New Insights into the Population Structure of *Anopheles gambiae* s.s. in the Gulf of Guinea Islands Revealed by *Herves* Transposable Elements

Patrícia Salgueiro¹, Marta Moreno², Frédéric Simard³, David O’Brochta⁴, João Pinto¹

¹ Centro de Malária e outras Doenças Tropicais/UEl Parasitoligia Médica, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal; ² Centro Nacional de Medicina Tropical, Instituto de Salud Carlos III, Madrid, Spain; ³ MIVEGEC (Maladies Infectieuses et Vecteurs: Ecologie, Genetique, Evolution et Controle), UMR IRD24-CNRS290-UM1-UM2, Institut de Recherche pour le Développement, Montpellier, France; ⁴ Department of Entomology and The Institute for Bioscience and Biotechnology Research, University of Maryland, College Park, Rockville, Maryland, United States of America

Abstract

Transposable elements (TEs) are mobile portions of DNA that are able to replicate and spread in the genome of many organisms. TEs can be used as a means to insert transgenes in insects, being stably inherited throughout generations. *Anopheles gambiae* is the main vector of human malaria in Sub-Saharan Africa. Given the extraordinary burden this disease imposes, the mosquito became a choice target for genetic control approaches with the purpose of reducing malaria transmission. In this study, we investigated the abundance and distribution of *Herves* TE in *An. gambiae* s.s. from Cameroon and four islands in the Gulf of Guinea, in order to determine their genetic structure. We have detected a population subdivision between Equatorial Guinea islands and the islands of São Tomé, Príncipe and mainland. This partitioning associates more with political rather than geographic boundaries, possibly reflecting different mainland source populations colonizing the islands.

Introduction

Over the past recent years, transgenic technologies have been proposed for malaria vector control [1,2,3]. One of the approaches in development consists in altering the vectorial capacity of wild populations of *Anopheles* mosquitoes by delivering and spreading transmission-blocking transgenes (e.g. refractoriness to *Plasmodium* infection) [4,5]. This approach requires gene drive systems and transposable elements (TEs) were one of the first candidate tools to be considered for population replacement [6,7].

Transposons or TEs are mobile genetic sequences that can replicate and change their relative position within the genome [8]. Most TEs used for insect germline transformation are Class II elements that transpose by a direct DNA ‘cut-and-paste’ mechanism [7,9,10,11]. Within the genome of the main Afrotropical malaria vector *Anopheles gambiae* sensu stricto several TEs have been catalogued [12,13]. Among these, the *Herves* element is a transpositionally active Class II TE isolated from *An. gambiae* s.s. and found in the sibling species *Anopheles arabiensis* and *Anopheles merus* [14]. *Herves* was inferred to be transpositionally active in natural populations of *An. gambiae* based on the high frequency of intact, transposase-encoding *Herves* elements observed and highly polymorphic insertion sites [15].

The dynamics of TEs within populations is determined by the transpositional activity of the element, the strength of self- and host-regulatory mechanisms and the genetic structure of host populations [16]. Sequence and insertion-site polymorphisms have allowed TEs to be used as markers for population genetic studies [17], including in *An. gambiae* [18,19,20].

Islands have been considered as candidate sites for experimental releases of transgenic mosquitoes for vector control [21,22,23], because the effects of migration from neighboring regions not under control are expected to be minimal. In this context, the islands of the Gulf of Guinea (West Africa) are potential sites that might benefit from malaria control strategies based on transgenic vectors. These islands are volcanic in origin and extend from the coast of Cameroon (Bioko island, 32 km off shore) to 350 km west of the coast of Gabon (Amöbôn island, Figure 1). Both Bioko and Annobón are part of Equatorial Guinea. Between these two islands, the archipelago of São Tomé and Príncipe is found, which comprises two islands located 140 km apart and about 250 and 225 km, respectively, off the northwestern coast of Gabon. The main malaria vector on these islands is *An. gambiae* s.s. [24,25]. In the island of Bioko, as well as in mainland (Equatorial Guinea and Cameroon), both M and S molecular forms, considered as incipient species within *An. gambiae* s.s. [26], occur in sympatry.
In São Tomé, Príncipe and Annobón only the M form is present [24,27]. Populations of An. gambiae s.s. on Bioko island were found to be genetically differentiated from mainland populations, using microsatellite DNA markers [28]. However, Moreno et al. [27] found a higher degree of genetic isolation of Annobón island when compared with Bioko, which is closest to mainland. Similarly, it has been shown that populations of An. gambiae s.s. from the islands of São Tomé and Príncipe are genetically isolated from continental populations [29,30]. The later authors suggested two main colonization events for An. gambiae s.s. into these islands, coincident with episodes of intense human migration [30].

