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Published

2010

Journal Title

Environmental Entomology

DOI

10.1603/EN07037

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Difference in Mosquito Species (Diptera: Culicidae) and the Transmission of Ross River Virus Between Coastline and Inland Areas in Brisbane, Australia

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Environ. Entomol. 36(6): 000–000 (2007)

ABSTRACT This study examined the distribution of major mosquito species and their roles in the transmission of Ross River virus (RRV) infection for coastline and inland areas in Brisbane, Australia (27°28′ S, 153°2′ E). We obtained data on the monthly counts of RRV cases in Brisbane between November 1998 and December 2001 by statistical local areas from the Queensland Department of Health and the monthly mosquito abundance from the Brisbane City Council. Correlation analysis was used to assess the pairwise relationships between mosquito density and the incidence of RRV disease. This study showed that the mosquito abundance of *Aedes vigilax* (Skuse), *Culex annulirostris* (Skuse), and *Aedes vittiger* (Skuse) were significantly associated with the monthly incidence of RRV in the coastline area, whereas *Aedes vigilax, Culex annulirostris,* and *Aedes notoscriptus* (Skuse) were significantly associated with the monthly incidence of RRV in the inland area. The results of the classification and regression tree (CART) analysis show that both occurrence and incidence of RRV were influenced by interactions between species in both coastal and inland regions. We found that there was an 89% chance for an occurrence of RRV if the abundance of *Ae. vigilax* was between 64 and 90 in the coastline region. There was an 80% chance for an occurrence of RRV if the density of *Cx. annulirostris* was between 53 and 74 in the inland area. The results of this study may have applications as a decision support tool in planning disease control of RRV and other mosquito-borne diseases.

KEY WORDS mosquito species, classification and regression trees, Ross River virus, coastline, inland

Ross River virus (RRV) infection is the most common vector-borne disease in Australia (Aaskov et al. 1981, Russell 2002, Gatton et al. 2004). RRV circulates enzootically in reservoir populations of marsupials in Australia (Aaskov et al. 1981, Russell 2002). Infection is asymptomatic in host animals, but although they are viremic, host animals can infect mosquitoes that feed on them. After the extrinsic incubation period, virus particles replicate to the point where the saliva of the mosquito is infective to the next nonimmune vertebrate host. If that mosquito bites a human, clinical disease may result. At least 20% of infected individuals develop an acute disease (Weinstein 1997, Harley et al. 2001, Russell 2002). Increased mosquito density enhances the opportunities for transmitting RRV between human and animals.

RRV has been isolated from many mosquito species, indicating wide susceptibility among mosquitoes (Russell 2002). *Culex annulirostris* (Skuse), which breeds in freshwater habitats, is a major RRV vector in inland regions, especially in irrigated areas (Kay 1979, Russell 1994, Dale and Morris 1996). Saltmarsh mosquitoes such as *Aedes vigilax* (Skuse) in Queensland represent the major threat along coastal regions (Dale et al. 1986, Mackenzie et al. 1994, Russell 1994, 1998). There is laboratory evidence that *Aedes notoscriptus* (Skuse) may also be a vector in the domestic urban situation (Ritchie et al. 1997, Watson and Kay 1998). However, their roles in the transmission of RRV in urban areas remain to be determined (Ritchie et al. 1997, Ryan et al. 1999, 2000).

Ecological data are often nonlinear and involve high-order interactions. The commonly used exploratory and statistical modeling techniques often fail to find meaningful ecological patterns from such data (De'ath and Fabricius 2000). Classification and regression tree (CART) analysis has been widely used as a powerful nonparametric approach. The classification trees aims to predict or explain response on a categorical-dependent variable. The regression tree aims to predict or explain response on a continuous-dependent variable (Breiman et al. 1984). This does not require the existence of linear relationships among the variables and has the ability to efficiently segment populations into homogenous subgroups. The method

