetic drift associated with the range expansion of *A. albopictus* to explain the differing variability of structural genes (allozymes) and mtDNA (Kambhampati and Rai 1991, Birungi and Munstermann 2002). Herein, an alternative hypothesis for these genetic data are presented, which focuses on the infection of *A. albopictus* by the intracellular parasite *Wolbachia*.

Wolbachia are maternally transmitted ricksettsia-like bacteria estimated to infect as many as 76% of all arthropod species (Jeyaprakash and Hoy 2000). Infection by *Wolbachia* parasites can have a wide variety of effects on host reproduction (Stevens et al. 2001).

1 E-mail: parmbrus@zoo.uvm.edu.
2 Uganda Virus Research Institute, PO Box 49, Entebbe, Uganda.
3 Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06520Ð8034.
4 GrifÞn Laboratory, Wadsworth Center, 5668 State Farm Road, Slingerlands, NY 12159.

Fig. 1. Geographic origin of populations of *A. albopictus* assayed for *Wolbachia* infection. Three populations were collected from Texas, Florida, and New Jersey as described in the text.

A. albopictus is known to be infected with two strains of *Wolbachia*, *w*AlbA and *w*AlbB (Zhou et al. 1998), both of which cause cytoplasmic incompatibility (CI) (Sinkins et al. 1995, Dobson et al. 2001). In an infected host population, CI confers a reproductive advantage to infected females relative to females that are unin-fected, thereby leading to a rapid spread, often re-ferred to as a "sweep," of the *Wolbachia* infection through the host population (Turelli and Hoffmann 1991, 1995). Because both *Wolbachia* and the host mtDNA are inherited in the host cytoplasm, a *Wol-bachia* infection will result in the homogenization of the host mtDNA with minimal effects to nuclear ge-netic diversity (Turelli et al. 1992, Rand et al. 1994, Ballard et al. 1996, Shoemaker et al. 1999).

We have used polymerase chain reaction (PCR) ampliÞcation of the *Wolbachia* surface protein (*wsp*) genetoassayfor *Wolbachia*infectionin18populations of *A. albopictus* from across a large portion of the current geographical range of this species (Fig. 1). We also sequenced 365 bp of the *w*AlbA gene and 474 bp of the *w*AlbB gene in a subset of seven of these pop-ulations. The results imply a relatively recent double infection of *A. albopictus* by *Wolbachia* and suggest a mitochondrial "sweep" by *Wolbachia* as an alternative hypothesis to explain the typical levels of structural gene diversity but low levels of mtDNA variation in *A. albopictus.*

Materials and Methods

Samples of *A. albopictus* were collected from the geographic localities indicated in Fig. 1. North Amer-ican samples were collected as larvae from three sites separated by 50Ð100 miles in Texas, Florida, and New Jersey, raised to adults, and stored in individual 0.5-ml tubes at 80 C. Samples from South America were collected as eggs, larvae and pupae, or adults, and were reared through Þve generations in the laboratory before being frozen as adults at 80 C. Hawaiian and Old World samples were collected as adults in the Þeld and stored in 95% ethanol during transport and then at 20 C in the laboratory.

DNA extractions for all Hawaiian and New World samples were performed by a phenol-chloroform pro-tocol described in Severson (1997), and for Old World collections as described by Birungi and Munstermann (2002). We isolated DNA from 10 adult females per population, except for the population from Hawaii, where we sampled 9 adult females. We sampled a limited number of individuals in each population to establish the presence or absence of *Wolbachia* infec-tion, because both theoretical considerations (Turelli and Hoffmann 1995) and empirical data (Turelli and Hoffmann 1991) indicate that once a *Wolbachia* in-fection enters a population, it is expected to spread to Þxation rapidly.

