Title: The *queenslandensis* and the *type* form of the dengue fever mosquito (*Aedes aegypti* L.) are genomically indistinguishable

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Short Title: Very pale *Ae. aegypti* are not genomically separate

Abstract

Background

The mosquito *Aedes aegypti* (L.) is a major vector of viral diseases like dengue fever, Zika and chikungunya. *Aedes aegypti* exhibits high morphological and behavioral variation, some of which is thought to be of epidemiological significance. Globally distributed domestic *Ae. aegypti* have been traditionally grouped into (i) the very pale variety *queenslandensis* and (ii) the *type* form. Because the two color forms co-occur across most of their range, there is interest in understanding how

freely they interbreed. This knowledge is particularly important for control strategies that rely on mating compatibilities between the release and target mosquitoes, such as *Wolbachia* releases and SIT. To answer this question, we analyzed nuclear and mitochondrial genome-wide variation in the co-occurring pale and *type Ae. aegypti* from northern Queensland (Australia) and Singapore.

Methods/Findings

We typed 74 individuals at a 1170 bp-long mitochondrial sequence and at 16,569 nuclear SNPs using a customized double-digest RAD sequencing. 11/29 genotyped individuals from Singapore and 11/45 from Queensland were identified as *var. queenslandensis* based on the diagnostic scaling patterns. We found 24 different mitochondrial haplotypes, seven of which were shared between the two forms. Multivariate genetic clustering based on nuclear SNPs corresponded to individuals' geographic location, not their color. Several family groups consisted of both forms and three *queenslandensis* individuals were *Wolbachia* infected, indicating previous breeding with the *type* form which has been used to introduce *Wolbachia* into *Ae. aegypti* populations.

Conclusion

Aedes aegypti queenslandensis are genomically indistinguishable from the *type* form, which points to these forms freely interbreeding at least in Australia and Singapore. Based on our findings, it is unlikely that the presence of very pale *Ae. aegypti* will affect the success of *Aedes* control programs based on *Wolbachia*-infected, sterile or RIDL mosquitoes.

Key words: *Aedes aegypti* var. *queenslandensis,* population genomics, RADseq, mosquito control

Author Summary

Aedes aegypti, the most important vector of dengue and Zika, greatly varies in body color and behavior. Two domestic forms of this mosquito, the very pale *queenslandensis* and the browner *type,* are often found together in populations around the globe. Knowing how freely they interbreed is important for the control strategies such as releases of *Wolbachia* and sterile males. To answer this question, we used RAD sequencing to genotype samples of both forms collected in Singapore and northern Queensland. We did not find any association between the mitochondrial or nuclear genome-wide variation and color variation in these populations. Rather, "paleness" is likely to be a quantitative trait under some environmental influence. We also detected several *queenslandensis* individuals with the *Wolbachia* infection, indicating free interbreeding with the *type* form which has been used to introduce *Wolbachia* into *Ae. aegypti* populations. Overall, our data show that the very pale *queenslandensis* are not genomically separate, and their presence is unlikely to affect the success of *Aedes* control programs based on *Wolbachia*-infected, sterile or RIDL mosquitoes.

Introduction

The mosquito *Aedes aegypti* (Linnaeus) is the most important arboviral vector in the tropics and subtropics [1]. Diseases transmitted by *Ae. aegypti,* like dengue fever and Zika, are on the rise [2], and some are reappearing. For instance, chikungunya has returned to the American tropics in 2013, after being absent for nearly 200 years [3]. Yellow fever was nearly eliminated thanks to an effective vaccine, but is now resurging in central and south Africa [4]. Such epidemiological trends highlight the need to persist with vector control efforts, which requires a thorough understanding of vector biology.

Nearly 60 years ago, Mattingly [5] noted that despite a vast body of literature, few mosquitoes have been "*the subject of misconception....in the minds of the general run of*

66 *entomologists*" like *Aedes aegypti* [5]. The species has a plethora of historical synonyms [6], mainly
67 as a result of having extensive variation in body color and scaling patterns [5][7] which was also
68 thought to correlate with behavioral differences (e.g. [8]). These issues urged Mattingly [5] to revise
69 the taxonomy of *Ae. aegypti* and create a foundation for the modern studies of this disease vector
70 [7].

71 Mattingly [5] proposed the intraspecific classification of *Ae. aegypti* into three forms.

72 (i) A very dark form that never has pale scales on the first abdominal tergite, avoids biting
73 humans, prefers natural breeding habitats and is confined to sub-Saharan Africa.
74 Mattingly gave this form a subspecies rank, *Ae. aegypti spp*. ***formosus*** (Walker).
75 (ii) *Ae. aegypti* sensu stricto or the ***type*** form, distinctly paler and browner than *spp.*
76 *formosus,* with pale scales restricted to the head and the first abdominal tergite. This
77 form prefers to bite humans and to use artificial breeding containers, and is globally
78 distributed.
79 (iii) A very pale form, *Ae aegypti* ***queenslandensis*** Theobold), with extension of the pale
80 scaling on the thorax, tergites and legs, that co-occurs globally with the *type* form.
81 Mattingly gave this form only a varietal rank (*Ae. aegypty* var. *queenslandensis*).

82 Because of great variation in color and scaling within and among *Ae. aegypti* populations,
83 McClelland [7] suggested that subdivision into forms seems oversimplistic and should be abandoned
84 unless correlation between genetic and color variation can be demonstrated [7]. His
85 recommendations have been largely disregarded [9] despite the fact that multiple genetic marker
86 systems (allozymes, microsatellites, nuclear and mitochondrial SNPs) have failed to find a clear
87 differentiation between forms and markers [10][11][12].

88 Recently, Chan et al. [13] suggested that the DNA barcoding technique can be used to
89 distinguish *queenslandensis* individuals from the *type* individuals in Singapore. The sequence

90 divergence of 1.5%-1.9% between the two forms [13], although lower than a commonly adopted
91 threshold of 3% for species delineation in insects [14], suggests that the two forms may not freely
92 interbreed. Historical records indicate that the two forms have co-occurred in Singapore and other
93 parts of south-east Asia and Australia for hundreds of generations [5][8]. In sympatry, genetic
94 isolation can be maintained largely through pre-zygotic isolation mechanisms like incompatibilities in
95 mating behavior [15]. For instance, molecular forms of the malarial mosquito, *Anopheles gambiae,*
96 fly together in mating swarms but rarely hybridize due to flight-tone matching between males and
97 females of the same form [16]. Similar incompatibilities in *Ae. aegypti* would have implications for
98 control strategies that rely on successful mating between the release and target mosquitoes, like
99 *Wolbachia*-based population replacement and suppression [17][18], releases of sterile males [19] or
100 males with a RIDL construct [20].

