The impact of biological invasion and genomic local adaptation on the geographical distribution of *Aedes aegypti* in Panama

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Abstract

Local adaptation is an important consideration when predicting arthropod-borne disease risk because it can impact on vector population fitness and persistence. However, the extent that vector populations are adapted to local environmental conditions and whether this can impact on species distributions generally remains unknown. Here we find that the geographic distribution of *Ae. aegypti* across Panama is rapidly changing as a consequence of the recent invasion by its ecological competitor, *Aedes albopictus*. Although *Ae. albopictus* has displaced *Ae. aegypti* in some areas, species coexist across many areas, raising the question: What biological and environmental factors permit population persistence?. Despite low population structure and high gene flow in *Ae. aegypti* across Panama, excepting the province of Bocas del Toro, we identify 128 candidate SNPs, clustered within 17 genes, which show a strong genetic signal of local adaptation. This putatively adaptive variation occurs across relatively fine geographic scales with the composition and frequency of candidate adaptive loci differing between populations in wet tropical environments along the Caribbean coast and the dry tropical conditions typical of the Pacific coast of Panama. Temperature and vegetation were important predictors of adaptive genomic variation in *Ae. aegypti* with potential areas of local adaptation occurring within the Caribbean region of Bocas del Toro, the Pacific coastal areas of Herrera and Panama City and the eastern Azuero Peninsula. Interestingly, several of these locations coincide with areas where *Ae. aegypti* and *Ae. albopictus* co-exist, suggesting that *Ae. aegypti* could have an adaptive edge under local environmental conditions that impacts on inter-specific competition with *Ae. albopictus*. Our results guide future experimental work by suggesting that locally adapted *Ae. aegypti* are able to persist on invasion by *Ae. albopictus* and, as a consequence, may fundamentally alter future arborviral disease risk and efforts to control mosquito populations.

Author Summary

Local environmental adaptation of mosquito vectors can alter the landscape of arthropod-borne disease by impacting on life history traits that increase their relative fitness thus promoting population persistence. We have identified a number of genomic loci in *Ae. aegypti* from Panama that exhibit a signal of natural selection associated with variation in the environment. Loci with a signal of local adaptation are predominately partitioned between wet and dry tropical environments with variation largely impacted by temperature and vegetation indices. Local adaptation in tandem with changes in the geographic distribution of *Ae. aegypti* due to the recent invasion of its ecological competitor, *Ae. albopictus,* has the potential to alter the landscape of arborviral disease.

Introduction

The establishment and persistence of vectors within new geographic locations poses a serious threat from emerging and endemic arboviral diseases [1,2]. For example, shifts in the distribution of ticks and *Culex* mosquitoes are linked to the rise of West Nile Virus and tick-borne encephalitis viruses within North America [3–5]. In addition, the introduction of invasive *Aedes* mosquitoes has facilitated the recent spread of Zika and Chikungunya viruses throughout the Americas [6,7]. Although introduced vector populations are unlikely to be at their fitness optimum when first confronted with a new environment, local adaptation may play a large role in disease dynamics as vectors adapt to their environment, increase their relative fitness and acquire new traits, thus potentially increasing the threat of human arboviruses. However, local environmental adaptation has not yet been characterised for any *Aedes* mosquito.

The importance of adaptation for human disease is exemplified in *Aedes aegypti*'s evolution to human commensalism and the establishment of a number of arboviruses worldwide [8]. This mosquito has undergone behavioural and genetic changes in comparison to its ancestral African form, including the evolution of house-entering behaviour and a preference for human odour and blood-feeding [9–11]. The adaptation of *Ae. aegypti* to exploit human environments has allowed for the spread of zoonotic arboviral diseases from forest animals to humans and promoted invasiveness through human-assisted dispersal [8]. Another human commensal, *Aedes albopictus*, is similar to *Ae. aegypti* across many ecological axes. The tiger mosquito has expanded from Asia within the last ~40 years and is now also globally distributed [12]. In many locations, *Ae. albopictus* has displaced resident *Ae. aegypti* [13,14], but the factors that facilitate co-occurrence are still unclear [15]. Identifying the abiotic and biotic factors important in *Aedes* species interactions, particularly whether the two *Aedes* mosquitoes coexist is critical. These interactions are likely to fundamentally reshape the arboviral disease landscape worldwide.

Here we characterize genome-wide variation in *Ae. aegypti* across Panama and use this data to explore the interplay between invasion history, the potential for local adaptation, and ecological change. Panama provides an ideal opportunity to begin to understand how these factors interact and, ultimately, affect the disease landscape by impacting on *Aedes* species distributions. Panama is a small country, measuring just 772 kilometres East to West and 185 km North to South, but provides a wealth of contrasting climatic conditions and discrete environments. This is largely owing to its situation as a narrow isthmus flanked by the Caribbean Sea and Pacific Ocean as well as the Cordillera Central mountain range, which acts as a North-South divide. Panama is also a hub of international shipping trade, providing an important route of *Aedes* mosquito invasion into the Americas. Panama's worldwide connections have potentially facilitated multiple introductions of the invasive *Ae. aegypti* mosquito dating back to the 18th century in association with the global shipping trade [8,16,17]. In addition, the Pan-American highway bisects the country, stretches almost 48,000

102 km throughout mainland America and provides important conduit for human-assisted dispersal of
103 *Aedes* mosquitoes [13,18].
104
105 We first investigate how genomic variation in *Ae. aegypti* is distributed across Panama. Secondly,
106 we evaluate the historical and current geographic distributions of both this mosquito and *Ae.*
107 *albopictus*. *Aedes albopictus* was first documented in Panama in 2002, providing the opportunity to
108 study how the interactions between the two species play out across a heterogeneous landscape.
109 Finally, we investigate whether local environmental adaptation could play a role in *Aedes* population
110 dynamics by identifying loci with a genomic signal of local adaptation that are associated with
111 discrete environmental conditions. These genomic regions might allow *Ae. aegypti* populations to
112 persist in competition with invading *Ae. albopictus*. How this scenario plays out in Panama will
113 provide insight into global species interactions and the spatial heterogeneity of viral transmission.
114
115 **Results**
116
117 **Characterisation of sequence variation in *Ae. aegypti*.** We processed 70 *Ae. aegypti* individuals with
118 hybridisation capture-based enrichment from 14 localities widespread across Panama. An average
119 number of 27,351,514 reads were mapped to the genome for each individual with 62 % of these
120 targeted to the designed capture regions. The mean coverage depth per individual was
121 approximately 74X. After applying stringent quality filters, 371,307 SNP's were identified throughout
122 all captured regions for downstream analyses.
123
124 **Global and local population structure of *Ae. aegypti*:** Our large SNP dataset allowed us to examine
125 population structure across both global and local scales. Comparison of global population structure
126 was achieved by comparing a subset of 2,630 of our SNP's from Panama that were shared with a
127 previously acquired *Ae. aegypti* SNP dataset from 26 other countries worldwide [19–23].