In the islands of the Gulf of Guinea, malaria remains a major cause of morbidity and child mortality. However, in recent years the situation has progressively changed. In São Tomé and Príncipe, a malaria control program has reduced the number of cases by half over the past decade [31]. This program included the use of Indoor Residual Spraying (IRS), Insecticide Treated Nets (ITNs) and artemisinin-combination therapies [32,33]. Similarly, the prevalence of childhood infection in the island of Bioko has been dropping considerably since the combined implementation of ITNs and IRS [34,35]. Under these circumstances, the implementation of a transgenic control component might be a promising complement to current malaria control measures, especially when insecticide-based measures are known to be difficult to sustain on the long-term due to issues such as financial constrains or insecticide resistance. However, there are still many issues related with the applicability of transgenic technologies to vector control that require further attention. One of these issues relates with the accurate estimation of the degree of isolation of island populations from mainland [30,36]. In addition, understanding the dynamics of TE elements in insular populations of An. gambiae is critical for developing predictive tools to be used in planning and managing release of transgenic mosquitoes.

In this study, we measured the abundance and site-occupancy frequency distribution of the Herves transposon in An. gambiae s.s. from Cameroon and the four islands in the Gulf of Guinea, in order to determine the genetic structure of this TE among physically isolated populations and relate with the potential applicability of transposons as gene drive systems for island vector populations.

**Materials and Methods**

**Mosquito DNA samples**

Mosquito specimens were collected from one continental population of Cameroon, and four island populations of the Gulf of Guinea: Bioko, Príncipe, São Tomé and Annobón (Table 1, Figure 1). Details on the localities and sampling methods have been described elsewhere [27,29]. Table 1 recalls collection dates and GPS position of collection sites, and Figure 1 displays the geographic linear distances between collection sites.

No specific permits were required for the described field studies. Mosquito collections were performed in villages either inside houses or outdoors. Informed verbal consent was obtained from household owners to perform the indoor sampling. This type of consent was chosen to avoid potential conflicts due to mistrust in signing official forms by the community. No specific ethics clearance was requested, since all mosquito collections were performed as part of routine mosquito surveillance implemented by the Malaria Control Programs of the regions sampled. The field studies did not involve endangered or protected species.

Extraction of DNA from single mosquitoes was performed by a phenol-chloroform protocol [37]. Species identification and determination of molecular forms were performed by PCR-RFLP.
Table 1. Collection data, site occupancy and genetic diversity of the sampled Anopheles gambiae populations.

| Country | island/mainland | Locality | Geographical coordinates | Year of collection | N  | k dun | hs     | 95% credibility interval of hs |
|---------|----------------|----------|--------------------------|-------------------|----|-------|--------|-----------------------------|
| S. Tomé and Principe | S. Tomé | Riboque | 0°19'N/6°43'E | 1997 | 14 | 15.7 | 0.299 | 0.238 0.356 |
|        | Principe | Rua dos Trabalhadores | 1°38'N/7°25'E | 2004 | 12 | 15.6 | 0.315 | 0.241 0.376 |
| Equatorial Guinea | Annobón | Annobón | 1°24'S/5°57'E | 2004 | 14 | 21.7 | 0.291 | 0.232 0.344 |
|        | Bioko | Malabo | 3°45'N/8°46'E | 2003 | 14 | 12.6 | 0.301 | 0.238 0.358 |
| Cameroon | mainland | Tiko | 4°05'N/9°21'E | 1999 | 14 | 15.4 | 0.273 | 0.210 0.331 |