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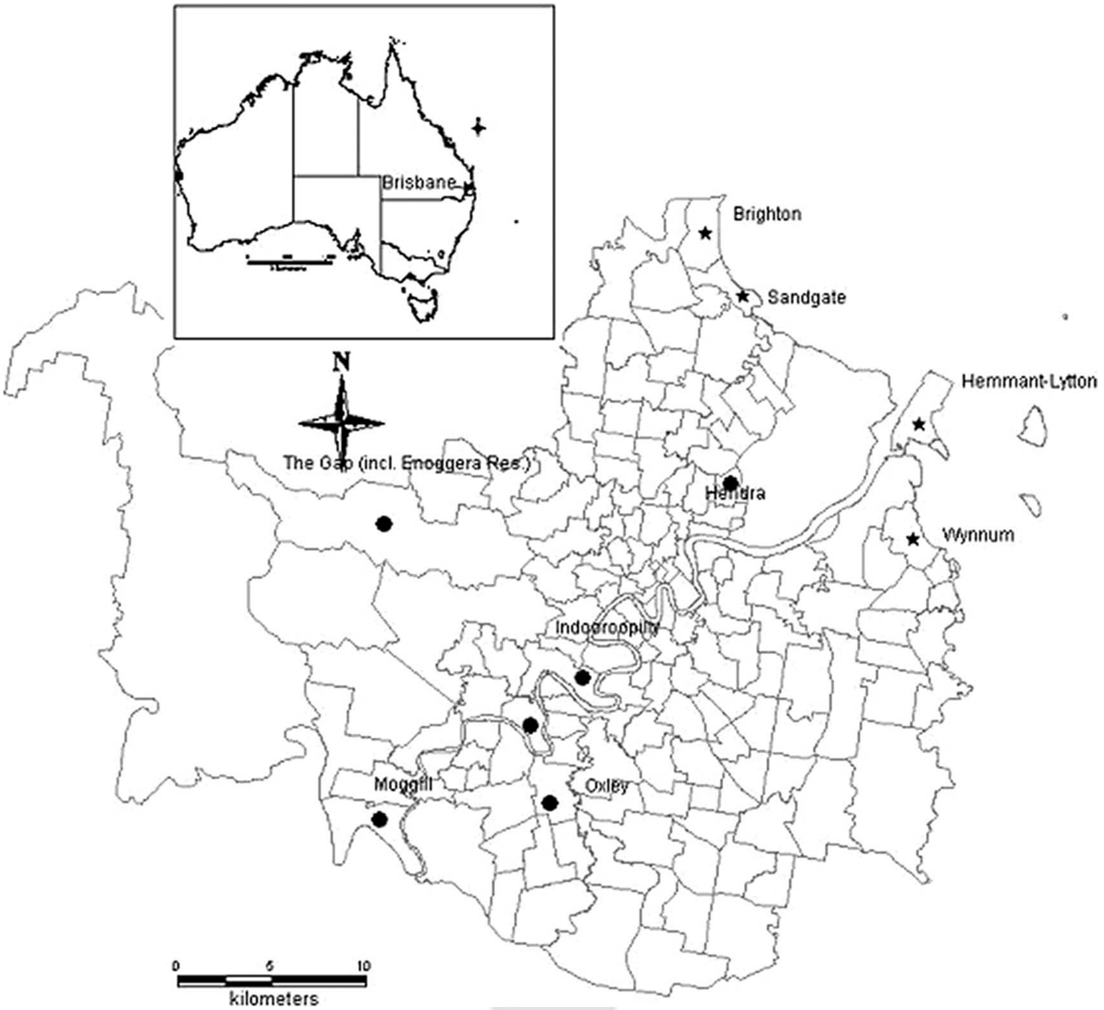


Fig. 1. Location of Brisbane, Australia, and 10 mosquito monitor stations in Brisbane (coastal locations marked with a star; inland ones with a filled circle).

has a strong capacity to disentangle the complex relations between variables, and therefore is a powerful statistical techniques ideally suited for both exploring and modeling such data, but have seldom been used in ecology (Breiman et al. 1984, Staub et al. 1992, Rejwan et al. 1999, De'ath and Fabricius 2000).

This study examined the distribution of mosquito species and their roles in RRV transmission between the coastline and inland areas in Brisbane, Australia.

Materials and Methods

Study Area

Brisbane (27°28′ S, 153°2′ E) is the third largest city in Australia after Sydney and Melbourne (Fig. 1). F1 Within the administrative boundaries of Brisbane City Council, which also determine the study area of this investigation, the population of Brisbane city was estimated at 883,449 on June 2001 (Australian Bureau of Statistics 2007). Brisbane consists of 163 statistical local areas (SLAs), which are the smallest special unit used for data aggregation by the Australian Bureau of Statistics. The SLA is based on the boundaries of incorporated bodies of each local government.

Data Collection

The monthly counts of the notified RRV cases in Brisbane by SLAs between 1998 and 2001 were obtained from the Queensland Department of Health. Monthly incidences of RRV disease of each of 10 SLAs with a mosquito collection station were calculated and were used as a response variable. The requirement for notification of RRV disease is based on demonstration of antibodies in blood sample during the acute and convalescent phases of the illness. A four-fold rise in antibody levels would confirm the clinical diagnosis. Data on the monthly mosquito density were obtained from the Brisbane City Council. Population data were supplied by the Australian Bureau of Statistics. Mosquito collections were made between November 1998 and December 2001 using standard Centers for Disease Control (CDC) light traps, with carbon dioxide and octenol, over one night, one to four times each month (Hu et al. 2006). The 10 mosquito monitoring stations were distributed in coastal, riparian, and inland habitats in Brisbane as shown in Fig. 1. There was no significant difference in trapping locations (height, nearby breeding areas, and vegetation) between coastal and inland areas. Light traps supplemented with carbon dioxide were set overnight in the backyards of houses in 10 SLAs of Brisbane. Monthly average mosquito densities at lag 1 and lag 2 mo were calculated and used as an independent variable. A time lag was defined as the time span between recorded date of mosquito density and the reported incidence of RRV (Chatfield 1975). In this study, monthly time scales were used, beause weekly or finer time scales for mosquito density data were not available.