128 FastStructure analysis revealed that the number of model components and model maximum
129 likelihood were maximised by assigning each individual to between K=4-6 populations (S1 Fig).
130 Similar to that reported previously, we found that the new world variation is composed of an
131 admixture of populations distinct from African and Asian sources at higher values of K [19,20] (S1
132 Fig). Individuals from Panama, Costa Rica, Colombia, the Caribbean islands and populations from
133 Arizona and Texas in South western USA were consistently composed of a similar composition
134 throughout each possible value of K (S1 Fig). Thus, *Ae. aegypti* from Panama were genetically similar
135 to those found throughout the Americas, consistent with a strong geographic component to the
136 distribution of genetic variation across the world [24].
137
138 Within Panama, the much larger dataset including all 371,307 SNP's, highlighted significant
139 population structure. There were two major genomic clusters (Fig 1B & 1C) that distinguished
140 individuals from Bocas del Toro province in the western Caribbean region compared to individuals
141 from all other regions across Panama, revealed on both FastStructure and PCA analysis of all SNP's.
142 In addition, *Ae. aegypti* from the eastern Azuero Peninsula also appeared somewhat genetically
143 discrete (Fig 1C). All areas of Panama, including sampling locations on the Azuero Peninsula had
144 similar levels of heterozygosity and therefore the population differences we observed are not
145 expected to result from a recent population bottleneck or from insecticide spraying treatment,
146 which is irregularly applied during epidemics to target adults only within the urban areas of Panama
147 (Fig 1B).
148
149 **The geographical distribution of *Ae. aegypti* in response to invasion by *Ae. albopictus*.** To
150 understand how the recent introduction of *Ae. albopictus* has shaped populations of *Ae. aegypti*
151 across Panama over the last decade, we coupled historical surveys of mosquito populations with
152 intensive sampling of focal populations over the last three years. Over the sampling period, there
153 has been significant changes in the geographic distribution of *Ae. aegypti* (Fig 2). Analysis of all

occurrence data throughout all years revealed that the presence of *Ae. aegypti* is positively and significantly associated with the presence of *Ae. albopictus* (GLM, Z = 18.93, *d.f* = 7390, P = 0.000), reflecting the ecological similarity of the two species and the continued expansion of *Ae. albopictus* throughout much of *Ae. aegypti*'s historical range. Although both species now co-exist in many areas throughout Panama, areas in the wet and humid western Azuero Peninsula, rural Chiriquí, Veraguas and the province of Panamá outside of Panama City (Gamboa and Chilibre), were solely inhabited by *Ae. albopictus*. This includes regions, from which *Ae. aegypti* was previously documented by the health authorities, confirming that *Ae. albopictus* has indeed replaced *Ae. aegypti* in these areas. The replacement of *Ae. aegypti* by *Ae. albopictus* was further supported by a general decrease in the proportion of positive sampling sites. This proportion has decreased for *Ae. aegypti* since 2005 from ~50 % to ~20 %, while the presence of *Ae. albopictus* has increased from 0 to ~65 % (S2 Fig). *Ae. aegypti* continued to be found in high abundance in Bocas del Toro and Darién, where *Ae. albopictus* has only recently arrived (Darién) or has not yet been documented (Bocas del Toro).

Genomic evidence for local adaptation in *Ae. aegypti* in response to environmental heterogeneity across Panama. The spatial environmental heterogeneity of Panama coupled with the recent population changes associated with the introduction of *Ae. albopictus* provides a framework to ask if there was any evidence that local adaption of *Ae. aegypti* might allow population persistence. If so, we would expect populations of *Ae. aegypti* to harbour genomic loci with a signal of selection that are correlated to the local environmental conditions. These loci are expected to be present in regions of *Aedes* co-existence.

As a first step, we applied redundancy analysis (RDA) to jointly identify candidate outlier loci and to assess how candidate variation was partitioned among the different environmental variables. In this analysis, we tested a number of environmental variables including Normalized Difference Vegetation Index (NDVI), average rainfall, average humidity, average minimum and maximum temperature, and

human population density. RDA identified 1,154 candidate SNP's with a genomic signal of local adaptation, which we used to visualise putatively adaptive variation on ordination plots. Overall, there was a partitioning of alleles dependant on dry tropical and wet tropical conditions. For example, the position of sampled individuals on the RDA ordination plots, in relation to the depicted environmental variables, revealed that the candidate genotypes of *Ae. aegypti* from the wet tropical regions of Almirante and Changuinola in Bocas del Toro province were positively associated with humidity and average rainfall. Those from the wet tropical region of Chiriquí Grande in Bocas del Toro were also positively associated with increasing NDVI vegetation index and negatively associated with higher temperatures (Fig 3A). In comparison, the candidate genotypes of individuals from dry tropical regions of Panamá province (i.e., Princesa Mía, Lluvia de Oro, Nuevo Chorrillo), Los Santos (i.e., La Villa de Los Santos, Pedasí), Darién (i.e., Metetí) and David in Chiriquí province were somewhat positively influenced by both temperature variables and negatively associated with wet and vegetated conditions. Putatively adaptive variation in individuals from the province of Colón (i.e., Sabanitas and Portobelo), locations which receive high rainfall but higher temperatures and lower vegetation cover than in Bocas del Toro province, were associated with intermediate temperature and vegetation conditions.

RDA is robust in detecting adaptive processes that result from weak, multilocus effects across a range of demographic scenarios and sampling designs [25]. However, a proportion of the 1,154 candidate loci identified through this single analysis were likely false positives. Thus, rather than reflecting local adaptation, the strongly skewed frequency differences could be reflective of demographic processes such as hierarchical population structure, isolation by distance, allele surfing on range expansion and background selection, as well as, coincidental associations of allele frequencies to environmental variation or even covariance to other environmental factors not included in the analysis [26]. To further refine our identification of putatively adaptive loci, we identified candidates using two additional methods, PCAdapt and Latent Factor Mixed Model

206 analysis (LFMM). Both are considered less sensitive to confounding demography due to their ability
207 to account for population structure or unobserved spatial autocorrelation in the data [27]. The three
208 methods identified different numbers of putatively adaptive loci. For example, compared to the
209 1,154 outlier SNP's identified by RDA, PCAdapt identified 352 SNP's (S3 Fig), whereas LFMM analysis
210 identified 3,426 outlier SNP's with a signature of selection widespread across the genome and
211 associated with the environment respectively (S4 Fig).
212
213 Across all three methods there were 128 SNP's consistently identified as outliers, providing greater
214 confidence that these loci are located in or close to genomic regions possibly involved in local
215 adaptation. These candidate SNPs fell into 15 distinct clusters, suggesting that linkage disequilibrium
216 was driving some of the observed patterns (S5 Fig). The 128 SNPs fell into 17 genes, 11 of which are
217 annotated as involved in structural functions, enzyme activity and metabolism (S1 Table). None of
218 these genes are known to be involved in the development of insecticide resistance in populations of
219 *Aedes* mosquitoes.
220
221 We further narrowed down which of the environmental variables contributed most to the
222 partitioning of genomic variation using a combination of Generalised Dissimilarity Modelling (GDM)
223 and Gradient Forests (GF) analyses. Both approaches allowed us to visualize the allelic turnover of
224 these putatively adaptive loci in relation to each environmental variable. The environmental
225 variables that contributed the greatest variance to both GDM and GF models on analysis of the 128
226 candidate loci were minimum and maximum temperature (S2 Table, S6 Fig). GDM analysis revealed
227 that an increase in average minimum temperature accompanied a large change in putatively
228 adaptive allele frequencies, visualised as a smooth curve accumulating in a steeper incline at the
229 higher temperature range (Fig 3B). In comparison, GF turnover plots show a steeper incline at the
230 mid-range for both average minimum and maximum temperature (S7 & S8 Fig). GDM analysis also
231 revealed a distinct frequency change in putatively adaptive alleles with increasing NDVI vegetation

index, although the change in allele frequency was relatively minor compared to that of minimum temperature (Fig 3B). In comparison, a low to negligible difference in allele frequency was observed in association with average rainfall, average humidity and human population density. Therefore, the variation in putatively adaptive allele frequencies between populations from dry tropical and wet tropical environments of Panama appears largely driven by differences in temperature and NDVI vegetation index.