Assays for *Wolbachia* infection were performed by PCR ampliÞcation of the *wsp* gene using diagnostic primers for the two *Wolbachia* strains *w*AlbA (primers 328 F, 691R) and *w*AlbB (primers 183 F, 691R) known to occur in *A. albopictus* as described by Zhou et al. (1998). First, direct sequencing of the PCR product conÞrmed that these primers were amplifying the ex-pected products (see sequencing methods below). Then a multiplex PCR reaction was used to assay for both the *w*AlbA and *w*AlbB fragments in a single ampliÞcation (Fig. 2). Each 25-*m*l reaction consisted of 21 *m*l sterile H_20, 1 *m*l of template DNA, 1 *m*l (10 mM concentration) of each of the primers 183 F, 328 F, and 691R (Zhou et al. 1998), and a single "ready-to-go" PCR bead (Amersham Pharmacia, Piscataway, NJ). Samples were denatured for 5 min at 94 C, followed

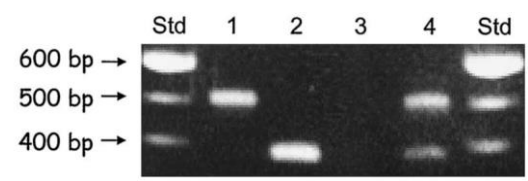

Fig. 2. Results of PCR ampliÞcation of *w*AlbB fragment (1), *w*AlbA fragment (2), a negative control (3), and a mul-tiplex ampliÞcation of both the *w*AlbA and *w*AlbB fragments in the same reaction (4). Std, 100-bp ladder (Gibco, Rock-ville, MD).

by 35 cycles of 1 min at 94 C, 1 min at 55 C, and 1 min at 72 C. A negative control in which 1 *m*l of sterile water was substituted for DNA template was included in each group of PCR reactions. PCR products were visualized by running 8 *m*l of product on a 1% agarose gel, staining with ethidium bromide, and viewing un-der a UV transilluminator (see Fig. 2).

We sequenced 365 bp of the *w*AlbA fragment and 474 bp of the *w*AlbB fragment from one individual from each of the following locations: Brazil, Japan, Madagascar, Malaysia, New Jersey, Taiwan, and Texas (Fig. 1). For each locality, PCR ampliÞcation was performed as described above except that the *w*AlbA and *w*AlbB fragments were ampliÞed in separate PCR reactions. PCR products were cleaned using "Centri-Spin" columns (Princeton Separations, Adelphia, NJ) according to the manufacturerÕs instructions. Cleaned PCR products were then dried, resuspended in 21 *m*l sterile water, and 1 *m*l of resuspended DNA was used as template for cycle-sequencing reactions with Dig-Dye chemistry (Applied Biosystems, Foster City, CA). Each sequencing reaction consisted of 7 *m*l ster-ile H_2O, 4 *m*l 5 sequencing buffer (200 mM Tris-base, 5 mM $MgCl_2$, pH 9.0), 2 *m*l glycerol, 2 *m*l template, 1 *m*l primer (10 mM 183 F, 328 F, or 691R, Zhou et al. 1998), and 4 *m*l Big-Dye mix. The temperature proÞle for the cycle-sequencing reaction consisted of 96 C for 1 min, followed by 30 cycles of 30 s at 96 C, 15 s at 56 C, and 4 min at 60 C. For each of the *w*AlbA and *w*AlbB fragments sequenced, forward and reverse se-quences were obtained to check for PCR replication errors.

Results

All of the adult females from all 18 populations tested positive for infection with both the *w*AlbA and *w*AlbB strains of *Wolbachia.* No evidence for contam-ination was detected in any of the negative controls (Fig. 2). We obtained 365 bp of *w*AlbA sequence and 474 bp of *w*AlbB sequence from seven geographically disparate populations of *A. albopictus.* Both the *w*AlbA and *w*AlbBsequencescontainedportionsofthedown-stream hypervariable region of the *wsp* gene (posi-tions 520Ð582; Ono et al. 2001). All *w*AlbA sequences were identical and exactly matched positions 230Ð594 of previously published *w*AlbA sequence obtained from a Houston population of *A. albopictus* (Zhou et al. 1998, Genbank accession no. AF020058). Similarly, all *w*AlbB sequences were identical and matched ex-actly positions 84Ð557 of previously published *w*AlbB sequences obtained from the same Houston popula-tion of *A. albopictus* (Zhou et al. 1998, Genbank ac-cession no. AF020059).