N number of individuals analyzed by TE display per locality; k – Number of unique chromosomal sites containing Herves; dcn – Diploid copy number of Herves [39]; hs – gene diversity with credibility intervals calculated by a Bayesian approach as implemented in HICKORY; Hs – the mean within-population expected heterozygosity (= Nei's gene diversity within populations).

doi:10.1371/journal.pone.0062964.t001

Transposable element display

PCR-based transposable element display was performed as described by Subramanian et al. [15]. Images of the scanned TE display gels were printed and bands were assigned to a molecular weight based on their mobility relative to the size markers using a standard curve developed for each gel. All samples from each site were analyzed on the same gel, allowing a single standard molecular weight curve to be used to score each dataset. On the basis of the combined results of three TE display experiments, a band was called as present or absent if it was unambiguously present in at least two of the three replicates. Determining the presence of bands in this way resulted in a single scoring matrix that was then used in subsequent analyses.

Data Analysis

Site occupancy frequency distributions were estimated using the TE display data. To obtain estimates of element frequencies and copy numbers per haploid genome we assumed Hardy–Weinberg equilibrium and followed Wright et al. [39], where the mean number of elements per haploid genome was calculated as described in [40]. In order to discard any locus under selection, we looked for outlier loci using the Dfdist approach with 100.000 simulations [41,42] in MCHEZA program [43].

Expected heterozygosity hs, mean within-population expected heterozygosity Hs and their 95% credibility values, were calculated using a Bayesian approach with 250.000 generations after a burn-in of 50.000 generations implemented in HICKORY 1.1 [44].

We calculated pairwise Fst estimates as a measure of genetic differentiation based on [45] and tested isolation by distance through the analysis of the correlation coefficient of Fst/(1-Fst) over pairwise geographical distances using Mantel tests [46].

Population Structure in Anopheles gambiae

Results

Site occupancy

We detected Herves elements in all specimens analyzed by TE display (N=81). A total of 35 sites were detected. Within each sample, element copy number (dcn) ranged from 4.5 to 7.5 per diploid genome (Table 1). The maximum number of elements per individual ranged from six in Principe to nine in Annobon.

The TEs with the highest site-occupancy frequency were: Herves element with 160 bp (Hv160, showing 6–13 occurrences per sample) and Hv130 (6–14 occurrences per sample) (Table S1, supporting information file). We have found a single element (Hv157) common to all six samples. The sample with the highest number of exclusive sites was Annobon (7, Table 2). The two temporal samples from S. Tomé shared 13 out of 15 possible sites, and these had 10 sites in common with the continental sample (Cameroon, Table 2).

Table 2. Distribution of shared Herves sites among populations of An. gambiae in the Gulf of Guinea.

| Country   | Cameroon | Bioko | Principe | S. Tomé 97 | S. Tomé 98 | Annobon |
|-----------|----------|-------|----------|------------|------------|---------|
| Cameroun  | 2        | 1     | 3        | 1          | 0          | 7       |
| Bioko     | 5        | 1     | 1        | 1          | 0          | 0       |
| Principe  | 6        | 4     | 3        | 1          | 0          | 0       |
| S. Tomé 97 | 10      | 5     | 8        | 1          | 0          | 0       |
| S. Tomé 98 | 10      | 5     | 9        | 13         | 0          | 0       |
| Annobon   | 10       | 7     | 9        | 9          | 9          | 7       |

Values in the diagonal represent the number of sites exclusive to the correspondent population.
doi:10.1371/journal.pone.0062964.t002

According to a previously described protocol [38]. A total of 81 An. gambiae s.s. of the M molecular form were analyzed in this study. Sample sizes for each locality are shown in Table 1.

