

1 **Title: The *queenslandensis* and the *type* form of the dengue fever mosquito**

2 **(*Aedes aegypti* L.) are genomically indistinguishable**

3

4 Authors: Gordana Rašić^{1*}, Igor Filipović¹, Ashley G. Callahan¹, Darren Stanford², Abigail Chan³, Sai

5 Gek Lam-Phua³, Huat Cheong Tan³, Ary A. Hoffmann¹

6 ¹Pest and Environmental Adaptation Research Group, Bio21 Institute, School of BioSciences, The

7 University of Melbourne, 3010 Parkville, Australia

8 ²Institute of Vector Borne Disease, Monash University, Australia

9 ³Environmental Health Institute, National Environment Agency, Singapore

10

11 *Corresponding author: gordana.rasic@unimelb.edu.au; rasic.gordana@gmail.com (GR)

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

13

14 **Abstract**

15 **Background**

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

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

25 **Methods/Findings**

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

34 **Conclusion**

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

39

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

41

42 Author Summary

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

55

56 Introduction

57 The mosquito *Aedes aegypti* (Linnaeus) is the most important arboviral vector in the tropics
58 and subtropics [1]. Diseases transmitted by *Ae. aegypti*, like dengue fever and Zika, are on the rise
59 [2], and some are reappearing. For instance, chikungunya has returned to the American tropics in
60 2013, after being absent for nearly 200 years [3]. Yellow fever was nearly eliminated thanks to an
61 effective vaccine, but is now resurging in central and south Africa [4]. Such epidemiological trends
62 highlight the need to persist with vector control efforts, which requires a thorough understanding of
63 vector biology.

64 Nearly 60 years ago, Mattingly [5] noted that despite a vast body of literature, few
65 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. aegypti* 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

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

116 **Sampling and identification**

117 In Singapore, all samples were collected as larvae from the domestic breeding containers at
118 nine locations during the second week of April 2015 (Figure 1, Table 1). These samples were
119 collected during routine inspection by enforcement officers of the National Environment Agency
120 (NEA), Singapore. Larvae were reared to the adult stage under standard laboratory conditions ($25^{\circ} \pm$
121 1°C , $80 \pm 10\%$ relative humidity and 12 h light/dark cycle). In Townsville (northern Queensland),
122 samples were collected as adults using Biogents Sentinel traps placed at 55 locations in January 2014
123 (Figure 1, Table 1). Adult mosquitoes were sexed and identified to form based on the key diagnostic
124 color and scaling features, following Mattingly [5] and McClelland [7]. Eleven out of 44 mosquitoes
125 (25%) from Singapore, and seven out of 99 mosquitoes (7%) from Townsville were identified as the
126 *queenslandensis* form (Table 1). Additional four *queenslandensis* individuals collected in Cairns
127 (northern Queensland) in December 2014 were included in the analyses (Table 1).

128 **RADseq genotyping**

129 DNA was extracted from 29 individuals collected in Singapore (18 female *type*, 11 female
130 *queenslandensis*) and 45 individuals from northern Queensland (17 male *type*, 17 female *type*, 11
131 female *queenslandensis*) (Table 1). Qiagen Blood and Tissue DNA kit (Venlo, Limburg, NL) was used
132 to extract DNA from a whole adult mosquito. 100 ng of DNA from each individual was used to
133 construct the double-digest RAD library following a previously validated protocol [21]. In short, 100
134 units of the two frequently cutting enzymes (*Mlu*I and *Nla*III, New England Biolabs, Beverly MA,
135 USA) were used to digest 100 ng of DNA during three hours of incubation at 37°C . 100 pM P1 and
136 300 pM P2 Illumina adapters with customized barcode sequences were ligated to the genomic
137 fragments using 100 units of T4 ligase at 16°C overnight (New England Biolabs, Beverly, MA, USA).
138 Pooled ligations were purified and size selected for fragments 300-450bp in length, using the 2%
139 Pippin Prep cassette (Sage Sciences, Beverly, MA, USA). The final libraries (one for each geographic

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

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

158 **Analyses of genetic diversity and structure**

159 The mitochondrial haplotype richness within and among groups (*Ae. aegypti* forms and
160 geographic regions) was calculated using the rarefaction method implemented in the program *HP-*
161 *rare* [28]. Phylogenetic relationship among mitochondrial haplotypes was estimated with the
162 maximum likelihood approach in the program *RAxML* (GTRM + G, rapid bootstrap heuristic algorithm
163 and thorough ML search) [29]. Haplotypes of three related *Aedes* species, for which the whole
164 mitochondrial genome sequences were available, served as outgroups: *Ae. albopictus* (NCBI:
165 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 ($\hat{\alpha}$) 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

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

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

203

204 **Nuclear genetic structuring**

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

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

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

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

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

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

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

259 ***Wolbachia* infection**

260 In addition to the absence of genetic structuring that corresponds to the two *Ae. aegypti*
261 *aegypti* forms, another line of evidence in support of ongoing interbreeding is the presence of
262 *Wolbachia* in both forms. We detected this endosymbiotic bacterium in three (out of four)
263 *queenslandensis* individuals from Cairns and 14 (out of 15) *type* individuals from Gordonvale, using a
264 light-cycler assay for *Wolbachia* detection [38]. *Wolbachia* is not naturally found in *Ae. aegypti*, but
265 was introduced into the populations in Gordonvale in 2011 and Cairns in 2013 in an effort to reduce
266 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.