Data Analysis

Correlations analysis adjusted for seasonality was used to assess the relationship between mosquito density and the incidence of RRV disease over moving averages at lags of 1 and 2 mo.

Classification and regression tree models were developed to predict the different vector competence of mosquito species for RRV disease between coastline and inland areas in Brisbane, Australia. The CART analysis consisted of three basic steps. The first step is built by means of recursive splitting of nodes. In each node, a predicted class is assigned whether or not that node is subsequently split into child nodes. The second step consists of stopping the tree-building process. A full tree has been produced, which probably greatly overfits the information contained. The resulting full tree is pruned, which results in the creation of a sequence of simpler trees, through the cutting of increasingly important nodes. Finally, optimal tree selections of the resulting simpler trees are obtained (Breiman et al. 1984, De'ath and Fabricius 2000, Hu et al. 2006). In our study, the incidence of RRV was used as a categorical (i.e., presence/absence) or numeric (i.e., morbidity) variable. The CART was constructed by repeatedly splitting the data on mosquito species density. At each split, the data were partitioned into two mutually exclusive groups, each of which was as homogeneous as possible. The groups were based on the abundance of mosquito species that were believed to be important for the RRV incidence. The splitting procedure was applied to each group separately. Splitting was continued until an overlarge tree was grown, which was pruned back to the desired size. Each group was characterized by either the presence/absence or incidence rate of RRV disease, group size, and the values of mosquito species abundance that define it. Trees are usually represented graphically, with the root node, which represents the undivided data, at the top, and the branches and leaves (each leaf represents one of the final groups) beneath. The CART analyses were performed using S-plus software (S-Plus Insightful 2003).

Results

Distribution of Major Mosquito Species Between Coastline and Inland Areas

There were at least 14 mosquito species collected for each monitoring station. Figure 2 provides descriptive information about mosquito abundance by species between coastline and inland areas. The four most common coastline mosquito species were *Aedes vigilax (39%)*, *Culex annulirostris* (31%), *Coquillettidia linealis* (*Skuse*) (23%), and *Aedes notoscriplus (2%)*, whereas inland, the four most common species were *Culex annulirostris* (58%), *Aedes vigilax (25%)*, *Aedess procax* (*Skuse*), (7%), and *Aedes notoscriplus* (4%).