For a visual representation of genetic differentiation patterns, we performed a factorial correspondence analysis (FCA) on the multilocus genotype of each individual, using the option that takes into account the population of origin (FCA over populations), as implemented in GENETIX v. 4.05.2 [49].
Population structure of Herves element

The analysis with DfiST showed no evidence of sites under selection and all subsequent analyses were thus performed using the 35 detected sites. The estimates of genetic diversity (Hs) within each sample ranged from 0.273 in Cameroon to 0.315 in S. Tomé and Príncipe (Table 1). These values were comparable among samples, judging from the overlapping credibility intervals.

Pairwise FST-values ranged from −0.080 (Príncipe vs. S. Tomé 1997) to 0.574 (Cameroon and S. Tomé 1997 vs. Annobón). Comparisons between Annobón and the other samples showed the highest and the only significant FST estimates (Table 3). The only exception was the comparison of Annobón and Bioko. The two temporal samples from S. Tomé showed no genetic differentiation between them. The FST values among Sáo Tomé and Principe islands (S. Tomé and Principe) and the continental Cameroon were negative. The comparison of these samples with Bioko and Annobón showed much higher FST values. No statistically significant correlation was detected between genetic differentiation and geographic distance (Mantel Test, r = −0.080, P = 0.541).

Figure 2 shows the first two components of the FCA performed with five clusters corresponding to our samples. The first axis of variation (33.2% on the FCP) visibly splits Annobón and Bioko from the other samples. Also continental Cameroon seems to be closer to Sáo Tomé and Principe islands, where the two temporal samples from S. Tomé clustered together. The AMOVA results corroborate the FCA (Table 4). It shows a strong genetic differentiation between Equatorial Guinea islands and the other samples: 56.3% of the total variation was attributed to variation among the two groups and −2.7% of the variance was attributed to population variation within groups (Table 4, group A, P < 0.001).

Discussion

We have detected a genetic partitioning between Equatorial Guinea islands (Annobón and Bioko) from the populations of Sáo Tomé and Principe and mainland. This was evidenced by both FCA and AMOVA and also by pairwise FST estimates. Geographic distance was not correlated with genetic differentiation and thus isolation by distance does not seem to influence Herves genetic structure. These results only partially agree with previous microsatellite-based studies conducted in the same geographic region. Reimer et al. [28] obtained significant FST estimates between Bioko island and Cameroon M-form populations ranging from 0.038 to 0.058. These estimates were comparable to FST estimates (0.023–0.042) obtained between Bioko island and mainland Equatorial Guinea [27]. However, these values were much lower than those recorded between Annobón and mainland (0.187–0.196) or Annobón and Bioko (0.212) [27]. A comparison between populations of Sáo Tomé and Principe and Gabon also revealed high FST values (0.110–0.250) but this study compared M-form island populations with S-form in mainland, which could have inflated differentiation [50]. A subsequent microsatellite analysis based on 13 loci comparing M-form populations from the four Guinean Gulf islands with mainland samples from central and southern Africa also revealed higher differentiation of Annobón (FST: 0.229–0.271), intermediate for Sáo Tomé and Principe (FST: 0.103–0.255) and lowest in Bioko (0.030–0.090), in agreement with their geographic distance relative to mainland (Pinto et al., unpublished observations).

The patterns of population structure disclosed by the Herves TE seem to reflect more political rather than geographic relations among islands. Bioko and Annobón, which belong to the same country, appear more closely related to each other and highly differentiated from mainland or Sáo Tomé and Principe, in spite of being the nearest and farthest islands from mainland, respectively. A possible explanation could be a higher passive mosquito dispersal promoted by the movement of Equatorial Guinean nationals between islands. This hypothesis would imply a higher degree of human-mediated dispersal between islands of the same nationality (i.e. Bioko-Annobón; Sáo Tomé-Principe) rather than between islands in closer geographic proximity (e.g. Bioko-Principe). However, the differences in mosquito biodiversity between the islands do not support this hypothesis and rather suggest that human-mediated mosquito dispersal between islands is likely to be rare. In Sáo Tomé and Principe, the anopheline fauna is represented by only two species: An. gambiae s.s. M-form and Anopheles coustani [24,51]. In contrast, at least Anopheles melas, Anopheles funestus, Anopheles smithii and both M and S forms of An. gambiae s.s. have been recorded in Bioko island [28,52]. These differences are consistent with a general island biogeography model found to occur in the islands of the Guinean Gulf [53], which would otherwise be violated if human-mediated anopheline dispersal was a common event.

