Aedes albopictus in North America: Probable Introduction in Used Tires from Northern Asia

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North American strains of *Aedes albopictus*, an Asian mosquito recently introduced into the Western Hemisphere, exhibit photoperiodic sensitivity and cold-hardiness characteristics similar to strains originating from temperate zone Asia. Trade statistics for used tire imports, the most likely mode of introduction, also indicate a north Asian origin. *Aedes albopictus*, an important vector of dengue and a potential vector of many other arboviral diseases, may therefore have the capability of infesting much of temperate North America.

THE MOSQUITO, *Aedes albopictus* (Skuse), an important disease vector native to Asia, has recently become established in the Western Hemisphere. In 1985, it was identified as the most abundant artificial container–breeding mosquito in Houston, Texas (*1*). Subsequent searches have revealed infestations in 11 other states in the United States, and in 3 states in Brazil (*2*). In Asia, *A. albopictus* is abundant in both tropical and temperate regions, extending northward to Beijing, China, and Honshu Island, Japan (*3*). If *A. albopictus* from tropical and temperate zones differ in their overwintering capability, then the origin of populations introduced into North America may determine the limits of its northward expansion.

We hypothesize that North American *A. albopictus* originates from northern Asia and not from the tropics. Strains collected in China, Korea, and Japan, like strains from Texas, Louisiana, Florida, Tennessee, and Indiana, are sensitive to photoperiod, whereas strains from tropical Asia, Mauritius, Madagascar, and northern Taiwan are not. In addition, five North American *A. albopictus* strains, like two Japanese strains, have significantly greater cold-hardiness than three tropical strains. Finally, used tires are imported in larger numbers from northern Asia than from tropical Asia. Used tires are the primary breeding site of North American *A. albopictus* (*2*) and are the most likely means of its introduction to the United States.

Though hibernal dormancy occurs in the egg stage of temperate zone *A. albopictus*, the photoperiodically sensitive stages are the pupae and adults (*4*). We tested 17 geographic strains for egg diapause after exposing insects to short or long photoperiod. Adults were fed on human or mouse blood, held for 4 to 5 days, and then provided with an oviposition substrate. Eggs were held for 6 to 8 days to allow completion of embryonation; percentage hatch was determined after flooding with deoxygenated water for 24 hours. Unhatched eggs were cleared with bleach to confirm embryonation (*5*).

Results (Fig. 1) show a clear photoperiodic effect on the North American and northern Asian strains but no such effect on any strain of tropical or subtropical origin. There are no known examples of a tropical arthropod having evolved de novo a photoperiodic mechanism after introduction to temperate latitudes (*6*). Since North American *A. albopictus* is a very recent introduction (*1*), we conclude that it originated from a population with a well-defined photoperiodic response, somewhere in northern Asia (*7*).

This conclusion is supported by experimental testing of cold-hardiness in *A. albopictus* eggs. Eggs of the closely related tropical species *A. aegypti* were tested for comparison. Embryonated eggs laid by adults that had been exposed to a long-day photoperiod (*8*) were cold-conditioned at 5°C for 2 weeks before a 24-hour exposure to −10°C. A set of control eggs was cold-conditioned but not frozen. After thawing, egg strips were flooded with deoxygenated water at 25°C for 24 hours. Hatched larvae were counted. The egg strips were blotted to remove excess water, stored at 25°C, relative humidity 85% for 1 to 7 days, reflooded, then similarly stored and reflooded up to eight times until no hatching was observed; the remaining unhatched embryonated eggs (determined by bleaching) were considered to be dead.

The percentage mortality due to exposure to subfreezing temperatures for the five North American strains and two Japanese strains was in no case greater than 22% (Fig. 2). *Aedes aegypti* and two *A. albopictus* strains from tropical Malaysia exhibited nearly 100% mortality, whereas a subtropical strain from Taiwan showed intermediate mortality. North American *A. albopictus*, therefore, have a degree of cold-hardiness similar to temperate zone Asian strains of this species.

Tires stored outdoors collect rainwater and are frequently infested with mosquitoes, particularly container-breeding species such as *A. albopictus* (*9*). The danger of importing exotic mosquitoes in shipments of used tires was first recognized at the end of World War II, when military equipment was returned to the United States from Asia, and there are several reports of the importation of *A. albopictus* in such cargoes (*10*). In