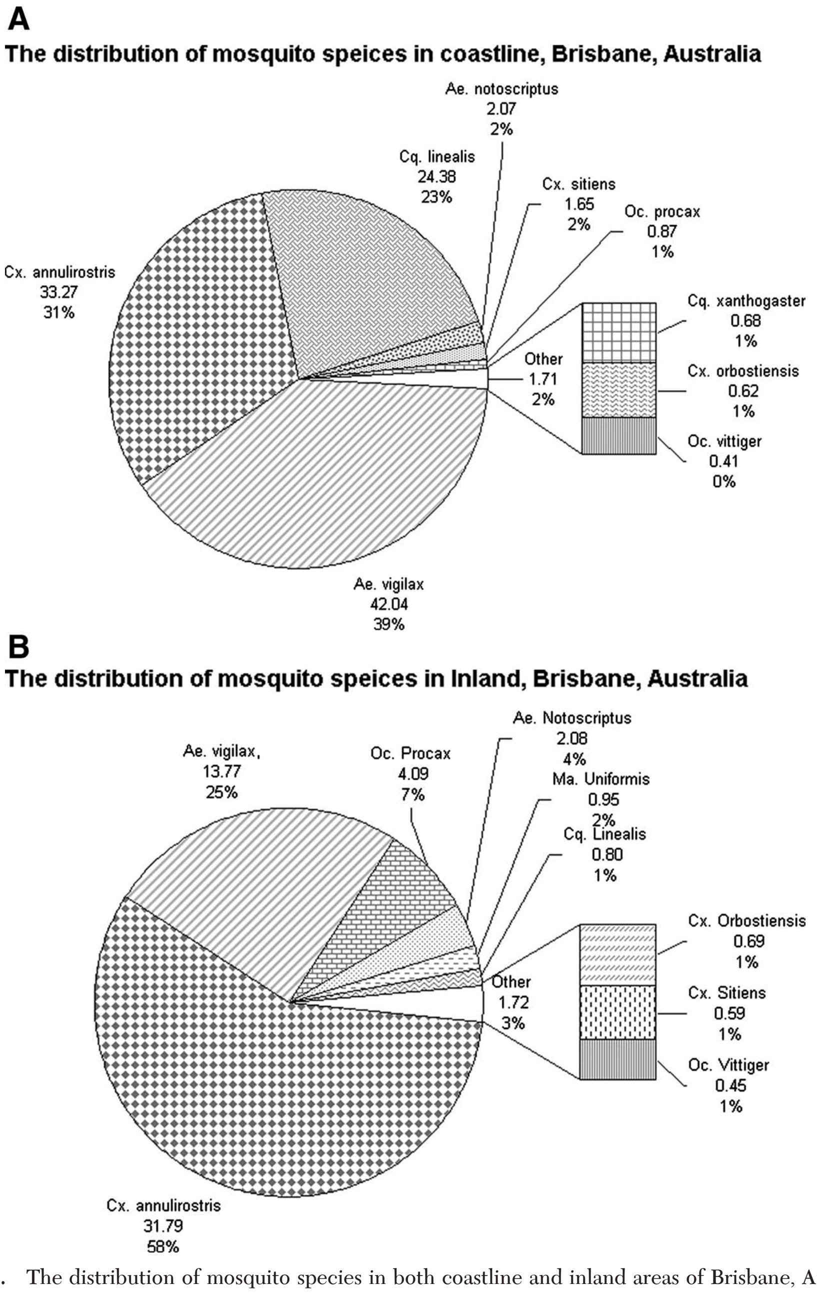
Correlation Analyses

Figure 3 shows the different response of RRV to relative abundances of mosquito species between coastline and inland areas in Brisbane, Australia. In general, there were quadratic relationships between mosquito density and the incidence rate of RRV. These relations seemed to be stronger in the coastline area than the inland area.

The results of the correlations analyses showed that the densities of most mosquito species were statistically significantly related to each other in both the coastline and inland areas. *Ae. vigilax* is statistically significantly associated with *Ae. vittiger* ($r = 0.525$) and *Cx. annulirostris* ($r = 0.597$) in the coastline area. There is also a statistically significant association between *Ae. vittiger* and *Cx. annulirostris* ($r = 0.614$) in the inland area (Table 1). The monthly incidence rates of RRV were significantly associated with *Ae. vigilax* ($r = 0.245$), *Cx. annulirostris* ($r = 0.261$), and *Ae. vittiger* ($r = 0.198$) in the coastline area and with *Ae. vigilax* ($r = 0.214$), *Cx. annulirostris* ($r = 0.261$), *Co. xanthogaster* (Edwards) ($r = 0.281$), and *Cx. sitiens* (Wiedemann) ($r = 0.174$) in the inland area (Table 1). These correlations do not indicate the total strength of the respective associations, because they only reflect the linear component of the quadratic relationships shown in Fig. 3.

CARTs

Presence/Absence Analysis. The results of CARTs analyses showed that a combination of a small number of species provided the best classification of presence of RRV and that, as expected, these species differed between coastline and inland areas (Fig. 4). In the coastline area, the most important species was *Ae. vigilax*, with *Ae. vittiger* becoming important when the abundance of *Ae. vigilax* was low. In the inland area, *Cx. annulirostris* was the most important species, but *Ae. vigilax* became important at low levels of *Cx. annulirostric.* Moreover, for both coastline and inland areas, the probability of presence of RRV was non-



AQ: 1 **Fig. 2.** The distribution of mosquito species in both coastline and inland areas of Brisbane, Australia.

linearly associated with the density of the primary species.

Figure 4 also shows the results of CART analyses of the presence/absence of RRV. The results show that there was 89% chance of an occurrence of RRV if the density of *Ae. vigilax* was moderately high between 64 and 90 in the coastline area. Similarly, there was an 80% chance of an occurrence of RRV if the density of *Cx. annulirostris* was moderately high between 53 and 74 in the inland area (Fig. 4). However, relatively high occurrences of RRV (50%) were also identified in the coastline area for lower levels of *Ae. vigilax* if *Ae. vittiger* was also present and in the inland area (67%) when there were high levels of both *Ae. annulirostris* and *Ae. notoscriptus.*

RRV Incidence Rate Analysis. Figure 5 shows the relationship between the monthly incidence of RRV and the density of mosquito species. The CARTs indicate the RRV incidence was most strongly associated with *Ae. vigilax* density in both the coastline and inland areas. However, in both areas, *Ae. vigilax* interacted with other species in affecting RRV incidence. A combination of high, but not extreme, *Ae. vigilax* and high *Cx. annulirostris* resulted in the high- F5