Another possibility is that the patterns of population structure reflect different historical mainland source populations from which Herves elements have been introduced into Sáo Tomé and Principe and Equatorial Guinea islands, respectively. Indeed, both historical and human mtDNA data suggest distinct origins and timings for the arrival of humans in Bioko (ca. 10,000 years BP) and Sáo Tomé island (during the 15th century) [54]. Given the synanthropic nature of An. gambiae s.s., it is likely that this mosquito also colonized the islands along with the human peopling [29,30]. Furthermore, sequencing analysis of mtDNA and rDNA markers suggested at least two independent introductions of An. gambiae s.s. in Sáo Tomé and Principe [30]. The study identified mainland populations from Ivory Coast/Ghana and from Angola as the most likely sources of introduction of this mosquito into Sáo Tomé and Principe. While no samples from these countries were included in the present analysis, the low levels of differentiation between Sáo Tomé and Principe islands and Cameroon may suggest An. gambiae M-form from Cameroon as a source population of Herves TEs in Sáo Tomé and Principe. These results reinforce the notion of multiple, yet sporadic, colonizations of this vector into these islands. Given these considerations, it is therefore likely that the strong differentiation revealed by Herves TE when comparing Bioko and Annobón with Sáo Tomé, Príncipe and Cameroon is the result of different continental origins of these.

Table 3. Estimates of pairwise genetic differentiation among populations of An. gambiae s.s. in the Gulf of Guinea, based on Herves elements.

|          | Came roon | Bioko | Prín cipe | S. To mé 97 | S. Tom é 04 | Annobón |
|----------|-----------|-------|----------|-------------|-------------|----------|
| Cameroon | -         | 0.488 | -        | -           | -           | -        |
| Bioko    | −0.080    | 0.472 | -        | -           | -           | -        |
| Príncipe | 0.077     | 0.488 | −0.080   | -           | -           | -        |
| S. Tomé  | −0.037    | 0.324 | −0.046   | −0.037      | -           | -        |
| S. Tomé  | −0.037    | 0.324 | −0.046   | −0.037      | -           | -        |
| Annobón  | 0.574     | 0.559 | 0.574    | 0.418       | -           | -        |

Significant FST estimates after Bonferroni correction in bold. doi:10.1371/journal.pone.0062964.t003
populations. In this context, the analysis of additional mainland samples would be required in order to further clarify the source populations of *An. gambiae* s.s. from Annobón and Bioko.

Finally, we cannot exclude the possibility that differences in the mode of evolution and inheritance may also contribute for the apparent discrepancy between patterns of population structure obtained by the *Herves* TE and by previous microsatellite based analyses. However, little is known about the mechanisms of regulation of *Herves* TE activity, which poses difficulties for comparing these markers.

The low differentiation observed between the two temporal samples from São Tomé provides no evidence for significant demographic changes in the mosquito population between 1997 and 2004 or for high rates of *Herves* transposition during this period. This result is consistent with the large estimates of current effective population size obtained for *An. gambiae* M-form in São Tomé island [29,50], comparable to those observed in mainland for the members of *An. gambiae* complex [55,56]. The relative demographic stability of *An. gambiae* suggested by this and previous studies in São Tomé and Príncipe is based on the analysis of samples for which collections pre-date the recent implementation of vector control measures that resulted in a significant malaria decrease on the islands [32,33]. In this context, it would be interesting to analyze the genetic variation of *Herves* TE in samples collected post-intervention as a means to assess the usefulness of TE in determining the impact of vector control in the genetic structure of *An. gambiae*.