280 Acknowledgements

281 We thank Carly Herbertson, Melanie Commerford and other Eliminate Dengue field assistants for
282 providing *Aedes aegypti* from Townsville and Cairns. We also thank the NeCTAR Cloud for providing
283 computational resources. This work is funded by the National Health and Medical Research Council
284 and the National Environment Agency, Singapore. The funders had no role in study design, data
285 analyses, interpretation or manuscript writing.

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.

292

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418

419

420 **Figures and Tables**

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

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

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

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

445 **Table 1. Sample information.** Sample ID, region (SNP – Singapore, QLD_T – Townsville, QLD_C –
 446 Cairns, Queensland), X, Y (longitude/latitude decimal degrees), collection (method/breeding
 447 container), sex (F – female, M – male), form (*t* – *type*, *q* – *queenslandensis* [5][7]), mitochondrial
 448 haplotype (mt hapl, Hap1-24), per individual proportion of heterozygous (het) nuclear loci, average
 449 (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	<i>q</i>	Hap5	0.195	32.2	0.03
B	SNP	103.7701	1.4418	Dish tray	F	<i>t</i>	Hap5	0.149	30.9	0.03
C	SNP	103.7701	1.4418	Dish tray	F	<i>q</i>	Hap5	0.201	33.1	0.02
D	SNP	103.7634	1.4228	Plastic tray	F	<i>t</i>	Hap6	0.252	34.3	0.01
E	SNP	103.7634	1.4228	Plastic tray	F	<i>t</i>	Hap24	0.253	30.6	0.01
F	SNP	103.7634	1.4228	Plastic tray	F	<i>t</i>	Hap6	0.260	26.4	0.01
G	SNP	103.7634	1.4228	Plastic tray	F	<i>t</i>	Hap24	0.244	32.0	0.01
H	SNP	103.7730	1.4456	Scupper drain	F	<i>t</i>	Hap6	0.214	28.5	0.02
I	SNP	103.7730	1.4456	Scupper drain	F	<i>q</i>	Hap11	0.260	33.8	0.01
J	SNP	103.7730	1.4456	Scupper drain	F	<i>t</i>	Hap6	0.159	35.9	0.03
K	SNP	103.7950	1.3099	Vase	F	<i>t</i>	Hap20	0.179	23.5	0.03
L	SNP	103.7950	1.3099	Vase	F	<i>t</i>	Hap19	0.106	29.8	0.04
M	SNP	103.7950	1.3099	Vase	F	<i>q</i>	Hap19	0.160	26.2	0.03
N	SNP	103.8282	1.3709	Fish tank	F	<i>t</i>	Hap14	0.250	33.3	0.03
O	SNP	103.8282	1.3709	Fish tank	F	<i>q</i>	Hap6	0.209	45.1	0.01
P	SNP	103.8282	1.3709	Fish tank	F	<i>t</i>	Hap15	0.250	29.4	0.02
Q	SNP	103.8282	1.3709	Fish tank	F	<i>t</i>	Hap6	0.233	22.2	0.02
R	SNP	103.8399	1.3714	Gully trap	F	<i>q</i>	Hap12	0.130	30.2	0.04
S	SNP	103.7431	1.3484	Flower vase	F	<i>q</i>	Hap6	0.256	30.6	0.01
T	SNP	103.7431	1.3484	Flower vase	F	<i>t</i>	Hap6	0.254	29.1	0.01
U	SNP	103.7660	1.3211	Corridor	F	<i>q</i>	Hap6	0.264	33.4	0.01
V	SNP	103.7660	1.3211	Corridor	F	<i>q</i>	Hap7	0.260	28.8	0.01
W	SNP	103.7660	1.3211	Corridor	F	<i>q</i>	Hap13	0.264	37.2	0.01
X	SNP	103.7660	1.3211	Corridor	F	<i>q</i>	Hap6	0.262	28.2	0.02
Y	SNP	103.7565	1.3147	Flower pot tray	F	<i>t</i>	Hap8	0.253	26.9	0.01
Z	SNP	103.7565	1.3147	Flower pot tray	F	<i>t</i>	Hap18	0.238	25.3	0.02
AA	SNP	103.7565	1.3147	Flower pot tray	F	<i>t</i>	Hap16	0.240	35.7	0.01
BB	SNP	103.7565	1.3147	Flower pot tray	F	<i>t</i>	Hap17	0.217	13.1	0.07
CC	SNP	103.7565	1.3147	Flower pot tray	F	<i>t</i>	Hap6	0.255	36.7	0.01
F13	QLD_T	146.7746	-19.2788	BG-Sentinel trap	F	<i>t</i>	Hap22	0.195	15.9	0.04
F19	QLD_T	146.7814	-19.2990	BG-Sentinel trap	F	<i>t</i>	-	0.240	15.6	0.04
F20	QLD_T	146.7605	-19.2862	BG-Sentinel trap	F	<i>t</i>	-	0.149	14.4	0.05
F21	QLD_T	146.7759	-19.2639	BG-Sentinel trap	F	<i>t</i>	-	0.217	20.0	0.02
F25	QLD_T	146.7833	-19.2806	BG-Sentinel trap	F	<i>t</i>	Hap9	0.201	13.8	0.05
F27	QLD_T	146.7820	-19.2774	BG-Sentinel trap	F	<i>t</i>	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