The geographic distribution of candidate adaptive alleles in relation to the present distribution of *Ae. albopictus*. Across our 128 candidate SNP's**,** we used GDM and GF analysis to visualise the change in frequencies across Panama, and therefore the geographical landscape features which increase or decrease the genomic signature of local adaptation in relation to the environment. GDM analysis presented a smoother turnover in the geographical distribution of putatively adaptive loci than that of putatively neutral loci as indicated by a smoother transition in the colour palette between proximal geographic locations (Fig 4A & 4B). For example, there was similarity in the colouring and therefore allele composition between wet tropical regions along the Caribbean coast (i.e., the mainland/islands of Bocas del Toro, Chiriquí, and both the inland and Caribbean coastal regions stretching from Bocas del Toro through Veraguas to Colón). Similarly, there was greater continuity between dry tropical areas including David in Chiriquí, the eastern Azuero Peninsula (i.e., La Villa de Los Santos and Pedasí), the Pacific coastal regions stretching from the Azuero Peninsula through Coclé to Panamá, and the Darién (i.e., Metetí), indicating that these environments share putatively adaptive alleles. Patterns in the data were less distinct for GF analysis but the geographical distribution of putatively adaptive variation agreed with the GDM analysis in that there was a continuity in the allele composition between the eastern Azuero Peninsula and dry tropical Pacific coastal regions, distinct from the wet tropical regions along the Caribbean coast (S9 Fig).

258 Allele frequency turnover as predicted under neutral conditions and a scenario of local adaptation
259 involving the candidate loci were compared across geographical space to identify locations that
260 show the greatest disparity. These reflected the populations within Panama expected to be
261 experiencing a strong genomic signal of local adaptation. Their comparison revealed multiple
262 patches of potential local adaptation widespread across Panama, with a palpable patch occurring in
263 the Azuero Peninsula, as indicated by a high distance between the patterns of predicted
264 compositional allele frequency turnover (Fig 4C). A genomic signal of local adaptation was not
265 identified in the region of Bocas del Toro. Since this region has a strong population structure and
266 distinct climate within Panama, it is likely that the co-correlation of population structure and
267 environmental variation across our sampling design hindered the inference of possible local
268 adaptation in this case. This conclusion was supported by FastStructure analysis of the 128
269 putatively adaptive loci, which revealed that *Ae. aegypti* from the wet tropical region Bocas del Toro
270 has a distinct allele composition composed of alleles assigned to a distinct composition of K
271 populations, including unique alleles in addition to those shared broadly across the dry tropical
272 regions of Panama (S10 Fig). Although the Talamanca mountain range was documented as a natural
273 geographical barrier to dispersal across the region of Bocas del Toro for some *Anopheles* mosquitoes
274 [28], this was not expected to hinder gene flow in *Ae. aegypti*, since human-assisted movement of
275 this mosquito occurs via the local transport network [18]. Partitioning of the genomic data into 6=K
276 populations revealed that Sabanitas on the Caribbean coast, which is subject to intermediate climate
277 conditions, shared some of the distinct alleles present in Bocas del Toro. Moreover, individuals from
278 the Azuero Peninsula, the driest and least vegetated region of Panama, were also somewhat distinct
279 from other sampled regions since they had reduced levels of admixture.
280
281 Comparison of the geographical distribution of putatively locally adapted *Ae. aegypti* as revealed by
282 GDM analysis and the species distribution data revealed that both *Ae. aegypti* and *Ae. albopictus*
283 tended to co-occur in regions where *Ae. aegypti* have divergent candidate loci, despite evidence for

284 species replacement elsewhere (Fig 5). Notably long-term co-existence was documented with the
285 Pacific regions of Panama City, Coclé, the eastern Azuero Peninsula and potentially David in Pacific
286 Chiriquí, where patches of local adaptation in *Ae. aegypti* were identified.
287
288 **Discussion**
289
290 We combined genomic and ecological data to investigate whether *Ae. aegypti* have a signal of local
291 adaptation to the environment, and to investigate whether this variation could influence species
292 persistence on invasion by the recently introduced competitor *Ae. albopictus*. We first documented
293 how fine-scale genomic variation within *Ae. aegypti* is distributed across a complex environment
294 [11]. On a regional scale, Panamanian populations of *Ae. aegypti* are genetically similar to other
295 Central and Caribbean American populations highlighting high dispersal potential and recent gene
296 flow in this invasive species; however, this similarity belies a more complex local genomic
297 architecture. Across Panama, genomic variation was not structured randomly, with the isolated
298 Bocas del Toro region showing significant overall population differentiation. Across the rest of
299 Panama, populations are more homogeneous suggesting high levels of gene flow, likely facilitated by
300 the dispersal of *Aedes* mosquitoes in used tyres that are traded along the Pan-American highway
301 [18]. Nonetheless, a subset of genomic variation was differentially distributed with evidence of
302 localised adaptation across a relatively small number of SNPs and over a relatively fine geographical
303 scale. Genomic variation in these SNPs was strongly correlated with temperature and NDVI
304 vegetation index. Both these abiotic variables were previously identified as important in predicting
305 large-scale *Aedes* distribution patterns [12]. Temperature is important for egg laying, development
306 and survival of *Ae. aegypti* in larval habitats [29] and likely to promote selection to thermal tolerance
307 at the adult stage to resist diurnal and inter-seasonal variation [30]. Vegetation is considered an
308 important variable that contributes to oviposition cues [31], feeding dynamics [32] and microhabitat
309 characteristics such as local moisture supply and shade [33,34]. Although correlational, the genomic