Discussion

Previous studies have documented high levels of allozyme variation within populations across the wide-spread distribution of *A. albopictus.* Average within population heterozygosities range from 0.22 to 0.31 in peninsular Malaysia and Borneo (Black et al. 1988b), from 0.12 to 0.42 in North America (Black et al. 1988a, Kambhampati et al. 1990), and from 0.13 to 0.23 in Italy (Urbanelli et al. 2000). These estimates are in the upper ranges of values typically reported for arthro-pods (Gillespie 1991).

In contrast to the high levels of structural gene diversity (e.g., allozymes), levels of mtDNA variation are notably low. For example, Kambhampati and Rai (1991) surveyed mtDNA restriction site variation within and among 17 *A. albopictus* populations from across almost all of its entire geographical distribution. They used 18 restriction enzymes and found only four haplotypes, one of which was common to all popula-tions and Þxed in 14 of the 17 populations. The three rare haplotypes were caused by a single restriction site gain from the ancestral haplotype. Birungi and Mun-stermann (2002) used single strand conformation polymorphism (SSCP) screening and direct sequenc-ing to survey variation in a 405-bp fragment of the nicotine adenine dinucleotide, reduced (NADH) de-hydrogenase subunit Þve gene from 20 to 50 individ-uals in each of 16 populations from the United States, Brazil, Madagascar, Malaysia, Indonesia, and Japan (764 total individuals). Birungi and Munstermann (2002) found only nine haplotypes, the most divergent of which differed by Þve substitutions.

Other authors have invoked explanations related to the range expansion of *A. albopictus* to explain either the low mtDNA variation within and among popula-tions in this species (Kambhampati and Rai 1991) or the contrasting levels of nuclear gene diversity (e.g., allozymes) and mtDNA variation (Birungi and Mun-stermann 2002). It is possible that the contrasting patterns of nuclear and mitochondrial diversity in the introduced ranges of *A. albopictus* could at least in part be caused by genetic drift during colonization, be-cause the effective population size of a mitochondrial gene is one-fourth that of a nuclear gene (Avise 1994). However, it is less clear how such a scenario would explain the lack of mtDNA variation both within and among populations in the ancestral range of *A. albo-pictus.*

We found that all females sampled in 18 populations across a wide geographic range (Fig. 1) were infected with both *Wolbachia* strains known to occur in this species, *w*AlbA and *w*AlbB of Zhou et al. (1998). Furthermore, all of the *w*AlbA sequences from seven geographically disparate populations were identical, and all of the *w*AlbB sequences from the same seven populations also were identical. Sequence identity from geographically disparate populations indicates that the double infection of *A. albopictus* by *Wolbachia* is sufÞciently recent that neutral substitutions have not become Þxed among geographic isolates of the *w*AlbA and *w*AlbB strains in the portion of the *wsp* gene that we examined. The *wsp* gene is a single copy gene coding for an outer surface membrane protein in *Wolbachia* (Braig et al. 1998) and is the most variable *Wolbachia* gene characterized to date (Zhou et al. 1998). The *wsp* gene shows enormous variation among strains infecting different hosts (up to 22% sequence divergence, Zhou et al. 1998) and has been reported to vary among geographic isolates from a single host species (Rokas et al. 2002).