450

451

452 **Supporting Information**

453

454 **S1 Table. Sample information for additional *Aedes aegypti*.** Mosquitoes from Rio de Janeiro (Brazil),

455 Gordonvale (northern Queensland), Ho Chi Minh city (Vietnam), used in the DAPC analysis.

456 **S2 File. Mitochondrial haplotypes.** FASTA file with mitochondrial sequences from *Aedes aegypti*

457 (Hap1-24), the Liverpool strain, and three outgroups used in the RAxML phylogenetic reconstruction.

458 Mitochondrial haplotypes were generated by concatenating 90 bp RAD sequences from: ND2 (1-90

459 bp), COXI (91-270 bp), ATP6 (271-450 bp), ND5 (451-630 bp), ND4 (631-720 bp), ND6 (721-810 bp),

460 cytB (811-1080 bp), ND1 (1081-1170 bp).

461 **S3 File. Pairwise nucleotide difference (p-distance) between mitochondrial haplotypes.** The

462 number of base differences per site from between sequences are shown. The analysis involved 28

463 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a

464 total of 1170 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

465 **S4 File. Pairwise Relatedness.** Estimates of relatedness (r) by Wang (2002) for *Aedes aegypti* pairs in

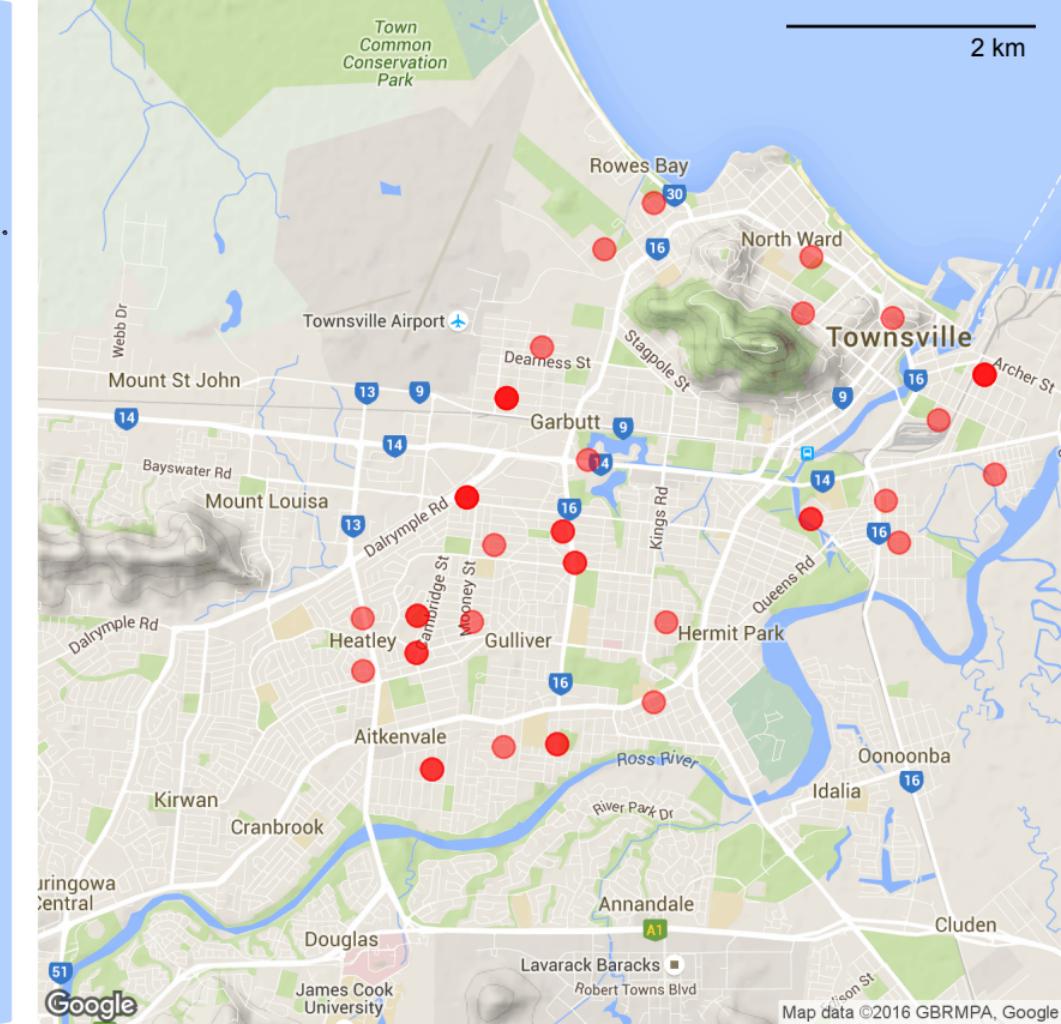
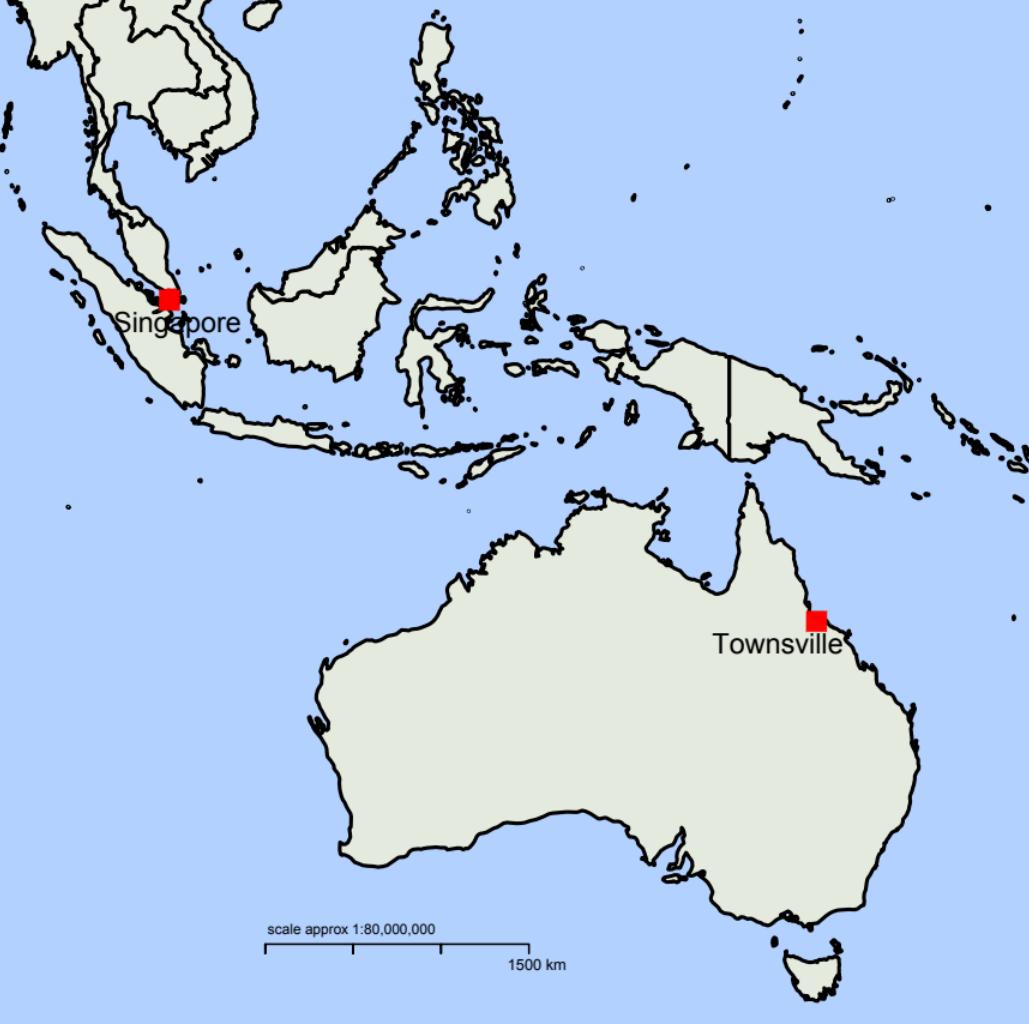
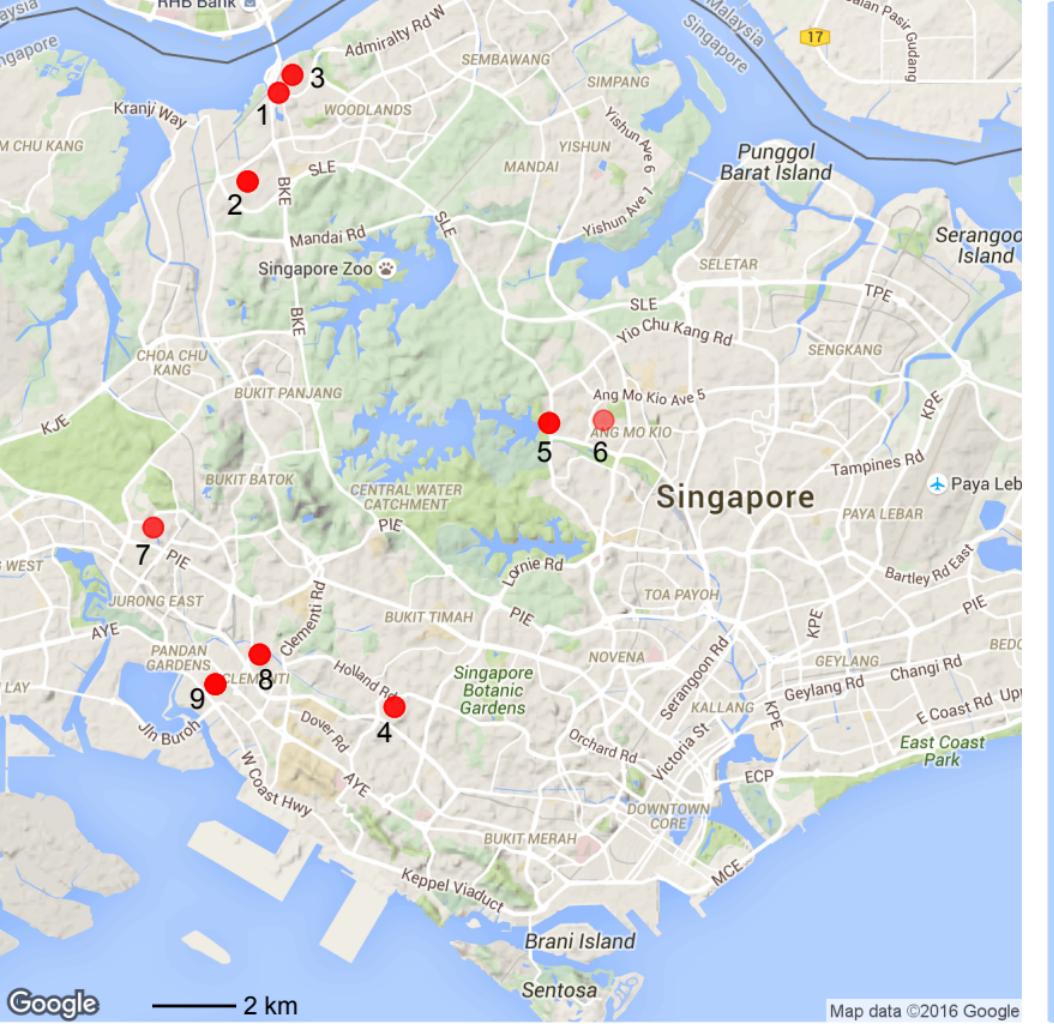
466 Singapore and Townsville. Reference: Wang, J. 2002. An estimator for pairwise relatedness using