310 patterns raise an important question: Is population persistence in the face of an ongoing invasion by
311 *Ae. albopictus* the result of local adaptation?
312
313 The possibility of climatically adapted populations of *Ae. aegypti* is not without precedence. Data on
314 a wide range of organisms with varying dispersal abilities [35–41] demonstrate that even well-
315 connected populations can adapt to environmental differences and habitat heterogeneity across
316 narrow spatial scales. Similar to other landscape genomics studies on plants [42–44], insects [45]
317 and vertebrates [46], we have found a signal of local environmental adaptation across a small
318 number of loci. The inability to identify more putative regions under selection may be the result of
319 the analytical difficulties weak multilocus signatures from the genomic differentiation introduced by
320 genetic drift and demography [25,47]. However, selection on just a few loci with large effects is
321 expected when migration is high since large effect loci are better able to resist the homogenising
322 effects of gene flow [48]. These few regions are expected to have a strong impact on fitness in one
323 environment over the other because the allele with the highest fitness is expected to spread to all
324 populations if this condition is not met [48].
325
326 The pattern of recent population distribution change in *Ae. aegypti* in response to the introduction
327 of *Ae. albopictus* was also consistent with local adaption. Similar to studies from the South Eastern
328 USA and Bermuda [49–54], we have found that species co-occurrence is condition dependant, with
329 the long-term persistence of *Ae. aegypti* occurring throughout many areas despite invasion by *Ae.*
330 *albopictus* 7 to 15 years ago. Previous studies have suggested *Ae. aegypti* is able to persist in dry
331 climate conditions and/or urban environments because they are better adapted [15 and refs within].
332 The eggs of *Ae. aegypti* are more tolerant to higher temperatures and desiccation in comparison to
333 the eggs of *Ae. albopictus*, which are able to survive lower temperatures through diapause [15,55].
334 Consistent with the prediction that local environmental adaptation contributes to *Ae. aegypti*
335 persistence, we found putatively adaptive loci within the dry tropical Pacific regions of Chiriquí

336 (David), Coclé, the eastern Azuero Peninsula and provincial Panamá where both species co-occur.
337 There was also genetic evidence for local adaptation in the isolated wet tropical region of Bocas del
338 Toro and Costa Abajo near Colon, but whether this variation will allow *Ae. aegypti* to resist invasion
339 by *Ae. albopictus* is unknown, given that *Ae. albopictus* was only recorded in Costa Abajo in 2018 and
340 has not yet reached Bocas del Toro. Alternatively, the present patterns of species co-existence could
341 simply reflect the abilities of *Aedes* species to exploit a different ecological niche without involving
342 local environmental adaptation. Nonetheless, this doesn't reconcile the fact that *Ae. aegypti* is no
343 longer found in many areas where candidate adaptive alleles were not detected. Our findings
344 provide us with clear testable hypotheses moving forward. For example, if the genomic regions we
345 identified are adaptive, then we expect genotype specific survival under different environmental
346 conditions, which can be tested in a common garden with reciprocal transplant experiment in the
347 presence of an ecological competitor.
348
349 The presence of locally adapted populations of *Ae. aegypti* could have a significant impact on the
350 future arboviral disease landscape. Climate variables, most notably precipitation and temperature
351 associated with altitudinal and latitudinal clines, are able to drive population differentiation in both
352 *Anopheles* mosquitoes and *Drosophila* flies [56–59]. In the former, the Anopheles gambiae species
353 complex is hypothesised to have radiated through ecological speciation driven by adaptation to
354 aridity and in response to larval habitat competition. This has led to a series of ecotypes with semi-
355 permeable species boundaries [60]. The resulting differences among ecotypes in anthropophily and
356 the adult resting behaviour has a significant impact on malaria transmission risk [61]. Thus, at the
357 most basic level, differentially adapted population variants of *Ae. aegypti* across Panama, could have
358 different abilities to vector arboviral disease [62–67]. In addition, environmental adaptation would
359 need to be considered in spatially predictive models. Currently, species geographic distribution or
360 disease prediction models incorporate a set of environmental parameters coupled with a predicted
361 outcome on mosquito biology and abundance without considering adaptive response [68,69].

362 Assuming that the whole population will respond to environmental precursors as a homogenous unit
363 is erroneous when local adaptation is present and considering adaptability as a parameter, in
364 combination with the environmental response, will improve the accuracy of future projections
365 [12,70]. Furthermore, the presence of locally adapted populations threatens the efficiency of gene
366 drive systems aimed at promoting disease resistance within mosquito populations. This is because
367 environmental differences between sites, as well as physical geographical barriers, will restrict
368 mosquito dispersal and therefore limit the spread of beneficial alleles or inherited bacteria [71].
369 However, if locally adaptive alleles are well-characterised, this knowledge could also potentially be
370 exploited. A more tailored approach could improve gene drive efficiency, since locally adapted
371 individuals are theoretically more likely to survive to pass on the intended benefit to the next
372 generation.
373
374 If local environmental adaptation is proven to influence *Aedes* co-occurrence, then this could
375 facilitate the emergence of sylvatic arboviral disease. *Ae. albopictus* is an opportunistic feeder, able
376 to utilise a wide range of peri-domestic habitats outside of its native range [72,73] and the species
377 could act as an efficient bridge vector for emergent zoonotic diseases from the forest [73]. The
378 addition of the specialised commensal *Ae. aegypti*, provides the opportunity for any emergent
379 epidemic to spread and be maintained within the urban population [8–11]. This scenario may have
380 happened recently, where yellow fever virus re-emerged from forest reservoirs in Brazil [74]. In this
381 case, the re-emergence was a function of both ecological changes and vaccination frequency. Unlike
382 yellow fever virus, there is no vaccination against dengue, Zika, or chikungunya, reinforcing the role
383 that ecological changes will likely play in future epidemics.
384
385 **Conclusion:** The identification of small number of putatively adaptive genomic intervals provides
386 exceptional experimental opportunities to determine 1) If these regions are in fact under selection,
387 2) How selection might be acting if our hypothesis is true. Defining species fitness in association with

388 our candidate loci will allow us to untangle the interplay between genomic process, the
389 environment, species competition and how these resolve the spatial distribution and abundance of
390 medically important *Ae. aegypti*. Advances will be used to improve the accuracy of disease
391 prediction models and characterise the genomic basis of adaptations with the capacity to alter the
392 epidemiological landscape.
393
394 **Materials and Methods**
395
396 **Mosquito Sampling** *Aedes* mosquitoes were collected through active surveillance and oviposition
397 traps placed across 35 settlements and nine provinces of Panama from 2016 and 2018 (S3 Table).
398 Immature stages of *Aedes* from each trap were reared to adulthood as separate collections in the
399 laboratory, identified using the morphological key of Rueda *et al.* [75] and stored in absolute ethanol
400 at -20°C.
401
402 **Genomics data.** DNA was extracted from 70 *Ae. aegypti* (Fig 1A), representing populations subject to
403 different environmental conditions using a modified phenol chloroform method [76]. To identify
404 putative regions involved in the local adaption of *Ae. aegypti*, 26.74 Mb of the AaeL3 exome were
405 targeted for capture. For each sample, 100 ng DNA was mechanically sheared to fragment sizes of ~
406 350-500 base pairs and processed to add Illumina adapters using the Kapa Hyperprep kit. Amplified
407 libraries were assessed on a Bioanalyser and Qubit before 24 uniquely barcoded individuals each
408 were pooled to a combined mass of 1 µg to create three libraries of 24 individuals for hybridization.
409 Sequence capture of exonic regions was performed on each pool according to the NimbleGen
410 SeqCap EZ HyperCap workflow and using custom probes designed by Roche for the regions we
411 specified (S1 Dataset).
412