101 To explore this further, we analyzed nuclear and mitochondrial genome-wide variation in the
102 co-occurring pale and *type Ae. aegypti* from Singapore and northern Queensland (Australia). The
103 RADseq approach we employed allows for detection of genetic structure and ancestry with power
104 unparalleled by previous genetic studies of the *Ae. aegypti* forms [18]. Any association between
105 genetic structuring (nuclear/mitochondrial) and the mosquito color/scaling would provide support
106 for the hypothesis of restricted interbreeding between the *type* and the *queenslandensis* form*,* with
107 implications for the implementation of biocontrol programs to suppress diseases transmitted by *Ae.*
108 *aegypti*.

109

110 ## Materials and Methods

111 ### Ethics statement

112 The collection of wild mosquitoes in the study areas does not require specific field ethics
113 approval. The sampling was not conducted on protected land, nor did it involve endangered or

protected species. Consent was obtained from residents at each location where collections occurred on private property.

Sampling and identification

In Singapore, all samples were collected as larvae from the domestic breeding containers at nine locations during the second week of April 2015 (Figure 1, Table 1). These samples were collected during routine inspection by enforcement officers of the National Environment Agency (NEA), Singapore. Larvae were reared to the adult stage under standard laboratory conditions (25° ± 1°C, 80 ± 10% relative humidity and 12 h light/dark cycle). In Townsville (northern Queensland), samples were collected as adults using Biogents Sentinel traps placed at 55 locations in January 2014 (Figure 1, Table 1). Adult mosquitoes were sexed and identified to form based on the key diagnostic color and scaling features, following Mattingly [5] and McClleland [7]. Eleven out of 44 mosquitoes (25%) from Singapore, and seven out of 99 mosquitoes (7%) from Townsville were identified as the *queenslandensis* form (Table 1). Additional four *queenslandensis* individuals collected in Cairns (northern Queensland) in December 2014 were included in the analyses (Table 1).

RADseq genotyping

DNA was extracted from 29 individuals collected in Singapore (18 female *type*, 11 female *queenslandensis*) and 45 individuals from northern Queensland (17 male *type,* 17 female *type*, 11 female *queenslandensis*) (Table 1). Qiagen Blood and Tissue DNA kit (Venlo, Limburg, NL) was used to extract DNA from a whole adult mosquito. 100 ng of DNA from each individual was used to construct the double-digest RAD library following a previously validated protocol [21]. In short, 100 units of the two frequently cutting enzymes (*MluCI* and *NlaIII*, New England Biolabs, Beverly MA, USA) were used to digest 100 ng of DNA during three hours of incubation at 37°C. 100 pM P1 and 300 pM P2 Illumina adapters with customized barcode sequences were ligated to the genomic fragments using 100 units of T4 ligase at 16°C overnight (New England Biolabs, Beverly, MA, USA). Pooled ligations were purified and size selected for fragments 300-450bp in length, using the 2% Pippin Prep cassette (Sage Sciences, Beverly, MA, USA). The final libraries (one for each geographic

region) were enriched with 12 PCR cycles with standard Illumina primers and then sequenced in two HiSeq2500 lanes with the 100 bp paired-end chemistry.

Raw fastq sequences were processed within our customized pipeline [21]. First, all reads were trimmed to the same length of 90 bp and removed if the base quality score was below 13 (FASTX Toolkit, *http://hannonlab.cshl.edu/fastx_toolkit/index.html*). High quality reads were then aligned to the reference mitochondrial genome [22] and the nuclear genome version AaegL1 [23] using the aligner *Bowtie* [24]. Uniquely aligned reads were passed to the *refmap.pl* program that runs the *Stacks* v.1.35 pipeline [25]. In addition to the samples from Singapore, Townsville and Cairns, we included previously sequenced individuals: 15 from Rio de Janeiro (Brazil) [26], 15 from Gordonvale (northern Queensland), and 15 from Ho Chi Minh City (Vietnam) (S1 file). This was done to compare the extent of genetic structuring within and among samples at a regional and global scale. Sexing of the larval samples from Brazil and Vietnam could not be done based on the external morphological features, so we employed a genetic sexing method based on the presence/absence of the male-specific RAD tags [27]. All 119 individuals were included in the creation of the RAD tag catalogues using the default *Stacks* parameters in the maximum likelihood model of SNP and genotype calling. The *populations* module was used to filter the catalogues and export data in the FASTA format (for the mitochondrial variation) and the variant calling format (VCF, for the nuclear variation).

Analyses of genetic diversity and structure

The mitochondrial haplotype richness within and among groups (*Ae. aegypti* forms and geographic regions) was calculated using the rarefaction method implemented in the program *HP-rare* [28]. Phylogenetic relationship among mitochondrial haplotypes was estimated with the maximum likelihood approach in the program *RAxML* (GTRM + G, rapid bootstrap heuristic algorithm and thorough ML search) [29]. Haplotypes of three related *Aedes* species, for which the whole mitochondrial genome sequences were available, served as outgroups: *Ae. albopictus* (NCBI: NC_006817.1), *Ae. notoscriptus* (NC_025473.1) [30] and *Ae. vigilax* (KP995260.1) [31]. Haplotype

166 sequence of the *Ae. aegypti* reference line (Liverpool, NC_010241.1) was also included in the
167 analysis.

168 Parameters of data quality and diversity (RAD tag depth, percentage of missing data,
169 heterozygosity averaged per individual) were compared between females of the two co-occurring
170 forms using independent sample *t*-test. The level of nuclear genetic structuring was estimated using
171 the non-spatial multivariate method DAPC [32] in the *R* package *adegenet* [33]. Rousset's genetic
172 distance ($\hat{a}$) and geographic distance between pairs of individuals were calculated in the program
173 *spagedi* [34]. Color distance between pairs of individuals was treated as a binary value: 0 (same
174 color/form) and 1 (different color/form).

175 ## Results & Discussion

176 ### Variation and phylogenetic relationship among mitochondrial haplotypes

177 From the mitochondrial RAD tag catalogue, we extracted 13 polymorphic tags that were
178 shared between at least 80% of individuals (60/74, Table 1). Tags were distributed across eight
179 different mitochondrial genes (*COXI, Cytb*, *ATP6, ND1-2, ND4-6*; S2 file). All 13 tags were
180 concatenated into a final 1170 bp long sequence that was treated as a mitochondrial haplotype. We
181 found 24 different haplotypes in samples from Singapore and Townsville. Haplotype richness did not
182 differ between the two forms in either location (Singapore *type* = 5.13, *queenslandensis* = 5.07;
183 Townsville *type* = 4.19, *queenslandensis* = 5.0). Moreover, seven haplotypes were shared between
184 the two forms (Table 1, Figure 2).