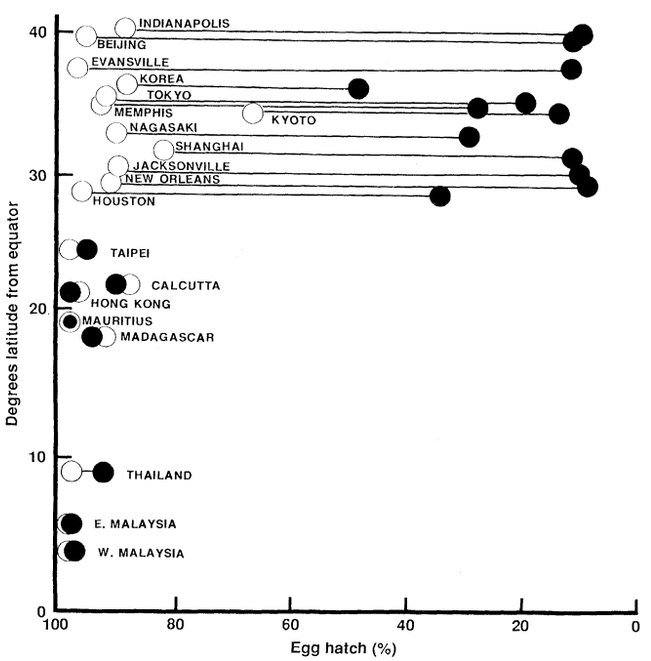

Fig. 1. Photoperiodic response of 20 geographic strains of *A. albopictus* in relation to latitude of origin. Each pair of symbols depicts the percentage hatch of eggs when sensitive stages were exposed to long (○) or short (●) days (*20*). The average number of eggs tested per population was 1600. The North American strains and East Malaysian strain had been colonized in the laboratory for two generations or less when tested; all other strains had been colonized for at least 20 generations. Results for populations from Shanghai, Nagasaki, and Kyoto are taken from the literature (*4*). The precise latitude of origin for strains from Beijing, Korea, and West Malaysia is unknown.

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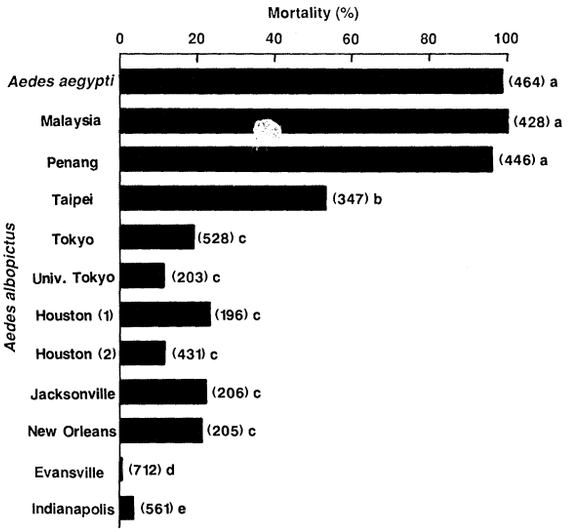

Fig. 2. Effect of a 24-hour exposure to −10°C on egg survival of ten strains of *A. albopictus* and the ROCK strain of *A. aegypti*. The North American and Penang, Malaysia, strains had been colonized in the laboratory for less than 2 generations when tested; all other populations had been kept in the laboratory for at least 20 generations. The ROCK strain of *A. aegypti* is from the Rockefeller Institute and has been kept in colony for at least 50 years. Houston (1) and Houston (2) denote separate experiments performed on the same population. Water for freezing was collected from abandoned tires, strained through a sieve (120-μm mesh) and stored at 4°C. Egg strips that had been cold-conditioned at 5°C for 2 weeks were dropped into tire water contained in untreated lambskin condoms and placed in a freezer. A thermister in the water of one of the condoms enabled us to record temperatures. When the temperature reached −2°C, the condoms and their frozen contents were wrapped in plastic, placed in sealed plastic bags, and plunged into an alcohol bath preset at −2°C. Temperature was lowered 0.7°C every 10 minutes and stabilized at −10°C for 24 hours. Following the cold treatment, the ice was thawed overnight at 4°C. Egg strips were removed and acclimated at 25°C for 24 hours before multiple hatching attempts. For each frozen egg strip, the percentage hatch of a paired control strip was determined. Shown here is the percentage mortality due to exposure to freezing temperatures calculated as the difference in the percentage egg hatch between the control and treatment eggs. At least 79% of eggs hatched on every control egg strip. Sample sizes (treatment plus control) are shown to the right of each bar; for each experiment, treatments with the same letter do not differ significantly ($P > 0.01$) as determined by G tests.

recent years, used tires have become an important article of commerce, both at the national and international level (*11*). They are traded for a variety of reasons, but mainly for reuse on vehicles. Differences in legislation and law enforcement concerning permissible tire wear and varying attitudes toward the use of recaps mean that tires which are not usable in one country are acceptable in others. Foreign exchange rates are another major factor in determining the origin and quantity of used tires imported from different countries. In the period 1970 to 1985, the United States imported 15.2 million used tires. Countries where *A. albopictus* is indigenous have supplied an increasingly dominant portion of this trade (Fig. 3), with a major portion originating in northern nations (*12*), where the species exhibits photoperiodic sensitivity and cold tolerance.