413 Low quality base calls (<20) and Illumina adapters were trimmed from sequence ends with
414 TrimGalore [77], before alignment to the *Ae. aegypti* AaeL5 reference genome with Burrows-
415 Wheeler aligner [78]. Read duplicates were removed with BamUtil. Sequence reads were processed
416 according to the GATK best practise recommendations, trained with a hard-filtered subset of SNPs
417 using online recommendations (https://gatkforums.broadinstitute.org/gatk/discussion/2806/howto-
418 apply-hard-filters-to-a-call-set). SNPs were called with a heterozygosity prior 0.0014 synonymous to
419 previously reported values of theta [24]. Filters applied to the resulting SNP dataset included a
420 minimum quality of 30, minimum depth of 30, minimum mean depth of 20, maximum 95 % missing
421 data across individuals and a minor allele frequency ≥ 0.01. Indels were additionally removed to
422 reduce uncertainty in true variable sites by poor alignment to the reference genome.
423
424 **Environmental Data.** Climate variables including average rainfall, average humidity, average
425 minimum and maximum temperature difference, average minimum temperature and average
426 maximum temperature were obtained for each collection site from interpolated raster layers
427 composed of values reported by Empresa de Transmisión Eléctrica Panameña (ETESA). All available
428 data points from 2010 to 2017 representing 50-60 meteorological stations across Panama were
429 averaged. NDVI vegetation indexes for Panama were obtained from MODIS Vegetation Indices 16-
430 day L3 Global 250m products (NASA, USA) with values averaged over all available images from 2010
431 to 2017. Human population density values were obtained from Instituto Nacional de Estadística y
432 Censo 2010. Raster layers for Generalised Dissimilarity Models and Gradient Forest analyses were
433 created for each variable by inverse distance interpolation across the extent of Panama to a
434 resolution of 0.05 pixels in QGIS version 2.18.15 [79].
435
436 The collinearity and covariance of the environmental data was assessed the R Stats package [80].
437 One variable, average minimum and maximum temperature difference was removed from analysis
438 because it was highly correlated with the other temperature variables (>0.8 correlation coefficient).

439 All other variable comparisons had a correlation coefficient below 0.7 and were retained for analysis
440 (S4 Table).
441
442 **Analysis of population structure.** FastStructure was also applied to all loci to infer the ancestry
443 proportions of K modelled populations [81]. The optimal model complexity (K*e) was chosen to be
444 two populations using the python script chooseK.py and confirmed by a PCA of all loci performed
445 with the R package PCAdapt [82](see Analysis of local environmental adaptation below).
446 FastStructure analysis with a logistic prior was also applied to 2,630 SNP's shared with a worldwide
447 SNP dataset representing *Ae. aegypti* from 26 different countries [19–23].
448
449 **Species distribution analysis.** Historical data on species distributions from 2005 to 2017 was
450 obtained from the Panamanian Ministry of Health (MINSA). This data was obtained through active
451 surveillance of settlements regardless of time of year. A binomial Generalised Linear Model was
452 performed to test for an association between the presence and absence of *Ae. aegypti* with the
453 presence and absence of *Ae. albopictus* using the species occurrence data obtained from both
454 MINSA and our own sampling using the Stats package in R [80]. The proportion of sampling sites
455 positive for *Ae. aegypti* and *Ae. albopictus* presence from 2005 through 2018 were calculated by
456 combining our mosquito surveillance data with that obtained from MINSA. Maps of the species
457 distribution of *Ae. aegypti* and *Ae. albopictus* were produced in QGIS [79].
458
459 **Analysis of local environmental adaptation.** To identify loci with a signal of selection differentiated
460 across regional environmental conditions, three methods with different underlying algorithms and
461 assumptions were applied. Two EAA approaches, redundancy analysis (RDA) and latent factor mixed
462 models (LFMM) were implemented to identify loci associated with environmental predictors. RDA
463 uses multivariate regression to detect genomic variation across environmental predictors as
464 expected from a multilocus signature of selection [25]. In comparison, LFMM is a univariate

465 approach which models background variation using latent factors, while simultaneously correlating
466 the observed genotype frequencies of individuals to each environmental variable [83]. Before
467 implementation of RDA, missing genotype values were imputed as the most common across all
468 individuals. Loci which are strongly correlated to environmental predictors were then identified
469 through multivariate linear regression of the genomic data with the environmental variables
470 followed by constrained ordination of the fitted values as implemented with the RDA function in the
471 R package Vegan [84]. Multi collinearity of the data was verified to be low as indicated by genomic
472 inflation factors ranging from 1.31-5.80. Candidate loci were then identified as those which
473 contribute most to the significant axes as determined by F statistics [85]. To account for population
474 structure, we applied two latent factors to our LFMM analysis based on the PCA and scree plots of
475 proportion of explained variance produced with PCAdapt (see below). As per recommendations to
476 improve power, we filtered our data before analysis to include only sites with an MAF > 5 % and
477 analysed our data with five separate LFMM runs, each with 20,000 cycles after an initial burn-in
478 period of 10,000 cycles. Median Z-scores were calculated from the five runs and Bonferroni
479 corrected for multiple tests, before loci significantly correlated with environmental variables were
480 identified based on a false discovery rate of 10 % using the Benjamini-Hochberg procedure outlined
481 in the program documentation. Visualisation of the Bonferroni adjusted probability values for the
482 loci correlated with each environmental factor revealed that the majority of probability values were
483 at a flat distribution while those correlated with environmental variables were within a peak close to
484 0, indicating that confounding factors were under control. In addition to the two EAA analyses,
485 PCAdapt was applied to identify loci putatively under selection pressure because they deviate from
486 the typical distribution of the test statistic Z [82]. Two K populations were chosen to account for
487 neutral population structure in the data based on scree plots of the proportion of explained variance
488 and visual inspection of PCA and STRUCTURE plots which revealed that populations from the region
489 of Bocas del Toro form a distinct genomic grouping (Fig 1, S11 Fig).
490

491 **Distribution of candidate loci across geographical space.** Both putatively neutral and adaptive
492 genomic variation was visualised across geographic space using Generalised Dissimilarity Modelling
493 (GDM) and Gradient Forests (GF) analysis [86]. GDM is a regression-based approach which maps
494 allelic turnover using non-linear functions of environmental distance in relation to F_{ST} genetic
495 distance. In comparison, GF uses a machine learning regression tree approach. Through subsetting
496 the genomic and environmental data, the algorithm determines the degree of change for each allele
497 along an environmental gradient and calculates the resulting split importance. Allelic turnover was
498 investigated for both a set of reference SNP's, not expected to be under selective pressure, as well
499 as the loci putatively involved in local adaptation as jointly identified by LFMM, PCAdapt analysis and
500 RDA. SNP's representative of neutral variation included those not identified as a candidate outlier by
501 any of the three methods. So as to reduce the dataset and avoid inclusion of strongly linked loci,
502 SNP's were thinned by a distance of 10 KB, an appropriate cut-off as indicated by the calculation of
503 R_2 linkage disequilibrium values for this dataset (S12 Fig).
504
505 To perform GDM analysis, the R program StAMPP [87] was used to generate the input FST matrixes
506 and BBmisc [88] used to rescale the distances between 0 and 1. Environmental and genetic distance
507 data were converted to GDM format and analysis performed using the R package GDM [89]. GF
508 analysis [90] was implemented on a matrix of minor allele frequencies for each SNP for both the
509 reference and candidate datasets, obtained through VCFtools [91]. Both SNP datasets only included
510 loci present in at least 11 of 14 populations to ensure robust regression. The model was fitted with
511 2,000 regression trees, a correlation threshold of 0.5 and variable importance computed by
512 conditional permutation with a distribution maximum of 1.37. Both analyses included Moran's
513 eigenvector map (MEM) variables which are weightings derived from the geographic coordinates of
514 sampling locations used to model unmeasured environmental variation and geographic distance
515 analogous to latent factors [86]. To visualise the patterns in allele variation across space, PCA was
516 used to reduce the variability into three factors. The difference in genomic composition was mapped