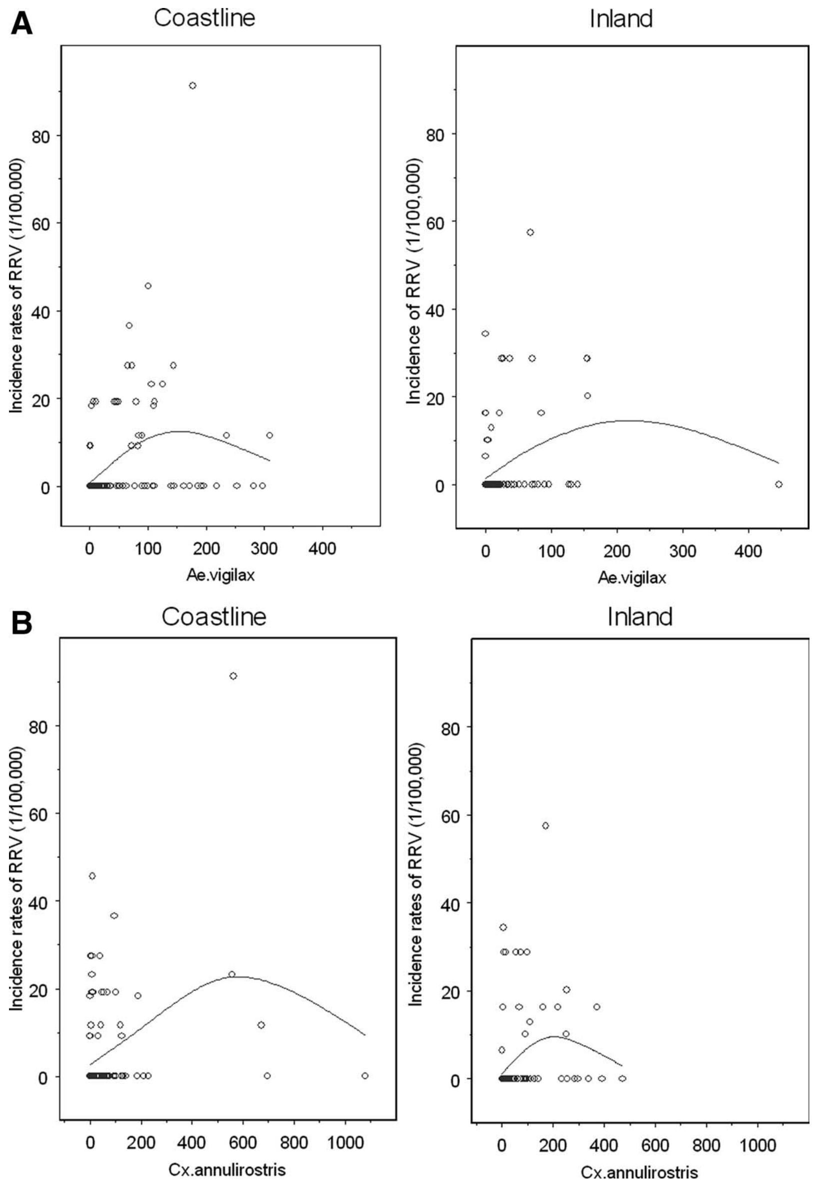


Fig. 3. The incidence of RRV disease in relation to the abundance of two commonly occurring mosquito species in coastline and inland areas of Brisbane, Australia.

est incidence (23 cases per 100,000 residences) in the coastline area. For the inland area, high densities of both *Ae. vigilax* and *Cx. sitiens* led to the highest incidence (22 cases per 100,000 residences).

Discussion

This study has identified some key mosquito species that played a significant role in the transmission of RRV in Brisbane, Australia. However, the relative importance of these mosquito species in the transmission of RRV varied with geographic area. There appeared to be different roles in the transmission of RRV by mosquito species between coastline and inland areas. Moreover, there were quite complex interactions between mosquito species and density that influenced the incidence of the disease.

Aedes vigilax is the major mosquito species in Brisbane. It breeds in the salt marshes and swamps. Tides are the major determinant of activity and abundance of *Ae. vigilax* (Dale et al. 1986, Russell 1994). Adults are most abundant in summer months and are active from mid-spring through autumn (Lee et al. 1984, Russell 1994, 1998, 2002). It is one of the most important carriers of Ross River and Barmah Forest viruses in coastal Australia and can also carry dog heartworm (Russell 2002).

Table 1. Correlation analysis between the density of mosquito species and RRV disease for both coastline (top value) and inland (bottom value, italics) areas