It is by no means certain that the present widespread distribution of *A. albopictus* in North America resulted from a single introduction, but the high proportion of used tires imported from temperate zone Asia and the biological characteristics of North American *A. albopictus* argue persuasively for at least one introduction of this mosquito from northern, rather than subtropical or tropical Asia.

Aedes albopictus is an efficient vector of dengue and other human viruses (*13*) and is susceptible to infection to a wide range of other arthropod-borne viruses, including yellow fever, Japanese and St. Louis encephalitis, and several California group Bunyaviruses (*14, 15*). Its aggressive man-biting habit (*16*) and its ability to colonize both treeholes in the forest habitat and man-made containers in the peridomestic environment (*10*), coupled with efficient transovarial virus transmission (*15, 17*), indicate that it has the potential to serve as both a maintenance and an epidemic vector of many arthropod-borne viruses in the Western Hemisphere. By September 1986, the range of *A. albopictus* had extended as far as 40°N in the United States (*18*). Our results imply that this northward expansion is not a transient phenomenon, but the beginning of the permanent establishment of *Aedes albopictus* at these latitudes. Climatic comparison of northern Asia and North America shows that *A. albopictus* may be capable of infesting most midwestern and eastern states (*19*).

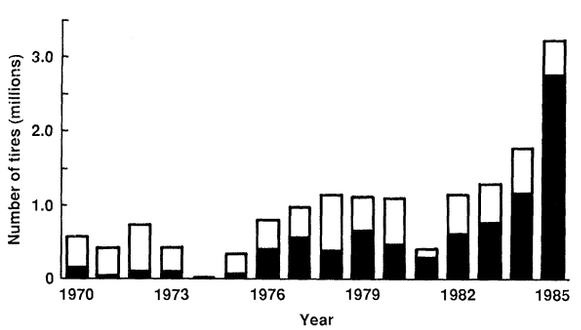

Fig. 3. Yearly imports of used tires into the United States (1970–1985). Shaded areas show imports from Asian countries where *A. albopictus* is indigenous (*12*).

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7. In contrast, a strain of *A. albopictus* from Espirito Santo State, Brazil, shows no evidence of photoperiodicity.
8. Diapause eggs were not tested because a 3- to 4-month period is required to break diapause. Our initial results (W. A. Hawley *et al.*, in preparation) indicate, however, that diapause adds little, if any, to the ability of *A. albopictus* eggs to survive this experimental treatment.
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20. Test conditions: pupae and adults exposed to 25° ± 1°C, light to dark cycle (L:D) of 18 to 6 hours (long day) or L:D 9:15 (short day) for strains from West Malaysia; East Malaysia; Mauritius; Hong Kong; Houston, TX; New Orleans, LA; Korea; Memphis, TN; and Tokyo or larvae, pupae, and adults exposed to 22° ± 1°C, L:D 16:8 (long day) or L:D 8:16 (short day) for strains from

Thailand; Madagascar, Calcutta, India; Taipei; Jacksonville, FL; Evansville, IN; Indianapolis, IN; and Beijing, China.
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Expression and Properties of Two Types of Protein Kinase C: Alternative Splicing from a Single Gene

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Two complementary DNA's, encoding the complete sequences of 671 and 673 amino acids for subspecies of rat brain protein kinase C, were expressed in COS 7 cells. The complementary DNA sequence analysis predicted that the two enzymes are derived from different ways of splicing and differ from each other only in the short ranges of their carboxyl-terminal regions. Both enzymes showed typical characteristics of protein kinase C that responded to Ca^{2+}, phospholipid, and diacylglycerol. The enzymes showed practically identical physical and kinetic properties and were indistinguishable from one of the several subspecies of protein kinase C that occurs in rat brain but not in untransfected COS 7 cells. Partial analysis of the genomic structure confirmed that these two subspecies of protein kinase C resulted indeed from alternative splicing of a single gene.