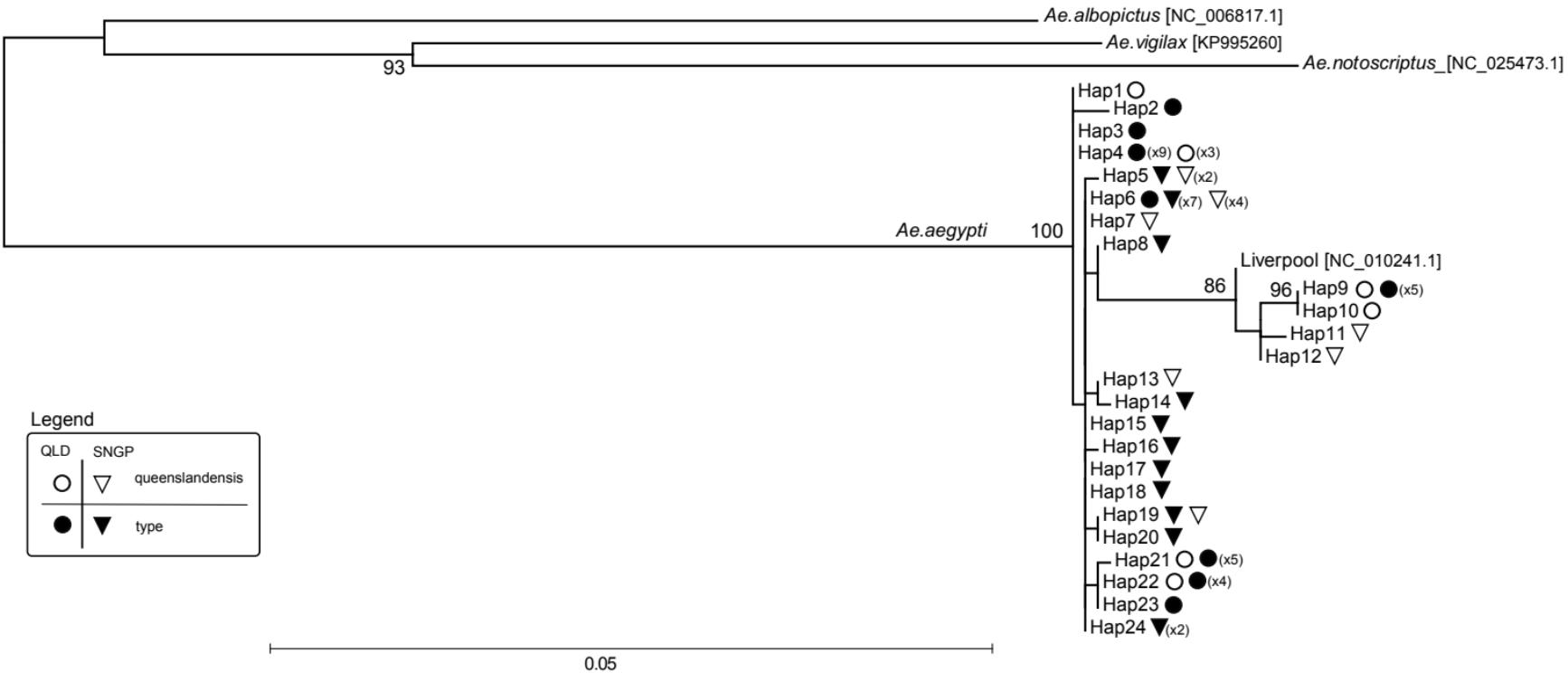
467 molecular markers. Genetics 160: 1203–1215.