	Incidence rates	*Ae. vigilax*	*Ae. vittig*	*Ae. noto-*	*Ae. proc-*	*Cq. lin-*	*Cq. xan-*	*Cx. annu-*	*Cx. austr-*	*Cx. quin-*	*Cx. sitie-*	*M. a un-*	*Cx. orb-*	*An. ann-*
Ae. vigilax	0.245 *0.214*													
Ae. vittiger	**0.198** *0.043*	**0.525** *0.316*												
Ae. notoscriptus	0.154 *0.191*	**0.326** *0.007*	**0.385** *−0.139*											
Ae. procax	−0.038 *0.053*	**0.364** *0.231*	**0.29** *0.201*	0.121 *0*										
Cq. linealis	−0.055 *−0.026*	**0.51** *0.735*	0.05 *0.203*	**0.257** *0.027*	0.004 *0.011*									
Cq. xanthogaster	0.053 *0.281*	**0.445** *0.161*	**0.335** *0.019*	0.163 *0.499*	**0.246** *0.106*	**0.444** *0.253*								
Cx. annulirostris	**0.261** *0.201*	**0.597** *0.267*	**0.487** *0.614*	0.143 *0.172*	**0.332** *0.314*	**0.235** *0.06*	**0.716** *0.292*							
Cx. australicus	−0.072 *−0.145*	−0.014 *−0.172*	−0.007 *−0.141*	−0.051 *−0.003*	−0.019 *−0.0151*	0.146 *−0.042*	0.079 *−0.089*	0.058 *−0.161*						
Cx. quinquefasciatus	−0.02 *0.102*	−0.027 *0.062*	−0.021 *0.263*	−0.039 *−0.149*	−0.009 *0.098*	−0.011 *−0.042*	−0.021 *−0.025*	−0.024 *0.127*	−0.041 *−0.112*					
Cx. sitiens	0.07 *0.174*	**0.215** *−0.017*	**0.343** *−0.119*	**0.512** *0.423*	**0.175** *−0.132*	−0.005 *0.005*	**0.284** *0.062*	**0.344** *−0.09*	0.09 *−0.024*	−0.035 *−0.082*				
Ma. uniformis	−0.032 *−0.12*	**0.198** *−0.069*	−0.045 *0.211*	0.023 *0.294*	0.063 *0.248*	**0.32** *−0.039*	**0.194** *0.326*	0.281 *0.204*	**0.202** *−0.06*	−0.004 *0.046*	**0.234** *−0.16*			
Cx. orbostiensis	0.058 *−0.081*	**0.383** *−0.043*	**0.387** *−0.053*	0.098 *0.51*	**0.23** *0.079*	**0.34** *0.09*	0.885 *0.231*	**0.682** *0.012*	0.025 *0.035*	−0.026 *−0.053*	**0.209** *−0.012*	0.114 *0.404*		
An. annulipes	0.004 *−0.026*	−0.055 *0.249*	−0.076 *0.038*	−0.145 *−0.067*	−0.053 *0.369*	−0.062 *0.073*	0.028 *0.032*	0.058 *0.146*	**0.284** *0.087*	0.013 *−0.069*	0.373 *−0.12*	0.404 *0.048*	−0.037 *0.151*	
An. atratipes	−0.027 *0.004*	−0.059 *−0.043*	−0.076 *0.433*	−0.101 *0.131*	−0.043 *0.058*	0.097 *−0.024*	−0.003 *0.274*	0.009 *0.389*	**0.192** *−0.062*	0.003 *0.534*	−0.023 *−0.066*	−0.014 *0.211*	0 *0.157*	0.067 *0.038*

Coefficients that are statistically significantly different from zero at the 5% level are in bold. The bold species are important species for RRV transmission.

Culex annulirostris is the most common and widespread mosquito species across Australia. *Cx. annulirostris* larval habitats include almost any type of surface water, either temporary or permanent, fresh or brackish, clean or polluted, and sunlit or shaded (Lee et al. 1989, Dale et al. 1996). There are indications that adult populations of *Cx. annulirostris* are distributed widely throughout Brisbane (Ritchie et al. 1997). It is most commonly found in freshwater wetlands. Its numbers can increase rapidly after flooding rains or heavy rains. It is a broad spectrum mammal feeder, which will also feed on birds. It is the most important carrier of RRV in inland Australia and is now recognized as an important RRV carrier in Brisbane (Hu et al. 2006).

Aedes vittiger, a large pale colored mosquito, can occur in waves after rain fills shallow grassy depressions where the eggs are laid and hatch after flooding. It is a persistent mosquito that will bite through clothes (Muller 2004).

Aedes notoscriptus is the most frequently encountered species around houses across Brisbane. It occurs in an unusually wide range of sizes from very small to medium. It breeds in natural and artificial containers such as tree cavities, gutters that do not drain, discarded tires and containers, bird baths, and pot plant trays (Muller 2004). Both Ross River and Barmah Forest viruses have been isolated from this species in Brisbane, and some evidence suggests that it is also capable of transmitting both viruses (Watson and Kay 1998, Muller 2004).

Culex sitiens is mainly distributed in coastal areas where it breeds in brackish tidal pools, especially after salinity is diluted by rainfall. The major breeding sites of the *Cx. sitiens* are the same as *Ae. vigilax. Cx. sitiens* are known to bite avidly in some situations, but in general, they do not disperse very far from their breeding sites (Muller 2004). *Cx. sitiens* has been circumstantially associated with virus activity in some localities and so may only play a limited role in the natural transmission of RRV (Fanning et al. 1992).

A number of studies have shown different spatial pattern of species abundances and their biological impacts (Walker 1990, Walther et al. 2002, Sweeney et al. 2007). There are differences in the ecological factors that influence species distribution through species-specific physiological thresholds of environmental factors (e.g., temperature and rainfall) (Walther et al. 2002). For example, Sweeny et al. used CART to model the malaria vectors distribution in northern Australia. They suggested that Malaria vectors overall patterns of occurrences for each species (*Anopheles farauti ss, A. farauti 2,* and *A. farauti 3*) were different and the atmospheric moisture was a critical variable for each species (Sweeney et al. 2007). Linard et al. (2007) showed that spatial variation in disease risk (i.e., Puumala virus and Lyme borreliosis infections) can be largely explained by environmental and socioeconomic factors.