517 across the landscape of Panama by assigning the three centred principle components to RGB
518 colours; similar genomic composition across space is indicated by a similar colour shade. The
519 difference in allele turnover for the reference and candidate dataset was characterised to explore
520 whether allelic turnover was greater than predicted under neutral expectations. Exploration was
521 achieved by comparing and visualising the compositional turnover of allele frequencies for both
522 reference and candidate SNP dataset across geographical space using a Procrustes superimposition
523 on the PCA ordinations.
524
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531
532 **Author contributions**
533
534 The study was designed by KLB, WOM and JRL, and conducted by KLB, WOM and JRL. Sample
535 preparation and laboratory procedures were conducted by KLB. KLB and JRL performed the data
536 analysis and figure preparation. KLB wrote the manuscript with contributions from WOM and JRL.
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Ethics Statement

Field research conducted in Panama was authorised under the Ministerio de Ambiente permit number SE/A-67-2016-9.

Data availability

SNP data is available in the Sequence Read Archive data repository XXX

Competing interests

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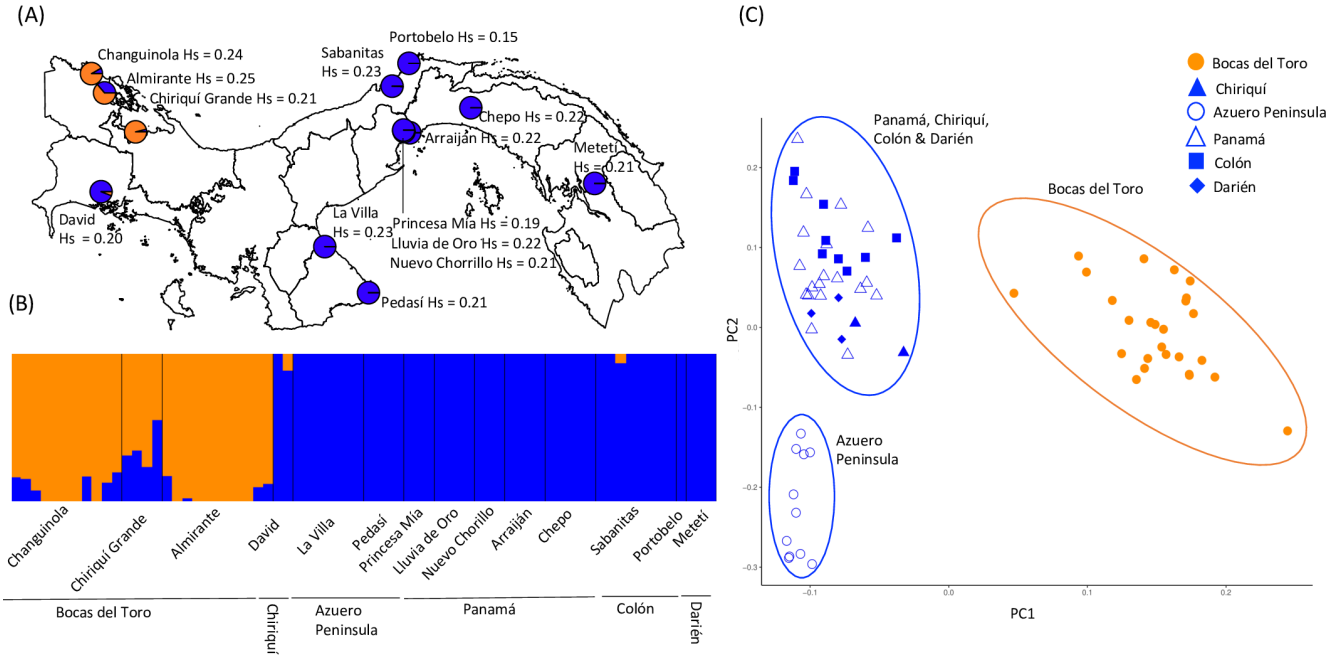

Fig 1. Strong local population structure within the context of regional homogeneity and global population structure: (A) FastStructure plot of K=6 populations comparing 2,630 SNP's in individuals of *Ae. aegypti* from Bocas del Toro and the rest of Panama to genetically similar populations originating from South-western USA, Caribbean islands, Costa Rica and Columbia. FastStructure assigns each individual to one or more K populations, as indicated by its colour. Genetically similar populations share the same colour or similar admixture composition on comparison. (B) Admixture proportions of K=2 populations in relation to sampling locations and population heterozygosity Hs of *Ae. aegypti* across Panama as determined by FastStructure for 371,307 SNP's. (C) PCA of all *Ae. aegypti* SNP's grouped by region.