468 **S1 Fig. Data quality parameters.** Boxplots of per-individual values for the proportion of uniquely

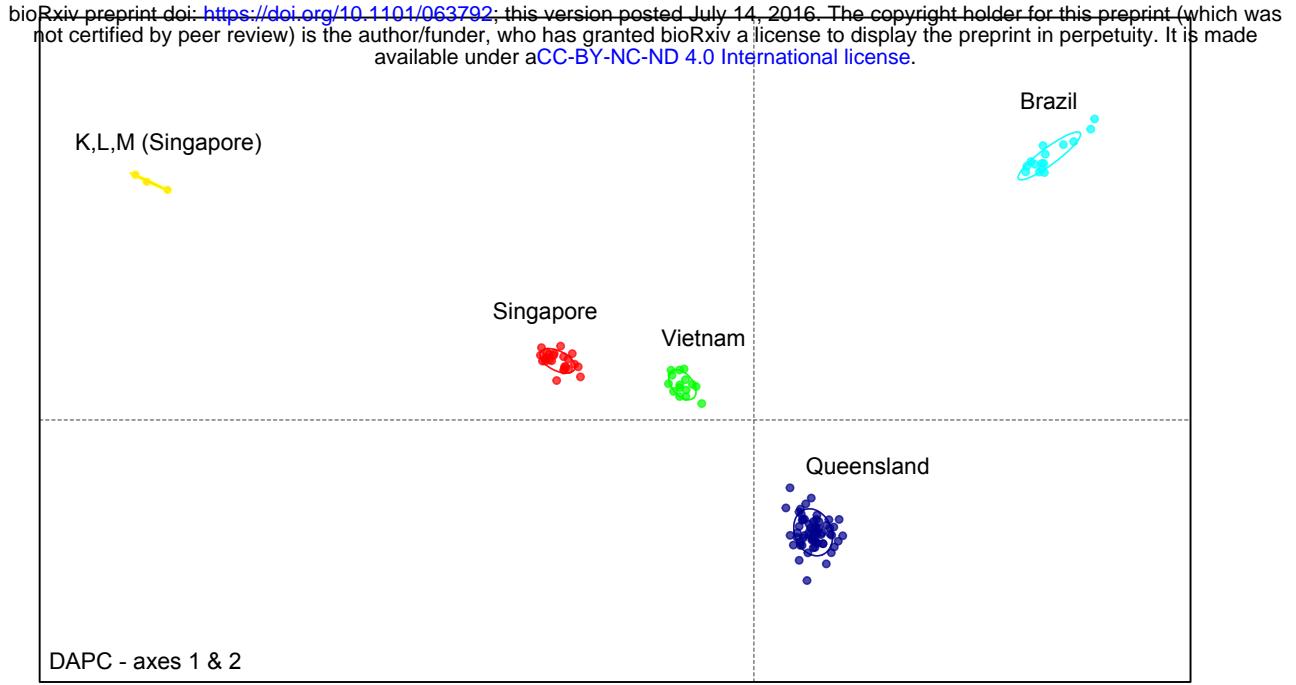
469 aligned reads, RAD tag read depth, proportion of heterozygous loci, proportion of missing data for

470 *Aedes aegypti* from Singapore (left) and Queensland (right).