Our data thus suggest an alternative hypothesis to explain the contrasting levels of nuclear and mitochondrial diversity within and among populations of *A. albopictus.* It is possible that a cytoplasmic "sweep" caused by the double infection of *A. albopictus* by *Wolbachia* has homogenized the host mtDNA. Such effects of a *Wolbachia* sweep are well documented (Turelli and Hoffmann 1991, 1995) and are consistent with the genetic data for *A. albopictus,* because these "sweeps" would not be expected to affect nuclear gene diversity. *Wolbachia* sweeps have been proposed to explain contrasting patterns of nuclear and mitochondrial variation in other Diptera, including *Drosophila* (Rand et al. 1994, Ballard et al. 1996, Shoemaker et al. 1999) and sand ßies (Ono et al. 2001).

Previous authors have reported populations of *A. albopictus* from North America, the Dominican Republic, Hawaii, Italy, Nepal, and Thailand to be infected by two strains of *Wolbachia* (Sinkins et al. 1995). *A. albopictus* invaded Hawaii sometime be-tween 1830 and 1896 (Joyce 1961), was present in Madagascar in 1904 (Ventrillon 1904), invaded North America and South America in the mid-1980s (Hawley et al. 1987), and invaded Italy in 1990 (Urbanelli et al. 2000). It therefore is likely that the double infection of *A. albopictus* by *Wolbachia* occurred some time before the spread of this species from its ancestral range into the Indian Ocean region and Western Hemisphere.

Sinkins et al. (1995) reported that populations from Mauritius and Samui island (Gulf of Thailand) were infected with only a single strain of *Wolbachia*. Both populations were collected pre-1970; therefore, it is possible that they lost one of the infections during the 25 yr they were maintained in the laboratory. One of the current authors (R.G.) has observed the loss of *Wolbachia* infection in laboratory cultures of *Drosophila*, and Sinkins et al. (1995) report the loss of *Wolbachia* infection from cell culture lines. However, it is also possible that these populations were founded before the double infection of *A. albopictus* by *Wol-bachia* was established and that they remained singly infected because of their geographic isolation on small oceanic islands. Recollecting *A. albopictus* from Mau-ritius and Samui island and assaying for *Wolbachia* infection status will help to distinguish between these possibilities and further reÞne our understanding of the infection of *A. albopictus* by *Wolbachia.* If these populations are still singly infected, our hypothesis regarding the role of *Wolbachia* would predict these populations should exhibit differences at the mtDNA level from doubly infected populations.

Acknowledgments

We thank Dan Sprenger, Jamie Scott, Phil Lounibos, Ricardo Lourenc¸o-de-Oliveira, and the Harris County Mosquito Control district for assistance in collecting *A. albopic-tus*. Phil Lounibos, John Edman, and three anonymous ref-erees provided helpful comments on an earlier version of this manuscript. This work was supported by grants from the NIH (F32AI09969-02 to P.A., R29AI40116 and R01AI54139 to J.E.C., and AI44793 to L.P.L. and L.E.M.) and the University of Vermont.

References Cited

Avise, J. C. 1994. Molecular markers, natural history, and evolution. Chapman & Hall, New York.

Ballard, J.W.O., J. Hatzidakis, T. L. Karr, and M. Kreitman. 1996. Reduced variation in *Drosophila simulans* mito-chondrial DNA. Genetics. 144: 1519Ð1528.

Birungi, J., and L. E. Munstermann. 2002. Genetic structure of *Aedes albopictus* (Diptera: Culicidae) populations based on mitochondrial ND5 sequences: evidence for an independent invasion into Brazil and United States. Ann. Entomol. Soc. Am. 95: 125Ð132.

Black, W.C.I., J. A. Ferrari, K. S. Rai, and D. Sprenger. 1988a. Breeding structure of a colonizing species: *Aedes albop-ictus* (Skuse) in the United States. Heredity. 60: 173Ð181.

Black, W.C.I., W. A. Hawley, K. S. Rai, and G.B.J. Craig. 1988b. Breeding structure of a colonizing species: *Aedes albopictus* (Skuse) in peninsular Malaysia and Borneo. Heredity. 61: 439Ð446.