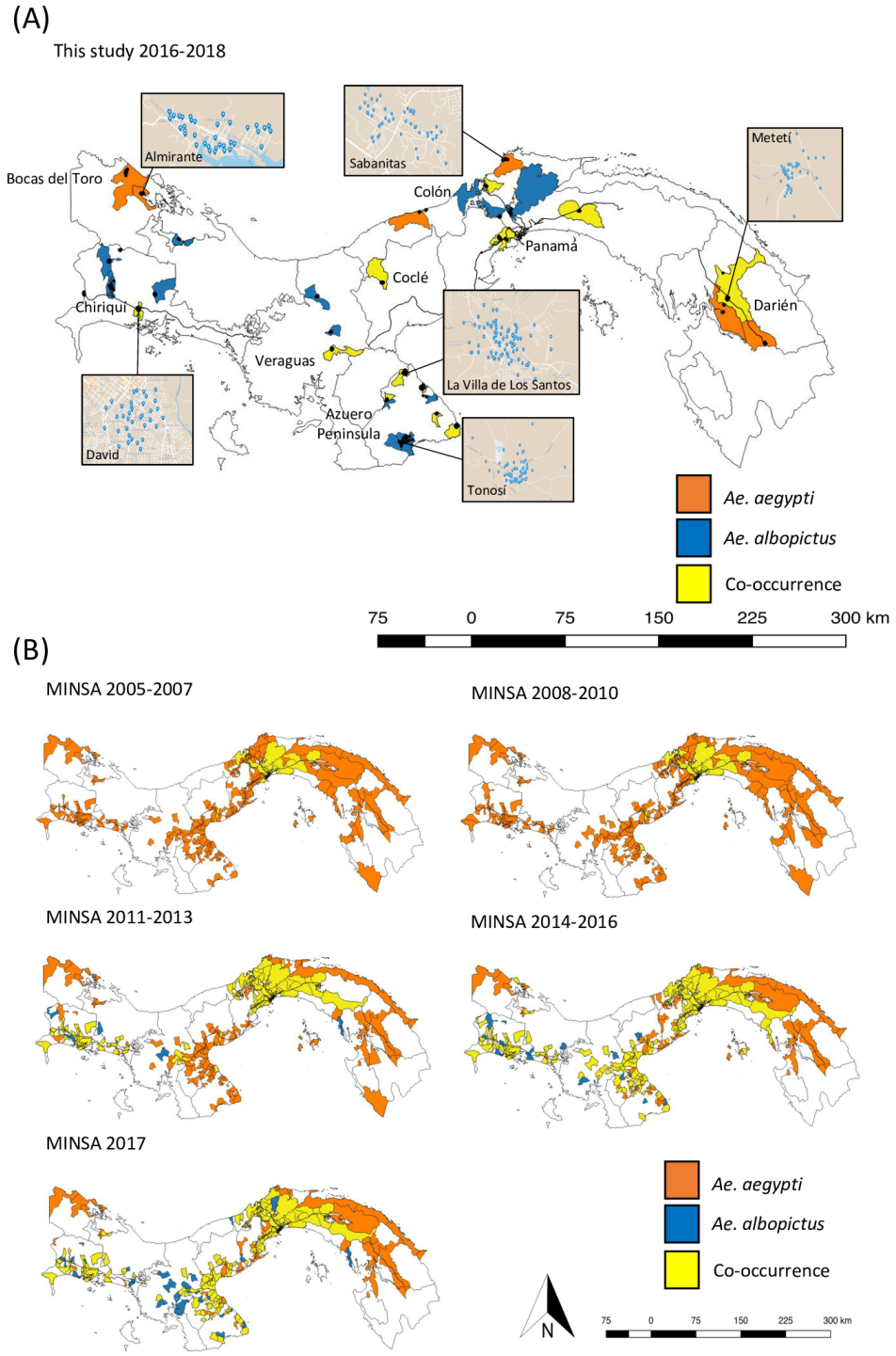

Fig 2. The species replacement and co-occurrence of *Aedes* species is condition dependent: (A) The presence of *Ae. aegypti* (orange), *Ae. albopictus* (blue) and species co-occurrence (yellow) recorded by extensive sampling with both active surveillance and oviposition traps during the wet season months from 2016 through to 2018 in comparison to (B) Species occurrence data recorded from 2005 through 2017 through active surveillance by the Ministry of Health in Panama.

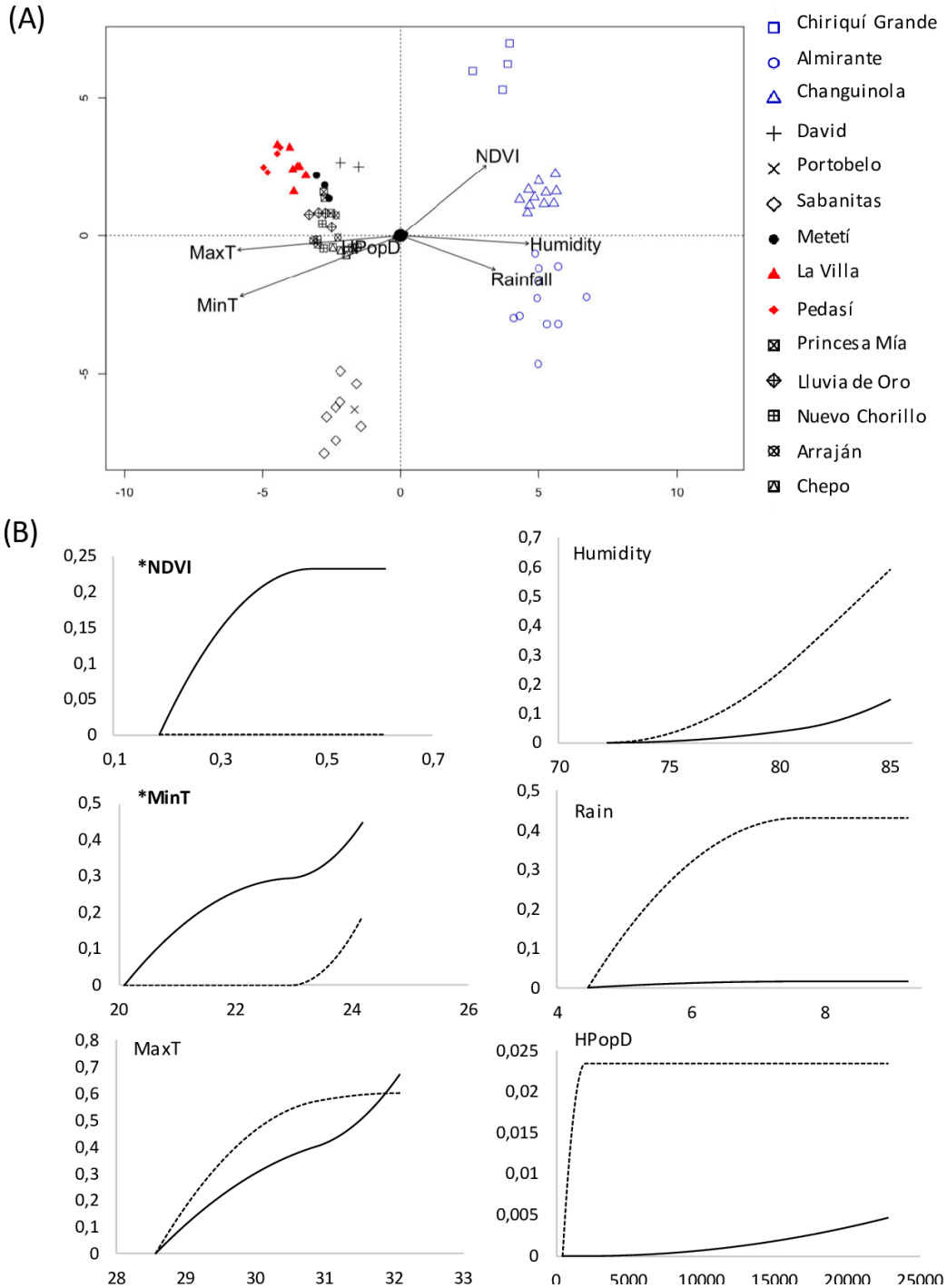

Fig 3. Putative adaptive variation in *Ae. aegypti* is partitioned between wet and dry tropical environments and associated with temperature and vegetation indices: (A) Ordination triplot of the first two constrained ordination axes of the redundancy analysis representing SNP's either positively or negatively associated with the environmental variables as depicted by the position of the arrows. *Ae. aegypti* from the wettest region (blue) and driest region (red) are highlighted. (B) Compositional turnover splines for GDM analysis for the reference loci that are putatively neutral (dashed line) and the 128 candidate loci with a signal of local adaptation (black line) in association with NDVI vegetation index (NDVI), average minimum temperature (MinT), average maximum temperature (MaxT), average humidity (Humidity), average rainfall (Rain) and human population