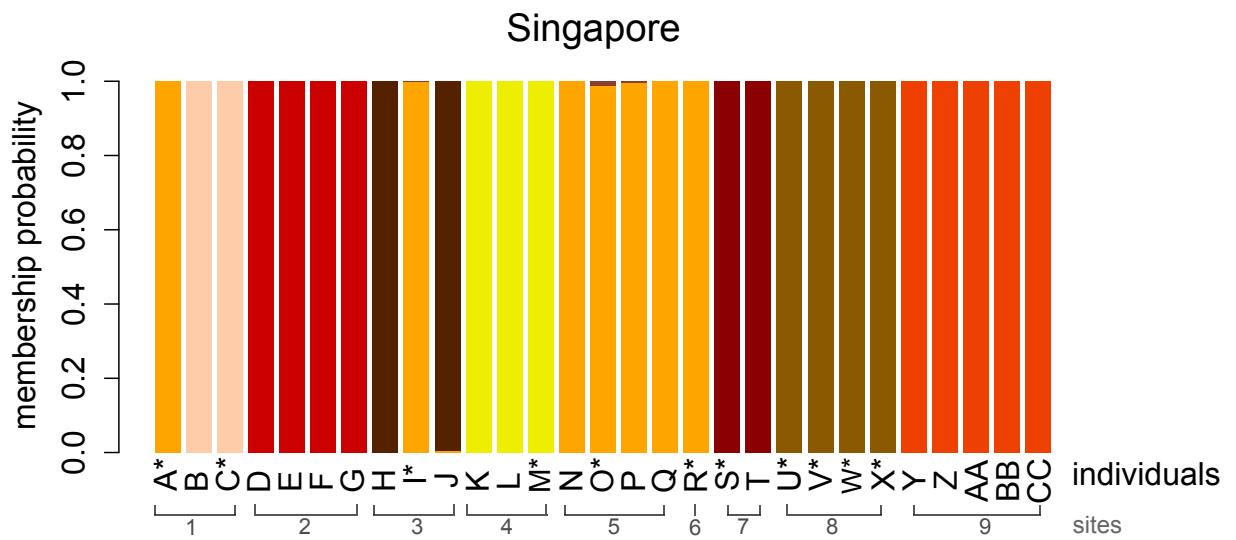




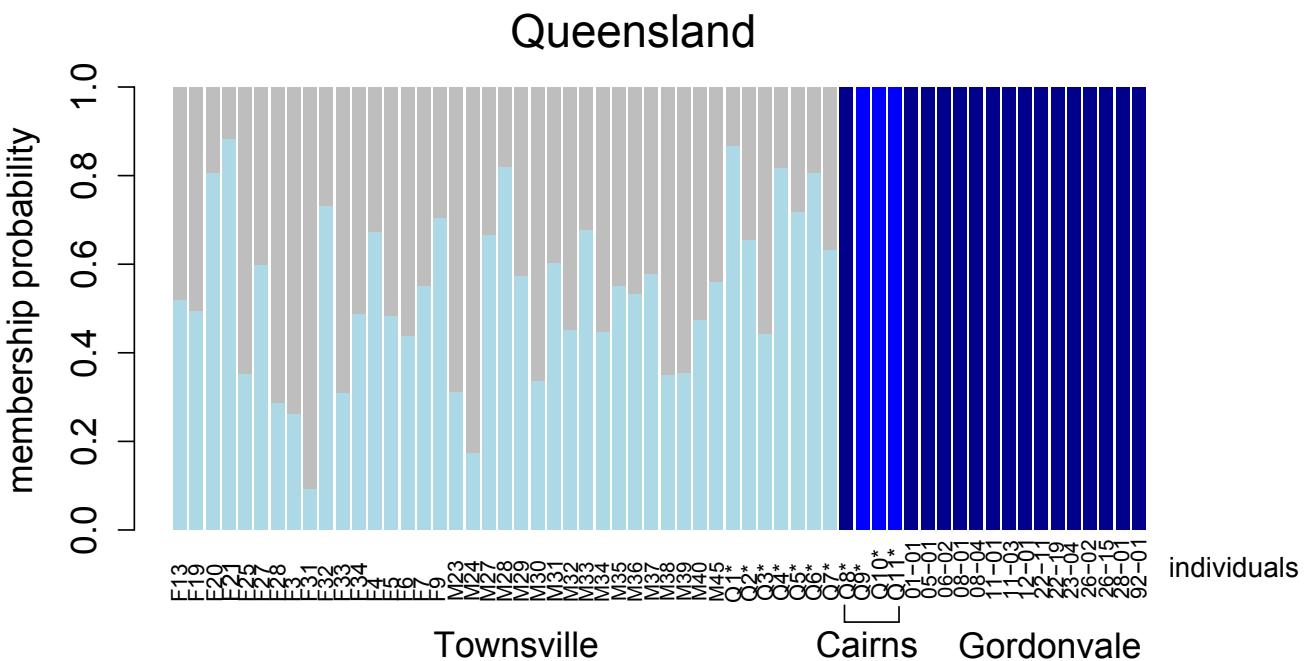
a)



b)