185 There were 207 distinctive alignment patterns and 8.17% of undetermined characters in the
186 dataset consisting of 24 haplotypes from Singapore and Queensland, one from the Liverpool strain
187 and three from other *Aedes* species (outgroups). Maximum likelihood phylogeny revealed two highly
188 statistically supported maternal lineages in *Ae. aegypti*: a basal clade (more similar to the outgroups)
189 and a clade arising from it (a derived clade) (Figure 2). Nucleotide distance (*p*-distance) between the
190 two clades ranged from 1.2% to 1.6% (S3 file). Importantly, haplotypes of the two *Ae. aegypti* forms

were found in both clades, indicating no association between mitochondrial variation and color variation (Figure 2).

While our results do not support the tentative patterns suggested by Chan et al. [13], they match those from the most comprehensive mitochondrial phylogeny of the African and global *Ae. aegypti* generated to date [35]. Using the ND4 variation, Moore et al. [35] showed that *Ae. aegypti* populations outside Africa represent "mixtures" of mosquitoes from the basal clade and the derived clade, with the basal clade likely originating from West Africa and the derived clade mainly from East Africa. Our analyses of the mitochondrial genome-wide variation revealed the same matrilineage structure in populations from Singapore and northern Queensland (Figure 2). A lack of mitochondrial distinctiveness between the *queenslandensis* and the *type* form is also in line with the findings of More et al. [35], who could not separate the *type* and *formosus* forms into distinct mitochondrial clades despite their assumed subspecies rank.

Nuclear genetic structuring

We extracted nuclear RAD tags that were shared between at least 80% of individuals in the entire dataset (Singapore, Townsville, Gordonvale, Ho Chi Minh City and Rio de Janeiro). To avoid redundant information from the highly linked markers, we randomly selected one SNP per tag with a minor allele frequency greater than 5%, which gave a total of 16,569 markers for downstream analyses.

Parameters of data quality and diversity did not significantly differ between the co-occurring *queenslandensis* and *type* individuals, including the average: percentage of reads uniquely aligned to the reference genome (Singapore: $t_{df,27} = 1.46$, $p = 0.15$; Townsville: $t_{df,26} = 0.782$, $p = 0.44$), locus depth (Singapore: $t_{df,27} = 1.66$, $p = 0.11$; Townsville: $t_{df,26} = -1.73$, $p = 0.095$), percentage of missing data (Singapore: $t_{df,27} = -0.67$, $p = 0.51$; Townsville: $t_{df,26} = 0.951$, $p = 0.35$), or heterozygosity (Singapore: $t_{df,27} = 0.46$, $p = 0.65$; Townsville: $t_{df,26} = -2.42$, $p = 0.023$) (Table 1, S1 figure).

Discriminant analysis of principal components (DAPC) showed a clear-cut differentiation of mosquitoes based on their geographic origin and not their color. When the entire dataset was considered, *Ae. aegypti* individuals formed genetic clusters that corresponded to their sampling region (i.e. Rio de Janeiro, Ho Chi Minh City, Singapore and northern Queensland) (Figure 3a). The only exceptions were three individuals in Singapore (K,L,M) that formed a distinct genetic group (Figure 3a). They were collected as larvae from the same breeding container, and two were identified as the *type* and one as the *queenslandensis* form (Table 1, Figure 3b). Given their high relatedness (Supplemental file 4) and shared mitochondrial haplotype, as well as high nuclear differentiation from other mosquitoes in the region, it is likely that individuals K, L and M are offspring of the incursion female(s) not local to Australia and Vietnam. These individuals were found near the city port (Figure 1), suggesting a possible route of introduction.

Further analysis of genetic structuring within Singapore revealed that family groups were sampled within the breeding containers, some of which had both color forms (Figure 3b). Highly related *queenslandensis* and *type* pairs were found at 4 locations (Figure 3b), including the incursion family group (K,L,M). Most of the related individuals (24/28 pairs), however, had the same color (Figure 4). These results suggest that the color/scaling pattern is likely to represent a quantitative trait under some environmental influence, which is in line with the recent discovery of multiple QTLs associated with the dorsal abdominal scaling pattern in *Ae. aegypti* [36].

Individuals from northern Queensland were grouped into three clusters corresponding to the three towns where the sampling occurred (Figure 3c). A single exception was one *queenslandensis* individual from Cairns that was grouped with the *type* individuals from Gordonvale (Figure 3c). The two forms in Townsville could not be distinguished based on their nuclear genome-wide variation (Figure 3c). We found four pairs of closely related individuals: two *queenslandensis* and two *type* pairs (Figure 4, S4 file). In other words, all related pairs detected in Townsville were of the same form.

A lower frequency of related individuals in Townsville when compared to Singapore is not surprising given that different sampling methods were employed in these locations. Collection of multiple larvae from the same breeding container increases the chance of sampling family groups, as seen in Singapore and parts of Rio de Janeiro [21]. On the other hand, when BG-sentinel traps are used, the likelihood of related individuals being collected is low. In Townsville, 12.5% of pairs from the same trap were close relatives. Sampling effects are reflected in an elevated level of pairwise genetic distance over geographic distance for mosquitoes from Singapore when compared to Townsville (Figure 4). Such differences in genetic patterns could be erroneously interpreted as differences in the underlying processes (e.g. different dispersal rates), and highlight that sampling methods are crucial when inferring processes within and among *Ae. aegypti* populations.

In summary, we did not find any association between nuclear genetic variation and color/scaling variation in *Ae. aegypti* from Singapore and northern Queensland. Our results are unlikely to be caused by a lack of power to detect genetic structure, given that more than 16,000 genome-wide SNPs allowed us to delineate family groups at a very fine spatial scale. In fact, several families had the *queenslandensis* and *type* members. Given that a recent study of global *Ae. aegypti* populations at 12 microsatellite loci found that *Ae. aegypti formosus* and *Ae. aegypti aegypti* constitute one genetic cluster in Africa [37], it is not surprising that the two *Ae. aegypti aegypti* varieties also form one genetic cluster.

Wolbachia infection

In addition to the absence of genetic structuring that corresponds to the two *Ae. aegypti aegypti* forms, another line of evidence in support of ongoing interbreeding is the presence of *Wolbachia* in both forms. We detected this endosymbiotic bacterium in three (out of four) *queenslandensis* individuals from Cairns and 14 (out of 15) *type* individuals from Gordonvale, using a light-cycler assay for *Wolbachia* detection [38]. *Wolbachia* is not naturally found in *Ae. aegypti*, but was introduced into the populations in Gordonvale in 2011 and Cairns in 2013 in an effort to reduce dengue transmission [39][40]. This was done by releasing *Wolbachia*-infected females and males

267 from the colony that originated from the *type Ae. aegypti* [41]. Because the infection is transmitted
268 from mother to offspring, the only way *queenslandensis* individuals could have become infected by
269 *Wolbachia* is by mating with infected, *type* females. Given the high *Wolbachia* frequency (>85%) in
270 Cairns and Gordonvale at the time of our sampling [42], finding the infection in 3/4 individuals
271 caught in Cairns, and 14/15 individuals caught in Gordonvale was expected.