PROTEIN KINASE C (PKC) HAS AN important role in cell surface signal transduction, and it modulates many Ca^{2+}-mediated physiological and pathological processes (*1*). The enzyme was initially thought to be a single entity, but analysis of its complementary DNA (cDNA) clones indicates that PKC is a complex family of closely related structures (*2–7*). Comparison of the predicted amino acid sequences reveals that at least four subspecies of the enzyme may be present in mammalian tissues, particularly in the brain. Although several laboratory groups have isolated the cDNA clones from the libraries prepared from the brains of different mammals including bovine (*2, 3*), human (*3*), rat (*4–6*), and rabbit (*7*), the reported sequences are nearly identical. These cloning experiments have been carried out independently, and thus different, sometimes contradictory, nomenclatures are adopted (Table 1). The three subspecies, α, β, and γ, designated by Coussens *et al.* (*3*) are shown to be encoded on distinct chromosomes, whereas the two subspecies, type I and type II, designated in our earlier report (*4*) are derived from alternative splicing of a single gene, as judged by the analysis of unspliced molecules. Therefore, we use the nomenclatures of α, βI and βII, and γ for the subspecies of PKC as indicated in Table 1.

The βI and βII cDNA's from both rat and rabbit brains encode 671 and 673 amino acid sequences of PKC subspecies, respectively; these species differ from each other only in the carboxyl terminal regions of approximately 50 amino acid residues (*4, 7*). We now report the expression of these cDNA's in COS 7 cells and some of the properties of these enzymes. Huang and his co-workers (*8*) have shown by column chromatography that rat brain PKC may be resolved into apparently three fractions. However, the correspondence of each fraction to the subspecies of PKC predicted by cDNA analyses remains to be explored. The two protein kinases isolated from the COS cells transfected by the βI and βII cDNA's were practically indistinguishable from each other in their physical and kinetic properties, and both appeared to correspond to one of the PKC subspecies present predominantly in brain tissues but not in untransfected COS cells.

The expression plasmids of PKC were constructed as shown in Fig. 1. The cDNA inserts of pTB652 and pTB653 were prepared from the βI and βII cDNA clones,

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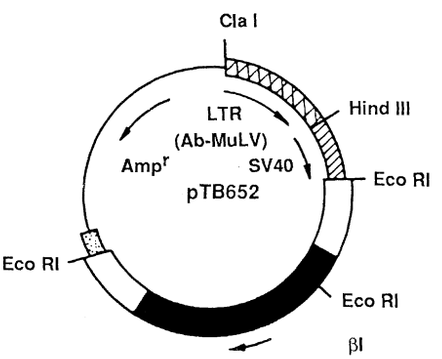

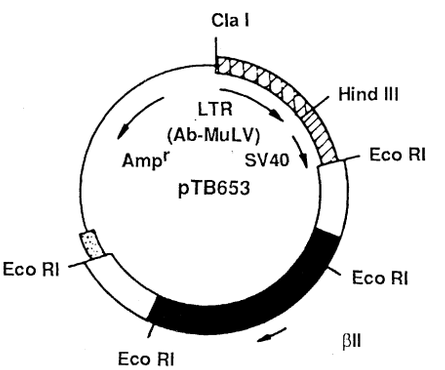

Fig. 1. The structures of two expression plasmids of PKC. The expression plasmid pTB389, containing a single cloning site of Eco RI downstream from the SV40 early promoter and late region introns, was constructed from Okayama and Berg vectors (*14*). The plasmid is essentially similar to pcDL1 (*15*). The Eco RI–Eco RI cDNA fragment (*4*) composed of λCKR152 (nucleotides 1 to 924) and λCKR108 (nucleotides 925 to 3190) of βI cDNA, and that composed of λCKR152 (nucleotides 1 to 924) and λCKR107 (nucleotides 925 to 3406) of βII cDNA were inserted into Eco RI site of pTB389. These cDNA inserts each contained a 5′-noncoding region of 684 nucleotides, the complete coding sequence of the βI or βII cDNA, and a 3′-noncoding region. To enhance promoter activity, we used the Cla I–Hind III fragment [(1.1 kb), which was derived from the original Cla I–Pst I fragment, and the Pst I site was effectively modified by the addition of the Hind III linker] of the Abelson murine leukemia virus (Ab-MuLV) long terminal repeat (LTR) (*16*) and inserted it upstream of the SV40 early promoter region. The resulting expression plasmids are referred to as pTB652 [(**left**) for βI type] and pTB653 [(**right**) for βII type], respectively. The plasmids pTB707 and pTB708 differed from pTB652 and pTB653, respectively, only in the length of the 5′-noncoding region (55 nucleotides). The pBR322 origin of DNA replication and the β-lactamase gene (Amp^r, ampicillin resistant) segments are represented by the solid lines. The LTR's of Ab-MuLV are the crosshatched boxes. The SV40 origin of DNA replication and the SV40 early promoter regions are indicated by hatched boxes. The 5′- and 3′-noncoding regions of PKC cDNA's are indicated by wide open boxes and the coding regions are indicated by wide solid boxes. The stippled boxes contain the polyadenylational signals. Not all the sites for a particular enzyme are shown.