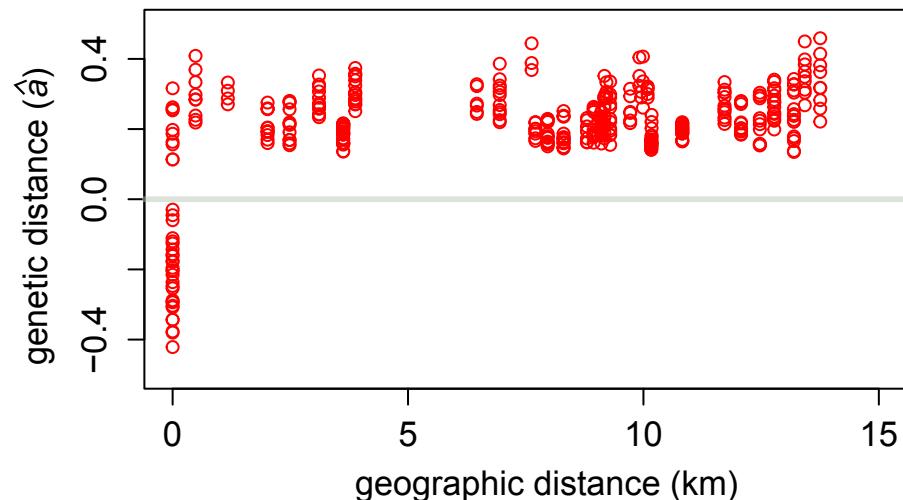


c)

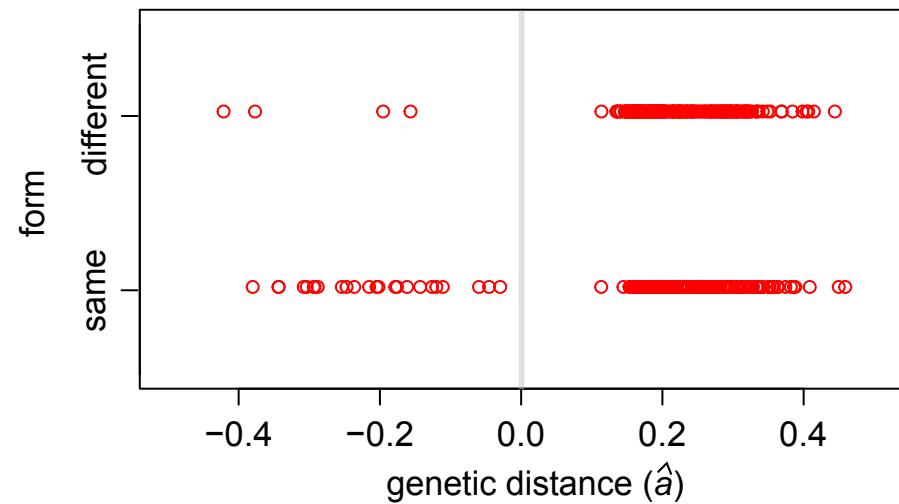


SINGAPORE

Geographic vs Genetic distance

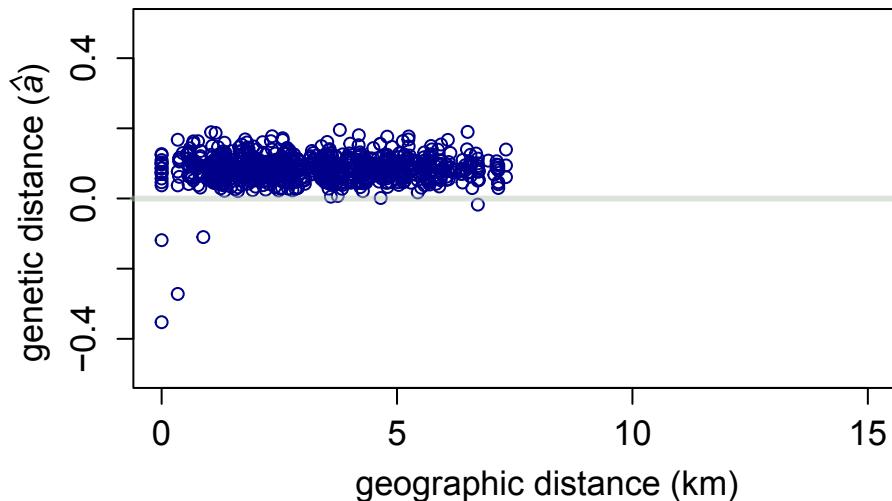


Genetic vs Color distance



TOWNSVILLE

Geographic vs Genetic distance



Genetic vs Color distance