272

273 Conclusion

274 Our analyses of mitochondrial and nuclear genome-wide variation and the *Wolbachia*
275 infection indicate that *Ae. aegypti queenslandensis* and *Ae. aegypti type* mosquitoes interbreed
276 freely, at least in Singapore and northern Queensland. These findings are of practical importance for
277 control strategies that rely on successful mating between the released and target mosquitoes. Our
278 results also re-enforce the recommendations by the early taxonomic authorities (Mattingly and
279 McClelland) that *Ae. aegypti queenslandensis* should not be ranked as a subspecies.

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286 Author Contributions

287 GR: generated, analyzed, interpreted data, and wrote the manuscript with the input from AAH and
288 IF; IF: generated and processed NGS data; AGC: done some field collections and molecular lab work;
289 DS: noted reoccurring very pale form in QLD, identified and pre-processed samples from QLD; AC; SG

290 L-P, HCT: identification and pre-processing of samples from Singapore; AAH: conceived the study. All

291 authors have read and approved the final manuscript version.

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Figures and Tables

Figure 1. Sampling sites. In Singapore (left), each sampling point represents one breeding container from which larvae were collected. In Townsville, each sampling point represents one BG-Sentinel trap from which adults were collected.

Figure 2. Mitochondrial Maximum likelihood phylogeny. Twenty four different mitochondrial haplotypes (Hap1-24) found in *Aedes aegypti type* and var. *queenslandensis* that co-occur in Singapore and northern Queensland, Australia. Sequences of the three outgroups (*Ae. albopictus, Ae. vigilax, Ae. notoscriptus*) and *Ae. aegypti* Liverpool strain were obtained from the NCBI nucleotide sequence/genome database, with the NCBI accession numbers listed in square brackets. The number of *Ae. aegypti* individuals with a given mitochondrial haplotype is listed in parentheses. A circle designates haplotypes found in Queensland, and a triangle those found in Singapore. Open symbols designate haplotypes found in the *queenslandensis* form, and filled symbols those found in the *type* form.

Figure 3. Nuclear genetic structuring (DAPC). Individuals marked with the asterix (*) in their sample ID were identified as *Aedes aegypti queenslandensis* based on the diagnostic scaling patterns [7]. (a) Scatterplot summarizing the individual DAPC scores (axes 1 and 2) in *Aedes aegypti* samples collected in Singapore, Queensland, Ho Chi Minh City (Vietnam) and Rio de Janeiro (Brazil); (b) Individual membership probability to the genetic groups in Singapore; (c) Individual membership probability to the genetic groups in northern Queensland.

Figure 4. Pairwise genetic *versus* geographic and color distance. Pairs of *Aedes aegypti* collected in Singapore (upper graphs) and Townsville (lower graphs). A value of zero for Rousset's genetic distance ($\hat{a}$) indicates a distance between a pair of individuals randomly drawn from a given sample, while a negative value indicates lower than average genetic distance between a pair (i.e. their higher relatedness). Color distance between pairs of individuals was treated as a binary value: 0 (same color/form) and 1 (different color/form).

Table 1. Sample information. Sample ID, region (SNP – Singapore, QLD_T – Townsville, QLD_C – Cairns, Queensland), X, Y (longitude/latitude decimal degrees), collection (method/breeding container), sex (F – female, M – male), form (*t* – *type*, *q* – *queenslandensis* [5][7]), mitochondrial haplotype (mt hapl, Hap1-24), per individual proportion of heterozygous (het) nuclear loci, average (aver) locus depth, and proportion of missing (miss) loci.

Sample ID	region	X	Y	collection site	sex	form	mt hapl	het loci	aver depth	miss loci
A	SNP	103.7701	1.4418	Dish tray	F	*q*	Hap5	0.195	32.2	0.03
B	SNP	103.7701	1.4418	Dish tray	F	*t*	Hap5	0.149	30.9	0.03
C	SNP	103.7701	1.4418	Dish tray	F	*q*	Hap5	0.201	33.1	0.02
D	SNP	103.7634	1.4228	Plastic tray	F	*t*	Hap6	0.252	34.3	0.01
E	SNP	103.7634	1.4228	Plastic tray	F	*t*	Hap24	0.253	30.6	0.01
F	SNP	103.7634	1.4228	Plastic tray	F	*t*	Hap6	0.260	26.4	0.01
G	SNP	103.7634	1.4228	Plastic tray	F	*t*	Hap24	0.244	32.0	0.01
H	SNP	103.7730	1.4456	Scupper drain	F	*t*	Hap6	0.214	28.5	0.02
I	SNP	103.7730	1.4456	Scupper drain	F	*q*	Hap11	0.260	33.8	0.01
J	SNP	103.7730	1.4456	Scupper drain	F	*t*	Hap6	0.159	35.9	0.03
K	SNP	103.7950	1.3099	Vase	F	*t*	Hap20	0.179	23.5	0.03
L	SNP	103.7950	1.3099	Vase	F	*t*	Hap19	0.106	29.8	0.04
M	SNP	103.7950	1.3099	Vase	F	*q*	Hap19	0.160	26.2	0.03
N	SNP	103.8282	1.3709	Fish tank	F	*t*	Hap14	0.250	33.3	0.03
O	SNP	103.8282	1.3709	Fish tank	F	*q*	Hap6	0.209	45.1	0.01
P	SNP	103.8282	1.3709	Fish tank	F	*t*	Hap15	0.250	29.4	0.02
Q	SNP	103.8282	1.3709	Fish tank	F	*t*	Hap6	0.233	22.2	0.02
R	SNP	103.8399	1.3714	Gully trap	F	*q*	Hap12	0.130	30.2	0.04
S	SNP	103.7431	1.3484	Flower vase	F	*q*	Hap6	0.256	30.6	0.01
T	SNP	103.7431	1.3484	Flower vase	F	*t*	Hap6	0.254	29.1	0.01
U	SNP	103.7660	1.3211	Corridor	F	*q*	Hap6	0.264	33.4	0.01
V	SNP	103.7660	1.3211	Corridor	F	*q*	Hap7	0.260	28.8	0.01
W	SNP	103.7660	1.3211	Corridor	F	*q*	Hap13	0.264	37.2	0.01
X	SNP	103.7660	1.3211	Corridor	F	*q*	Hap6	0.262	28.2	0.02
Y	SNP	103.7565	1.3147	Flower pot tray	F	*t*	Hap8	0.253	26.9	0.01
Z	SNP	103.7565	1.3147	Flower pot tray	F	*t*	Hap18	0.238	25.3	0.02
AA	SNP	103.7565	1.3147	Flower pot tray	F	*t*	Hap16	0.240	35.7	0.01
BB	SNP	103.7565	1.3147	Flower pot tray	F	*t*	Hap17	0.217	13.1	0.07
CC	SNP	103.7565	1.3147	Flower pot tray	F	*t*	Hap6	0.255	36.7	0.01
F13	QLD_T	146.7746	-19.2788	BG-Sentinel trap	F	*t*	Hap22	0.195	15.9	0.04
F19	QLD_T	146.7814	-19.2990	BG-Sentinel trap	F	*t*	-	0.240	15.6	0.04
F20	QLD_T	146.7605	-19.2862	BG-Sentinel trap	F	*t*	-	0.149	14.4	0.05
F21	QLD_T	146.7759	-19.2639	BG-Sentinel trap	F	*t*	-	0.217	20.0	0.02
F25	QLD_T	146.7833	-19.2806	BG-Sentinel trap	F	*t*	Hap9	0.201	13.8	0.05
F27	QLD_T	146.7820	-19.2774	BG-Sentinel trap	F	*t*	Hap22	0.255	20.6	0.02