Braig, H. R., W. Zhou, S. Dobson, and S. L. O'Neill. 1998. Cloning and characterization of a gene encoding the major surface protein of the bacterial endosymbiont *Wol-bachia.* J. Bacteriol. 180:2373Ð2378.

Dobson, S. L., E. J. Marsland, and W. Rattanadechakul. 2001. *Wolbachia*-induced cytoplasmic incompatibility in sin-gle- and superinfected *Aedes albopictus* (Diptera: Culicidae). J. Med. Entomol. 38: 382Ð387.

Fonseca, D. M., S. Cambell, W. J. Crans, M. Mogi, I. Miyagi, T. Toma, M. Bullians, T. G. Andreadis, R. L. Berry, B. Pagac, M. R. Sardelis, and R. C. Wilkerson. 2001. *Aedes* (Finlaya) *japonicus* (Diptera: Culicidae), a newly recognized mosquito in the United States: analyses of genetic variation in the United States and putative source populations. J. Med. Entomol. 38: 135Ð146.

Forattini, O. P. 1986. IdentiÞcacaˆo de Aedes (Stegomyia) albopictus (Skuse) no Brasil. Rev. Sau´de Publ. 20: 244Ð 245.

Gerhardt, R. R., K. L. Gottfried, C. S. Apperson, B. S. Davis, P. C. Erwin, A. B. Smith, N. A. Panella, E. E. Powell, and R. S. Nasci. 2001. First isolation of La Crosse virus from naturally infected *Aedes albopictus.* Emerg. Inf. Dis. 37: 805Ð811.

Gillespie, J. H. 1991. The causes of molecular evolution. Oxford University Press, New York.

Hawley, W. A., P. Reiter, R. S. Copeland, C. B. Pumpuni, and G.B. Craig Jr. 1987. *Aedes albopictus* in North America: probable introduction in used tires from northern Asia. Science. 236:1114Ð1116.

Holick, J., A. Kyle, W. Ferraro, R. R. Delaney, and M. Iwaseczko. 2002. Discovery of *Aedes albopictus* infected with West Nile virus in southeastern Pennsylvania. J. Am. Mosq. Control Assoc. 18: 131.

Ibanˆez-Bernal, S., B. Brisenˆo, J.-P. Mutebi, E. Argot, G. Rodriguez, C. Martinez-Campos, R. Paz, P. de la Fuente-San Ramon, R. Tapia-Conyer, and A. Flisser. 1997. First record in America of *Aedes albopictus* naturally infected with dengue virus during the 1995 outbreak at Reynosa, Mexico. Med. Vet. Entomol. 11:305Ð309.

Jeyaprakash, A., and M. A. Hoy. 2000. Long PCR improves *Wolbachia* DNA ampliÞcation: wsp sequences found in 76% of sixty-three arthropod species. Insect Mol. Biol. 9: 393Ð405.

Joyce, C. R. 1961. Potentialities for accidental establishment of exotic mosquitoes in Hawaii. Proc. Hawaii Entomol. Soc. 17: 403Ð413.

Kambhampati, S., and K. S. Rai. 1991. Mitochondrial DNA variation within and among populations of the mosquito *Aedes albopictus.* Genome. 34: 288Ð292.

Kambhampati, S., W. C. Black IV, K. S. Rai, and D. Sprenger. 1990. Temporal variation in genetic structure of a colo-nizing species: *Aedes albopictus* in the United States. Heredity. 64: 281Ð287.

Kambhampati, S., W. C. Black IV, and K. S. Rai. 1991. Geographic origin of the US and Brazilian *Aedes albopictus* inferred from allozyme analysis. Heredity. 67:85Ð94.

Lee, C. E. 2002. Evolutionary genetics of invasive species. Trends Ecol. Evol. 17: 386Ð391.

Lounibos, L. P. 2002. Invasions by insect vectors of human disease. Annu. Rev. Entomol. 47: 233Ð266.