In this study, locally fixed *Herves*-occupied sites were rare and sites occupied in only a few individuals were very common in the sampled populations. This suggests that *Herves* remains active within these island mosquito populations, as was also observed in previous analyses of other mainland African *An. gambiae* popula-

![Figure 2. Projection of 81 individual Herves genotypes of Anopheles gambiae on the principal axes of a factorial component analysis.](image)

Each colour corresponds to a sampled island population as indicated in the legend. Inertia percentage values are presented for each factorial component (FC1 and FC2).

doi:10.1371/journal.pone.0062964.g002

### Table 4. Apportionment of molecular variance measured among populations of *An. Gambiae*.

| Groups Tested | Total variance | % among groups | P    | % among populations within groups | % within populations |
|---------------|----------------|----------------|------|-----------------------------------|---------------------|
| A             | 0.30           | 56.3           | <0.001 | 2.7                              | 46.5                |
| B             | 0.21           | -11.8          | n.s.  | 44.5                             | 67.4                |
| C             | 0.26           | 48.3           | <0.05 | -3.1                             | 54.8                |
| D             | 0.23           | 41.8           | n.s.  | -2.74                            | 60.9                |

P represents the significance of the variation among groups (random > observed). Variation was partitioned in the tested structures.

A: group 1: Cameroon, Príncipe, S. Tomé (1997, 2004), group 2: Annobón and Bioko;
B: group 1: Cameroon, group 2: all other populations;
C: group 1: Cameroon, group 2: S. Tomé and Príncipe, group 3: Annobón and Bioko.
D: group 1: Cameroon, group 2: Príncipe, group 3: S. Tomé, group 4: Annobón and group 5: Bioko.

doi:10.1371/journal.pone.0062964.t004
tions [15,40]. Following WHO’s recommendations [22], transgenic mosquitoes use should involve several phases of field release under conditions that limit spread into the environment. In this context, remote islands are considered as suitable candidate sites. Accordingly, the most recent open-field trials to attempt transgenic mosquito vector control involved island mosquito populations [23]. In agreement with previous studies based on other genetic markers [27], the analysis of Herves TE suggests Annobón as probably the most adequate of the four islands of the Gulf of Guinea for experimental release of transgenic vectors: it is the most remote island with An. gambiae displaying the highest degree of genetic isolation and the highest amount of Herves insertion site polymorphism. However, the patterns of population structure on the islands of the Guinean Gulf disclosed in this study by Herves TE are more likely to reflect different origins of mosquito colonization rather than levels of contemporary gene flow. These results should therefore be interpreted with caution. Further analyses involving comparisons with more mainland samples and combining different genetic markers are required to clarify the degree of isolation of these islands.

References

1. Collins F, Sakai R, Vernick K, Paskewitz S, Seeley D, et al. (1986) Genetic selection of a Plasmodium refractory strain of the malaria vector Anopheles gambiae. Science 234: 607–610.

2. Miller LH, Sakai RK, Romans P, Gwadz RW, Kantoff P, et al. (1987) Stable integration and expression of a bacterial gene in the mosquito Anopheles gambiae. Science 237: 779–781.

3. Marshall JM, Taylor CE (2009) Malaria Control with Transgenic Mosquitoes. PLoS Med 6: e20.

4. Ito J, Ghosh A, Moreira LA, Wimmer EA, Jacobs-Lorena M (2002) Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. Nature 417: 452–453.

5. Kim W, Koo H, Richman AM, Seeley D, Vizioli J, et al. (2004) Ecotypic expression of a Cecropin transgene in the human malaria vector mosquito Anopheles gambiae (Diptera: Culicidae): effects on susceptibility to Plasmodium. J Med Entomol 41: 447–453.