F28	QLD_T	146.7759	-19.2639	BG-Sentinel trap	F	*t*	Hap6	0.252	12.4	0.06
F3	QLD_T	146.7759	-19.2639	BG-Sentinel trap	F	*t*	Hap22	0.253	13.0	0.06
F31	QLD_T	146.8167	-19.2743	BG-Sentinel trap	F	*t*	Hap23	0.260	20.4	0.03
Mf32	QLD_T	146.7918	-19.2442	BG-Sentinel trap	M	*t*	Hap4	0.248	18.7	0.02
Mf33	QLD_T	146.8181	-19.2786	BG-Sentinel trap	M	*t*	Hap2	0.228	16.3	0.03
F34	QLD_T	146.7663	-19.2898	BG-Sentinel trap	F	*t*	Hap21	0.218	19.9	0.02
F4	QLD_T	146.7679	-19.3015	BG-Sentinel trap	F	*t*	Hap22	0.255	15.1	0.05
F5	QLD_T	146.8078	-19.2554	BG-Sentinel trap	F	*t*	Hap9	0.239	14.9	0.04
F6	QLD_T	146.7864	-19.2489	BG-Sentinel trap	F	*t*	Hap4	0.248	19.2	0.02
F7	QLD_T	146.7717	-19.2740	BG-Sentinel trap	F	*t*	Hap22	0.221	9.8	0.13
F9	QLD_T	146.7756	-19.2993	BG-Sentinel trap	F	*t*	Hap9	0.221	14.7	0.04
M23	QLD_T	146.7663	-19.2898	BG-Sentinel trap	M	*t*	Hap4	0.252	21.9	0.01
M24	QLD_T	146.8272	-19.2616	BG-Sentinel trap	M	*t*	Hap9	0.260	22.1	0.01
M27	QLD_T	146.7717	-19.2740	BG-Sentinel trap	M	*t*	Hap21	0.265	21.0	0.02
M28	QLD_T	146.7717	-19.2740	BG-Sentinel trap	M	*t*	Hap4	0.268	20.4	0.01
M29	QLD_T	146.7605	-19.2916	BG-Sentinel trap	M	*t*	Hap21	0.259	24.2	0.01
M30	QLD_T	146.7722	-19.2866	BG-Sentinel trap	M	*t*	Hap21	0.285	22.6	0.01
M31	QLD_T	146.7814	-19.2990	BG-Sentinel trap	M	*t*	Hap22	0.238	20.4	0.02
M32	QLD_T	146.8223	-19.2662	BG-Sentinel trap	M	*t*	Hap21	0.243	22.0	0.03
M33	QLD_T	146.8283	-19.2717	BG-Sentinel trap	M	*t*	Hap22	0.261	27.4	0.01
M34	QLD_T	146.7797	-19.2588	BG-Sentinel trap	M	*t*	Hap22	0.268	22.0	0.01
M35	QLD_T	146.7679	-19.3015	BG-Sentinel trap	M	*t*	Hap4	0.262	20.3	0.02
M36	QLD_T	146.8174	-19.2558	BG-Sentinel trap	M	*t*	Hap4	0.230	15.9	0.04
Fm37	QLD_T	146.7931	-19.2866	BG-Sentinel trap	F	*t*	Hap22	0.257	25.5	0.01
M38	QLD_T	146.8087	-19.2496	BG-Sentinel trap	M	*t*	Hap3	0.265	20.4	0.01
M39	QLD_T	146.7820	-19.2774	BG-Sentinel trap	M	*t*	Hap9	0.258	18.7	0.03
M40	QLD_T	146.7833	-19.2806	BG-Sentinel trap	M	*t*	Hap22	0.227	11.6	0.08
Fm45	QLD_T	146.7917	-19.2947	BG-Sentinel trap	F	*t*	Hap22	0.250	25.0	0.01
Q1	QLD_T	146.7847	-19.2702	BG-Sentinel trap	F	*q*	Hap4	0.225	18.3	0.02
Q2	QLD_T	146.7664	-19.2860	BG-Sentinel trap	F	*q*	Hap10	0.234	14.7	0.05
Q3	QLD_T	146.8272	-19.2616	BG-Sentinel trap	F	*q*	Hap22	0.243	16.1	0.04
Q4	QLD_T	146.8086	-19.2762	BG-Sentinel trap	F	*q*	Hap4	0.229	13.7	0.04
Q5	QLD_T	146.8272	-19.2616	BG-Sentinel trap	F	*q*	Hap21	0.232	14.3	0.04
Q6	QLD_T	146.8086	-19.2762	BG-Sentinel trap	F	*q*	Hap4	0.203	10.3	0.10
Q7	QLD_T	146.7664	-19.2860	BG-Sentinel trap	F	*q*	Hap9	0.233	16.8	0.03
Q8	QLD_T	145.7488	-16.9389	BG-Sentinel trap	F	*q*	Hap1	0.254	17.8	0.03
Q9	QLD_C	145.7562	-16.9310	BG-Sentinel trap	F	*q*	Hap4	0.206	11.0	0.09
Q10	QLD_C	145.7562	-16.9310	BG-Sentinel trap	F	*q*	Hap4	0.163	13.0	0.05
Q11	QLD_C	145.7562	-16.9310	BG-Sentinel trap	F	*q*	Hap4	0.194	13.9	0.05