Classification and regression tree models have been used to study spatial relationships between environmental predictors and species distribution (Walker 1990, Franklin 1998, Barbosa and Caldas 2007, Sweeney et al. 2007). For example, Franklin modeled the distribution of shrub species in relation to climate and terrain-derived variables in southwest of Califor-

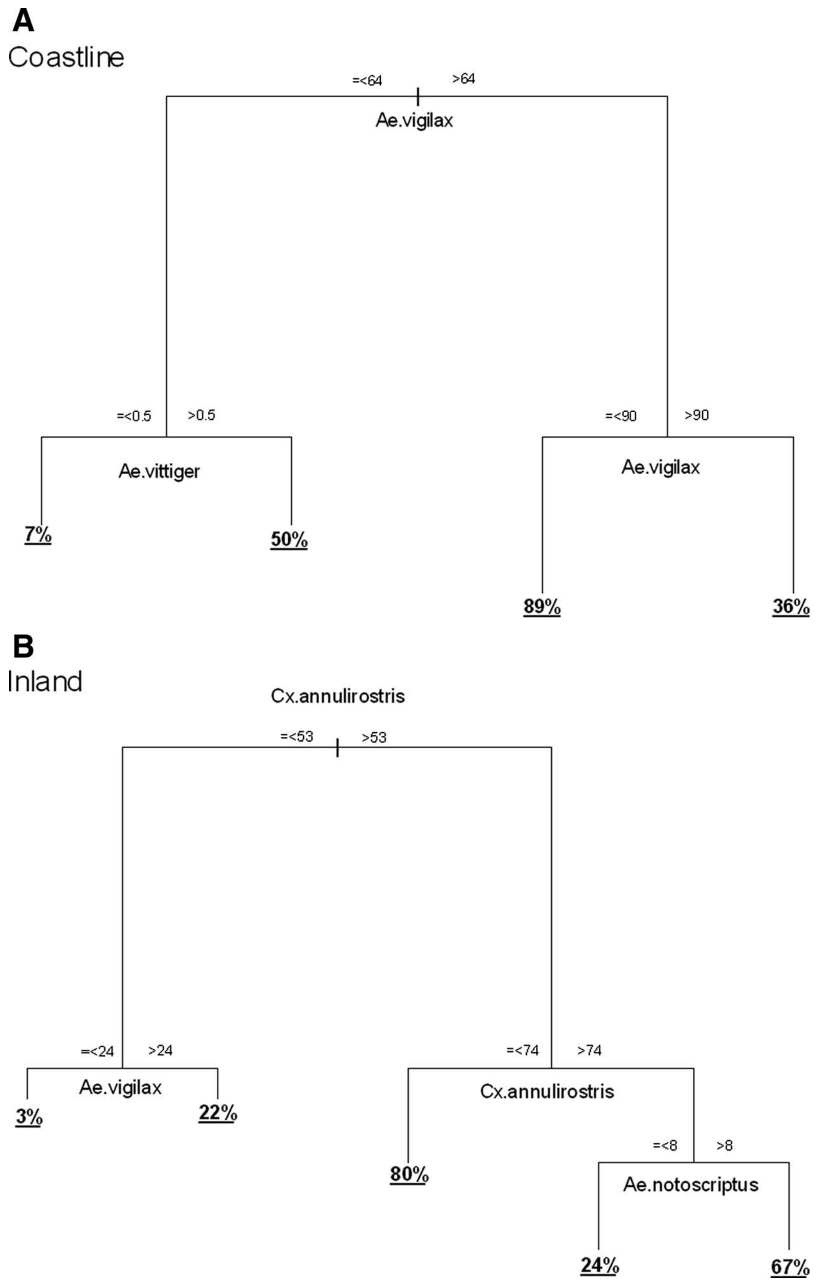


Fig. 4. The relationship between average monthly mosquito density and RRV disease (present/absent) in coastline and inland areas (numbers within branches are the mosquito species; underline numbers are the chance [%] of an occurrence of RRV).

nia using CART, a generalized linear model (GLM) and generalized additive model (GAM). They conclude that the CART model produced a similar accuracy for a given species compared with GLM and GAM but prediction errors of CART models were lower than the other two methods (Franklin 1998). The comparative advantage is partly caused by the nonparametric, robust model underlying the tree. Classification and regression tree makes binary splits of the independent variables to construct a decision tree and can rank the independent variables in descending order of their contribution to tree construction. Classification and regression tree models do not distort the model when outliers appear. The most powerful feature of CART models is that they can isolate local interactions in relationship (Breiman et al. 1984).