863 density (HPopD). A change in allele frequency relative to the reference loci is seen in the putatively
864 adaptive alleles with increasing values of NDVI and MinT, marked in bold with an asterix.
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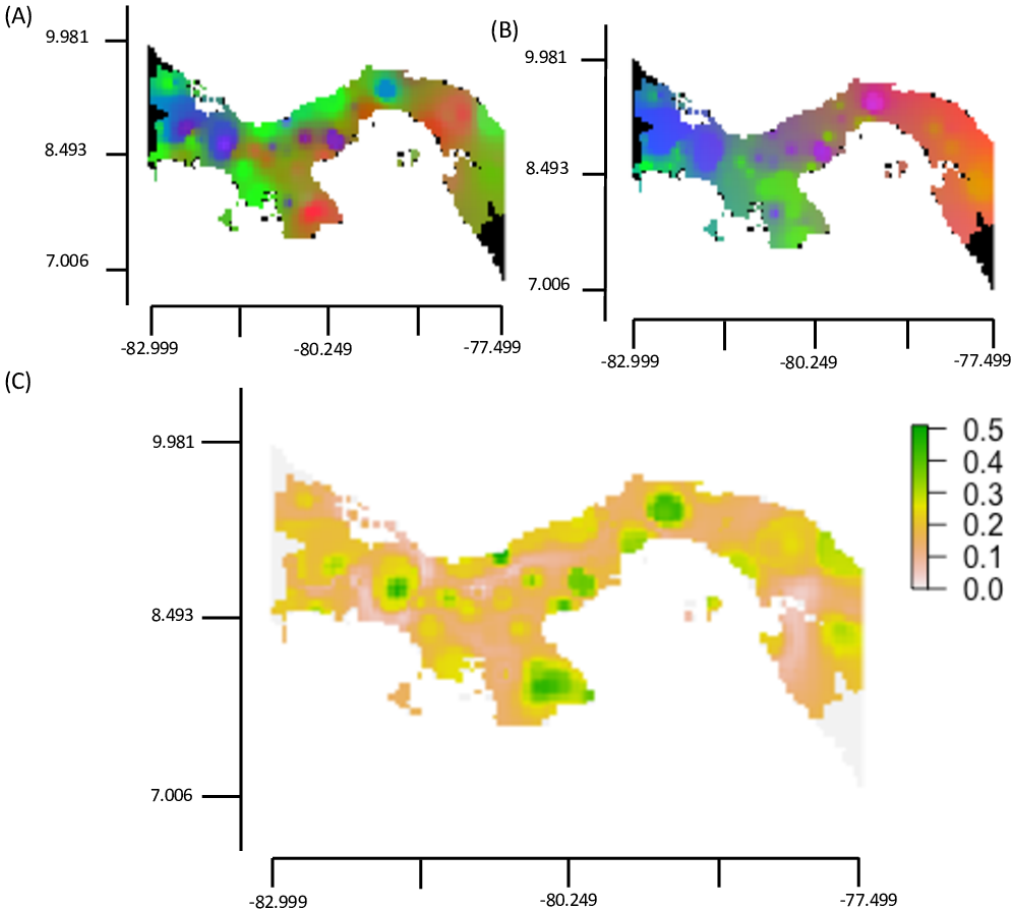

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868 **Fig 4. Patches of local adaptation are revealed on comparison of putative neutral and adaptive**
869 **variation across geographical space:** RGB maps of compositional allele frequency turner over across
870 geographical space based on GDM analysis of (A) putatively neutral loci, (B) the 128 candidate loci
871 with a signal of local adaptation and (C) the difference in allele compositional turnover between the
872 putatively neutral reference loci and putatively adaptive candidate dataset using a Procrustes
873 superimposition on the PCA ordinations. On maps (A) and (B), the dissimilarity between allele
874 composition is depicted by an increasing divergent colour spectrum. Locations with a similar allele
875 composition are a similar colour. On map (C), the scale represents the distance between the allele
876 compositional turnover of the reference and candidate SNP datasets, with higher distances
877 indicating areas that are potentially experiencing local adaptation.
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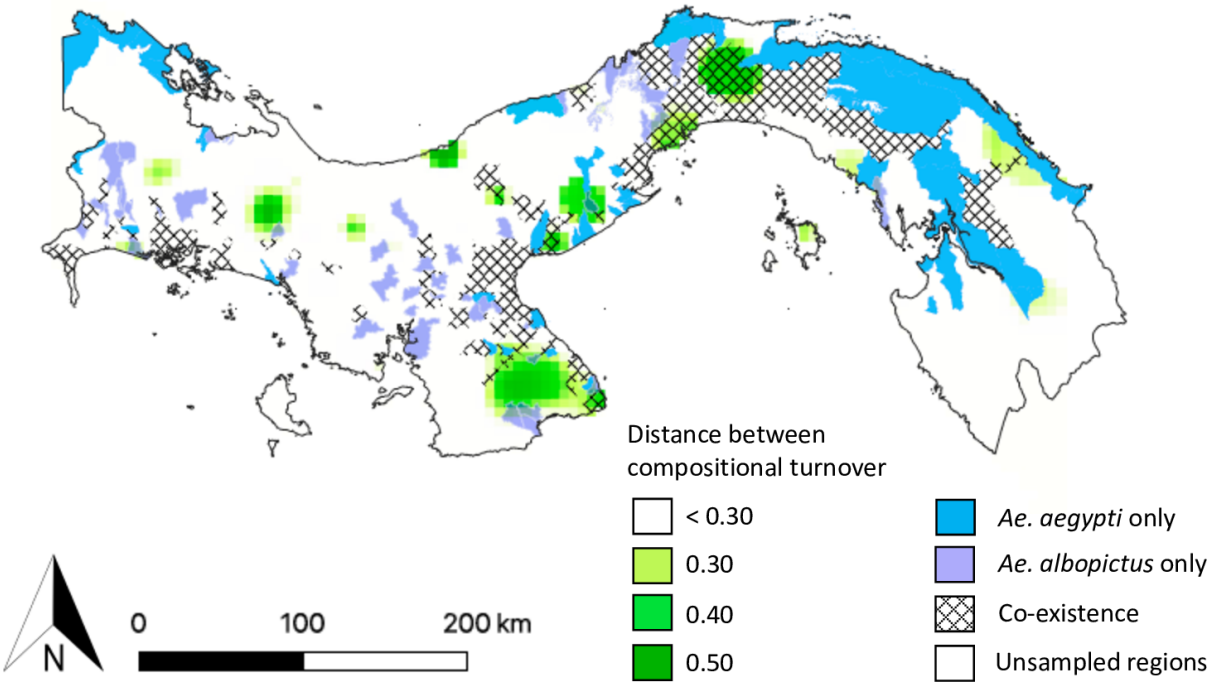

Fig 5. Putatively adaptive loci are predicted in *Ae. aegypti* populations within areas of species co-existence. Corregimientos with *Aedes* co-occurrence (dashed areas) are shown based on the most recent species distributions recorded during this study in 2018 and in other sampled regions by MINSA in 2017. The co-occurrence data is overlaid onto the compositional turnover of the reference and candidate SNP dataset from Figure 4., with values greater than 0.30 shown. Green coloured areas represent regions with a greater predicted distance between the allele composition of the reference and candidate datasets, indicating the potential presence of locally adapted *Ae. aegypti*. Corregimientos where only *Ae. aegypti* (blue) and *Ae. albopictus* (purple) were present are also indicated.