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Supporting Information

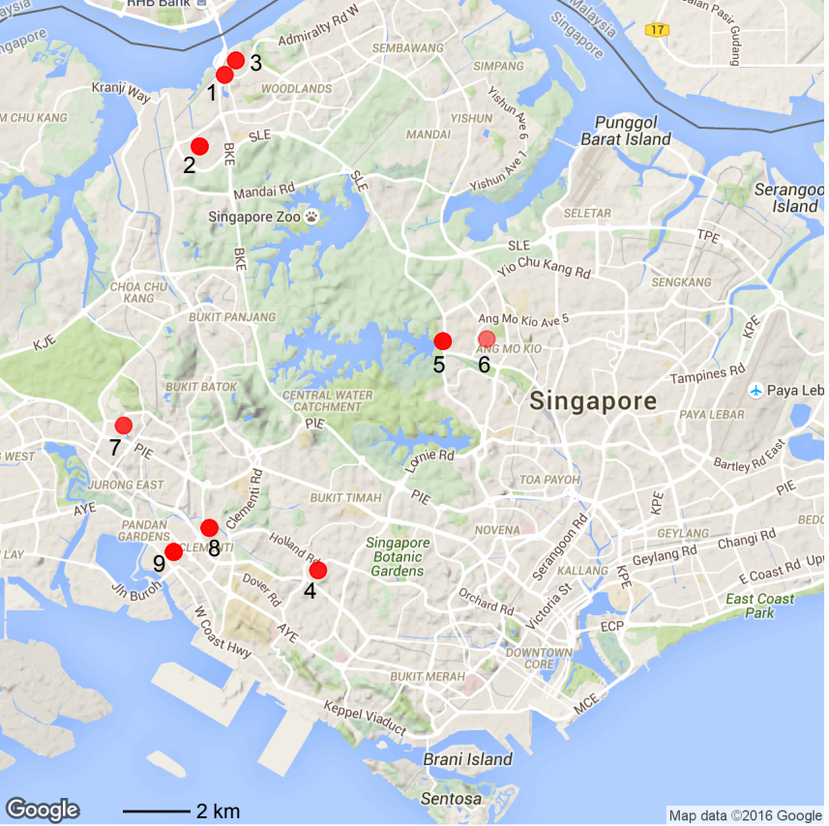
S1 Table. Sample information for additional *Aedes aegypti*. Mosquitoes from Rio de Janeiro (Brazil), Gordonvale (northern Queensland), Ho Chi Minh city (Vietnam), used in the DAPC analysis.

S2 File. Mitochondrial haplotypes. FASTA file with mitochondrial sequences from *Aedes aegypti* (Hap1-24), the Liverpool strain, and three outgroups used in the RAxML phylogenetic reconstruction. Mitochondrial haplotypes were generated by concatenating 90 bp RAD sequences from: ND2 (1-90 bp), COXI (91-270 bp), ATP6 (271-450 bp), ND5 (451-630 bp), ND4 (631-720 bp), ND6 (721-810 bp), cytB (811-1080 bp), ND1 (1081-1170 bp).

S3 File. Pairwise nucleotide difference (*p*-distance) between mitochondrial haplotypes. The number of base differences per site from between sequences are shown. The analysis involved 28 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1170 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

S4 File. Pairwise Relatedness. Estimates of relatedness (*r*) by Wang (2002) for *Aedes aegypti* pairs in Singapore and Townsville. Reference: Wang, J. 2002. An estimator for pairwise relatedness using molecular markers. Genetics 160: 1203–1215.