In this study, the CART method was used to examine the major mosquito species and their roles in

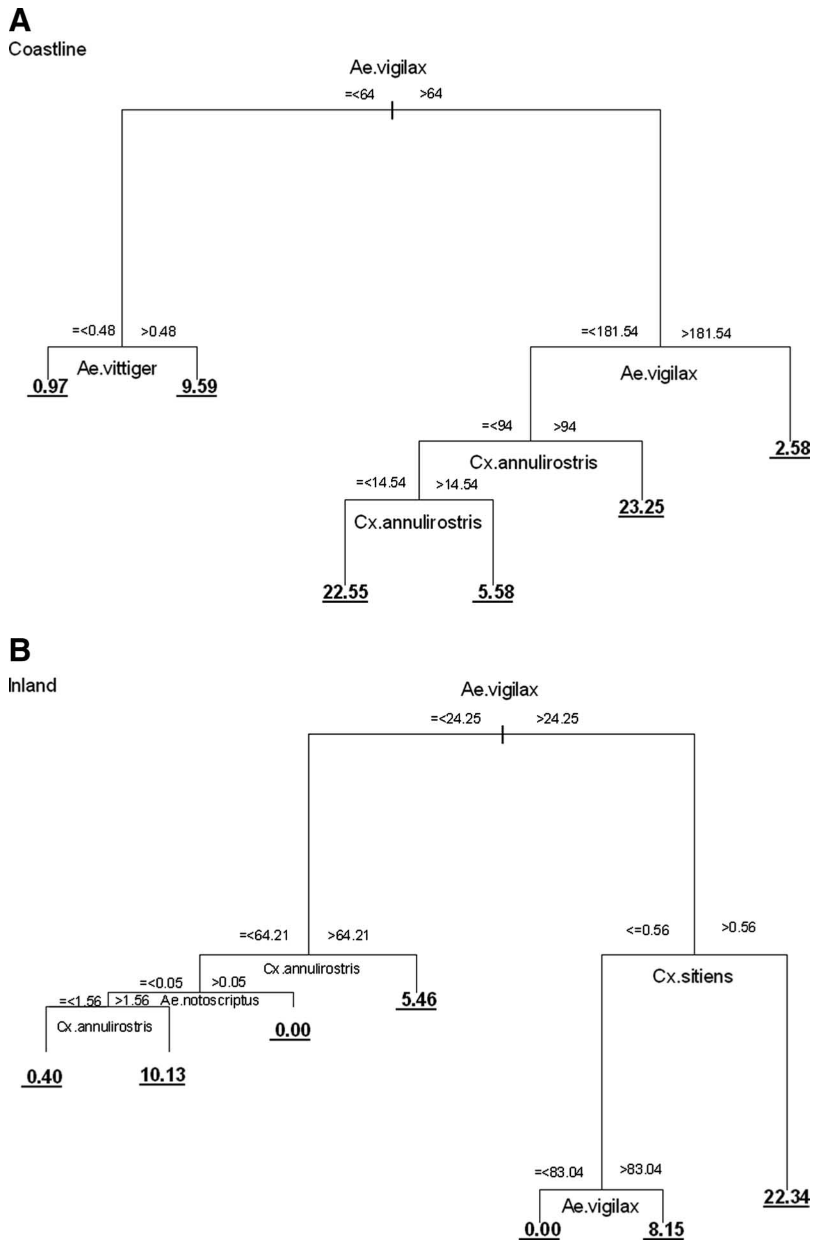


Fig. 5. The relationship between average monthly mosquito density and RRV disease (incidence rates) in coastline and inland areas (numbers above branches are the mosquito species density; underline numbers are the RRV incidence rates [1/100,000]).

determining the RRV transmission cycles in coastline and inland areas of a major city. The results of this study showed complex roles of mosquito species in the transmission of RRV across different geographic areas. The results found that in both coastline and inland areas the probability of presence of RRV was nonlinearly associated with the density of the primary mosquito species. Classification and regression trees showed that there was an 89% chance for an occurrence of RRV if the abundance of *Ae. vigilax* was between 64 and 90 in the coastline region. There was an 80% chance for an occurrence of RRV if the density of *Cx. annulirostris* was between 53 and 74 in the inland area. The results of this study may have applications as a decision support tool in planning disease control of RRV and may be useful in planning further research of RRV ecology.

The limitations of this study must also be acknowledged. Mosquito density data were only available from 10 monitoring stations, and therefore, the results of this study may not be generalizable to other low-risk areas. Additionally, difference may exist between the geographic distribution of notified RRV cases and the locations in which infections actually occur. Other factors (e.g., virus strains, human behavior, and host immunity) are also involved in the transmission cycles of RRV, but the availability of these data is limited.

In conclusion, the results of this study indicate that the density of mosquito species was an important factor in the transmission cycles of RRV, but its impact seems to differ between coastline and inland areas. *Ae. vigilax* and *Cx. annulirostris* were two major mosquito species associated with RRV transmission in Brisbane. However, in both areas, *Ae. vigilax* and *Cx. annulirostris* interacted with other species in affecting RRV incidence. Public health authorities need to pay more attention to the monitoring and control of the major vectors of RRV in Brisbane, particularly during epidemic seasons.

Acknowledgments

We thank the Queensland Department of Health, Brisbane City Council, and Australian Bureau of Statistics for providing the data on notified RRV cases, mosquito density, and population growth, respectively. The study was partly funded by the NHMRC (Project 242210) and Australian Research Council (0559655). S.T. is supported by a NHMRC research fellowship.

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Received for publication 13 February 2007; accepted 15 August 2007.