Mitchell, C. J., M. L. Niebylski, N. Karabatsos, D. Martin, and J.-P. Mutebi. 1992. Isolation of eastern equine enceph-alitis from *Aedes albopictus* in Florida. Science. 257:526Ð 527.

Ono, M., H. R. Braig, L. E. Munstermann, C. Ferro, and S. L. O'Neill. 2001. *Wolbachia* infections of phlebotomine sand ßies (Diptera: Psychodidae). J. Med. Entomol. 38: 237Ð241.

Rand, D. M., M. Dorfsman, and L. M. Kann. 1994. Neutral and non-neutral evolution of *Drosophila* mitochondrial DNA. Genetics. 138: 741Ð756.

Rokas, A., R. J. Atkinson, G. S. Brown, S. A. West, and G. N. Stone. 2002. Understanding patterns of genetic diversity in the oak gallwasp *Biorhiza pallida:* demographic history or selective sweep? Heredity. 87: 294Ð304.

Savage, H. M., V. I. Ezike, C.A.N. Nwankwo, R. Spiegel, and B. R. Miller. 1992. First record of breeding populations of *Aedes albopictus* in continental Africa: implications for arboviral transmission. J. Am. Mosq. Control Assoc. 8: 101Ð103.

Severson, D. W. 1997. RFLP analysis of insect genomes, pp. 309Ð320. *In* J. M. Crampton, C. B. Beard, and C. Louis (eds.), The molecular biology of insect disease vectors. Chapman & Hall, New York.

Shoemaker, D. D., V. Katju, and J. Jaenike. 1999. *Wolbachia* and the evolution of reproductive isolation between *Dro-sophila recens* and *Drosophila subquinaria.* Evolution. 53: 1157Ð1164.

Shroyer, D. A. 1986. *Aedes albopictus*andarboviruses:aconcise review of the literature. J. Am. Mosq. Control Assoc. 2: 424Ð428.

Sinkins, S. P., H. R. Braig, and S. L. O'Neill. 1995. *Wolbachia* superinfections and the expression of cytoplasmic incompatibility. Proc. Roy. Soc. Lond. B. 261:325Ð330.

Stevens, L., R. Giordano, and R. F. Fialho. 2001. Make-killing, nematode infections, bacteriophage infection, and virulence of cytoplasmic bacteria in the genus *Wolbachia.* Annu. Rev. Ecol. Syst. 32: 519Ð545.

Tsutsui, N. D., A. V. Suarez, D. A. Holway, and T. J. Case. 2000. Reduced genetic variation and the success of an invasive species. Proc. Nat. Acad. Sci. 97: 5948Ð5953.

Turelli, M., and A. A. Hoffmann. 1991. Rapid spread of an inherited incompatibility factor in California *Drosophila.* Nature (Lond.). 353: 440Ð442.

Turelli, M., and A. A. Hoffmann. 1995. Cytoplasmic incompatibilityin *Drosophila simulans:*dynamicsandparameter estimates from natural populations. Genetics. 140: 1319Ð 1338.

Turelli, M., A. A. Hoffmann, and S. W. McKechnie. 1992. Dynamics of cytoplasmic incompatibility and mtDNA variationinnatural *Drosophila*populations. Genetics. 132: 713Ð723.

Urbanelli, S., R. Bellini, M. Carrierri, P. Sallicandro, and G. Celli. 2000. Population structure of *Aedes albopictus* (Skuse): the mosquito which is colonizing Mediterranean countries. Heredity. 84: 331Ð337.

Ventrillon, E. 1904. Description de culicides de Madagas-car. Bull. Mus. Hist. Nat. Paris. 12: 143Ð145.

Zhou, W., F. Rousset, and S. O'Neill. 1998. Phylogeny and PCR-based classiÞcation of *Wolbachia* strains using *wsp* gene sequences. Proc. Roy. Soc. Lond. B. 265: 509Ð519.

Received for publication 25 November 2002; accepted 20 January 2003.