S1 Fig. Data quality parameters. Boxplots of per-individual values for the proportion of uniquely aligned reads, RAD tag read depth, proportion of heterozygous loci, proportion of missing data for *Aedes aegypti* from Singapore (left) and Queensland (right).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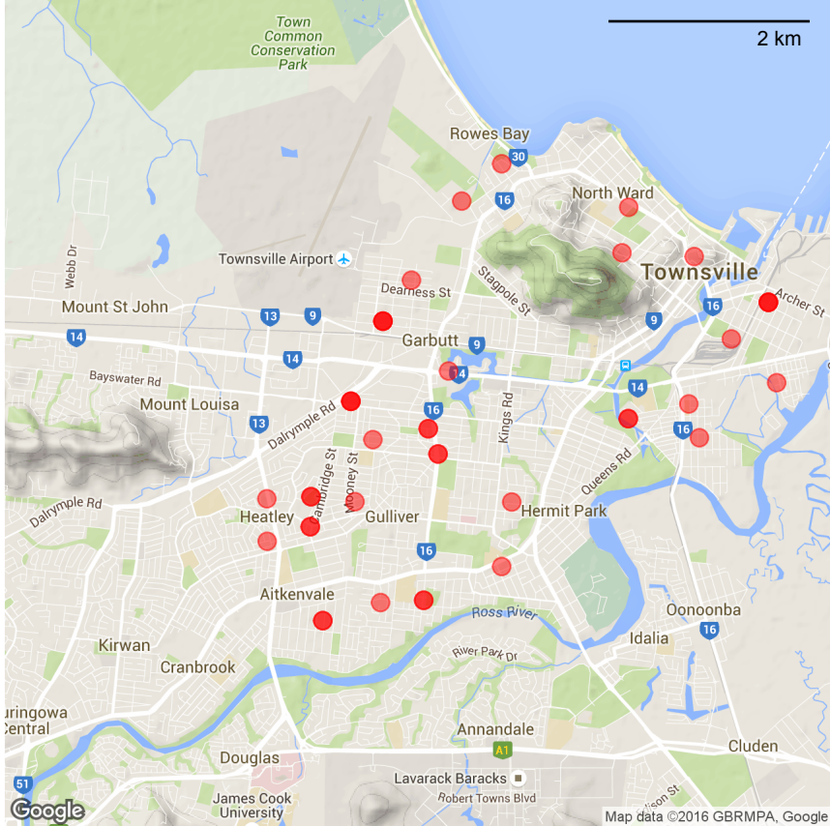
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Kirwan
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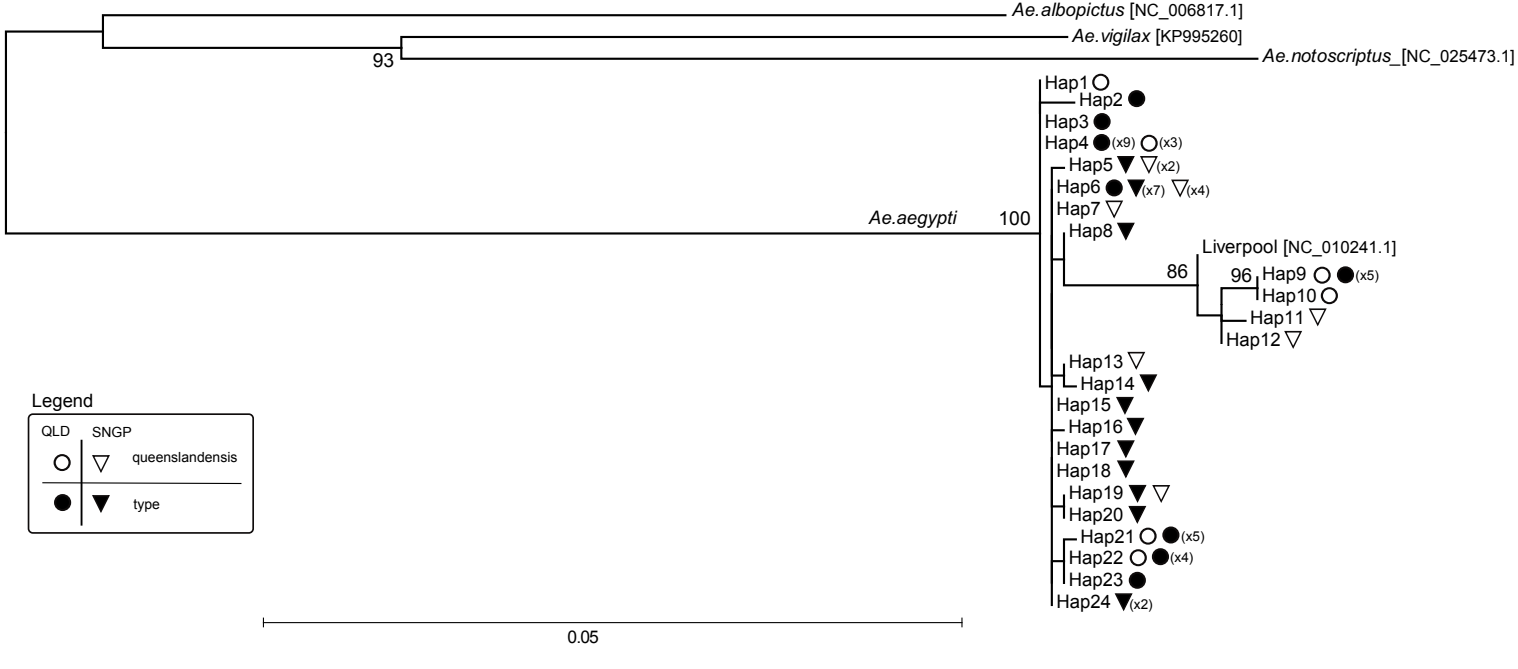
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Hap1
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Hap6 (x7) (x4)
Hap7
Hap8
Liverpool [NC_010241.1]
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Hap9 (x5)
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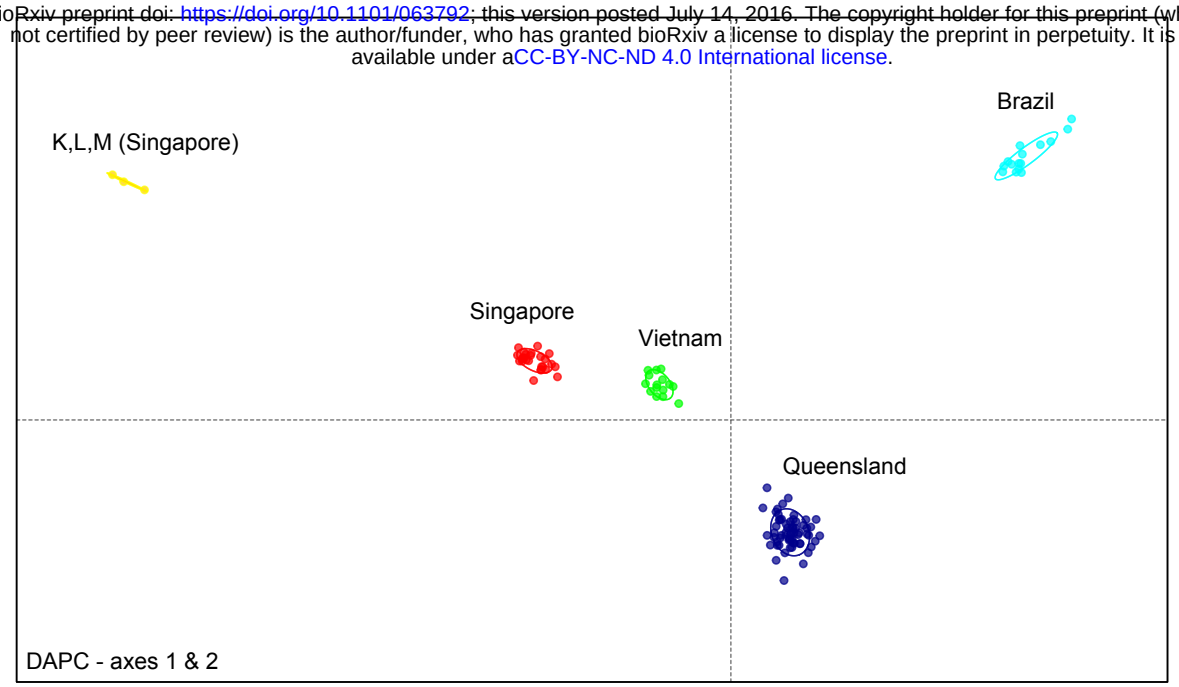
a)



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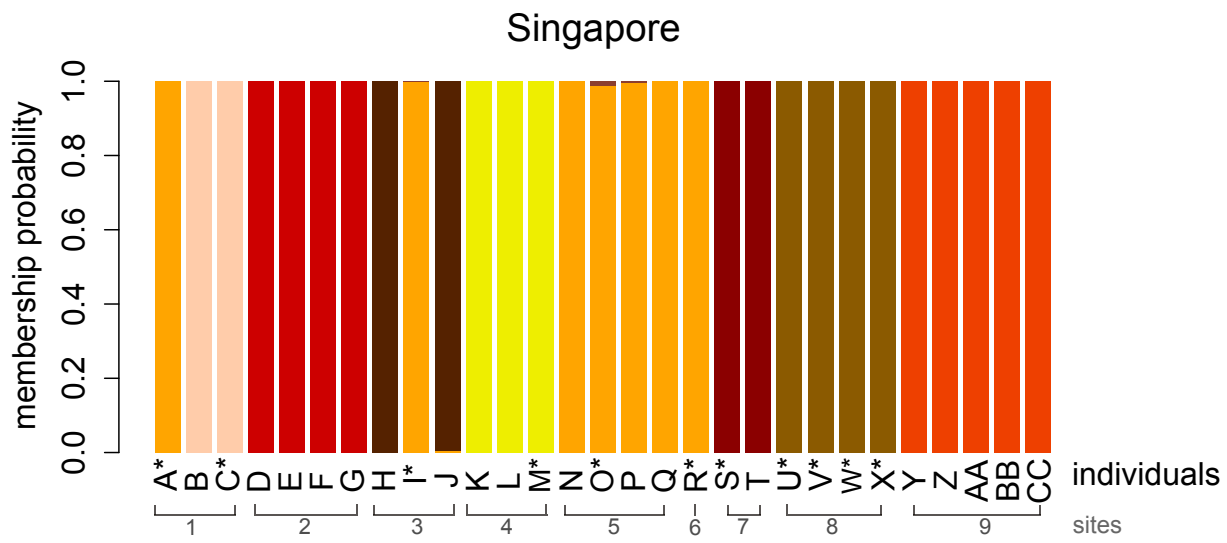

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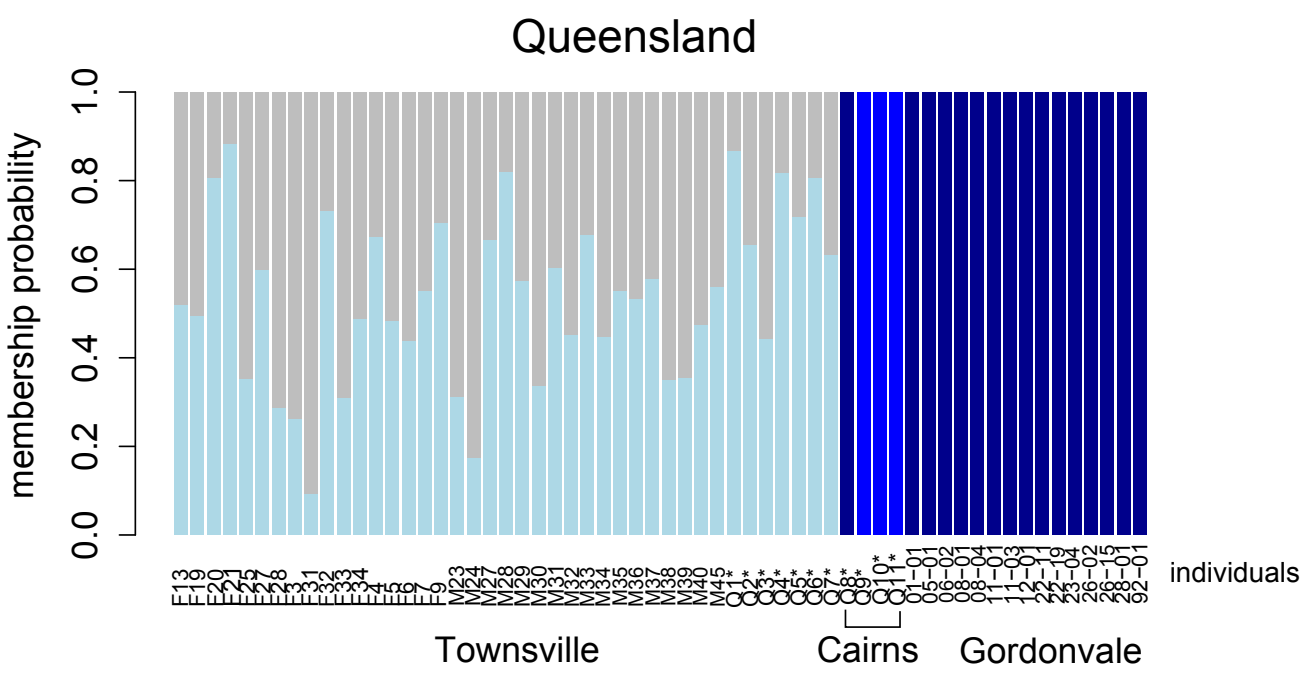
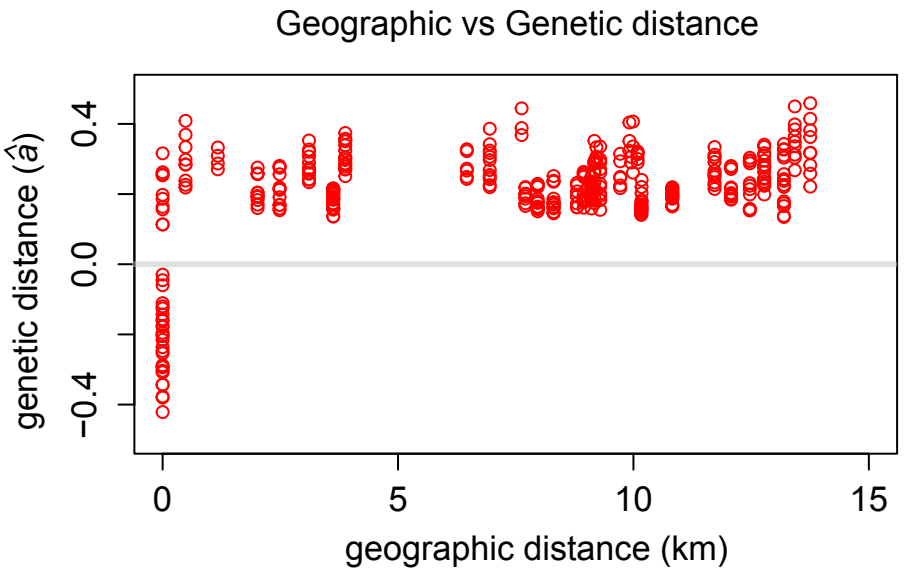
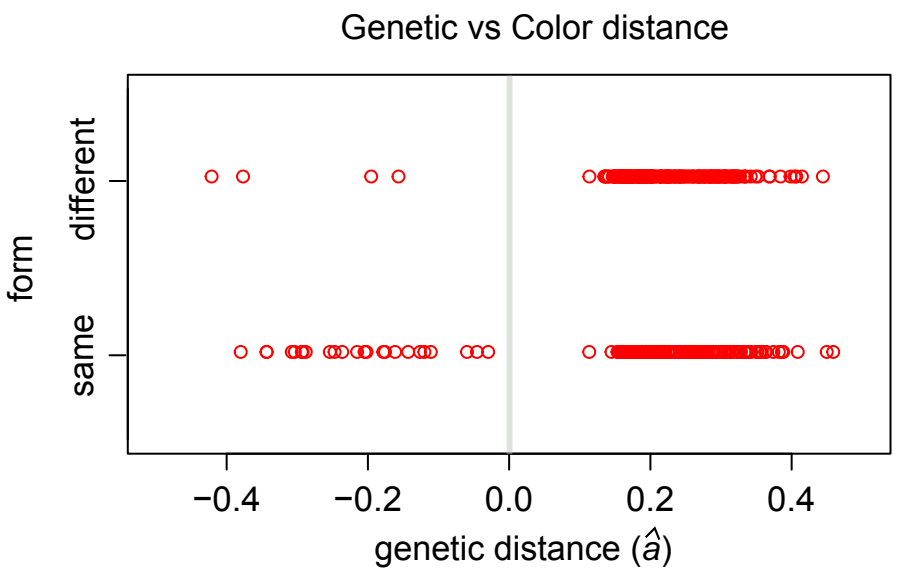


SINGAPORE





TOWNSVILLE