6. Ribeiro JMC, Kidwell MG (1994) Transposable Elements as population drive mechanisms: specification of critical parameter values. J Med Entomol 31: 10–16.

7. Sinko SP, Gould F (2006) Gene drive systems for insect disease vectors. Nat Rev Genet 7: 427–435.

8. Craig NL, Craigie R, Geltell M, Lambowitz A, editors (2002) Mobile DNA II. Washington D.C.: American Society for Microbiology Press. 1204 p.

9. Fenneman BJ (1909) Eukaryotic transposable elements and genome evolution. Trends Genet 5: 107–107.

10. Fraser MJ (2012) Insect Transgenesis: Current Applications and Future Prospects. Annu Rev Entomol 57: 267–289.

11. Fryer BH, O’Brocha DA (2012) Transposable elements for insect transformation. In: Gilbert LI, editor. Insect Biochemistry and Molecular Biology. London, United Kingdom: Academic Press. 90–133.

12. Struchiner CJ, Massal E, Tu Z, Ribeiro JMC (2009) The tempo and mode of evolution of transposable elements as revealed by molecular phylogenies reconstructed from mosquito genomes. Evolution 63: 3136–3146.

13. Fernández-Medina RD, Struchiner CJ, Ribeiro JMC (2011) Novel transposable elements from Anopheles gambiae. BMC Genomics 12: 260–260.

14. Arentsburger P, Kim YJ, Orselli J, Alkhairi C, O’Brocha DA, et al. (2005) An active transposable element, Herves, from the African malaria mosquito Anopheles gambiae. Genetics 169: 697–708.

15. Subramanian RA, Arentsburger P, Atkinson PW, O’Brocha DA (2007) Transposable element dynamics of the hAT element Herves in the human malaria vector Anopheles gambiae s.s. Genetics 170: 2477–2487.

16. Decelis RE, Chen S, Biémont C (2005) The dynamics of transposable elements in structured populations. Genetics 169: 467–474.

17. Lepetit D, Brehm A, Foullet P, Biémont C (2002) Insertion polymorphism of retrotransposable elements in populations of the inular, endemic species Drosophila melanogaster. Mol Ecol 11: 347–354.

18. Barnes MJ, Lohe NF, Coulibaly MB, Sagnon NF, Costantini C, et al. (2005) SINE insertion polymorphism on the X chromosome differentiates Anopheles gambiae molecular forms. Insect Mol Biol 14: 353–363.

19. Boudevitch M, Simard F, Antonio-Nkondjio C, Jomo-Amene H, Fontenaille D, et al. (2007) Insertion polymorphism of transposable elements and population structure of Anopheles gambiae M and S molecular forms in Cameroon. Mol Ecol 16: 441–452.

20. Ennault C, Boulesteix M, Duchemin J, Kolff A, Chaudhri F, et al. (2009) High genetic differentiation between the M and S molecular forms of Anopheles gambiae in Africa. PLoS ONE 3: e19608.

21. Alphley L, Beard CB, Billingsley P, Coetzee M, Crisanti A, et al. (2002) Malaria control with genetically manipulated insect vectors. Science 298: 119–121.

22. WHO (2009) Progress and gaps for the use of genetically modified mosquitoes to inhibit disease transmission. Geneva: WHO. 66 p.

23. Harris AF, Nimmo D, McKenney AR, Kelly N, Scaife S, et al. (2012) Field performance of engineered male mosquitoes. Nat Biotechnol 29: 1034–1037.

24. Pinto J, Sousa CA, Gil V, Ferreira C, Gonçalves L, et al. (2000) Malaria in São Tomé and Príncipe: parasite prevalences and vector densities. Acta Trop 76: 185–193.

25. Berzosa P, Cano J, Roche J, Rubio J, García L, et al. (2002) Malaria vectors in Bioko Island (Equatorial Guinea): PCR determination of the members of Anopheles gambiae Giles complex (Diptera: Culicidae) and pyrethroid knockdown resistance (kdr) in An. gambiae sensu stricto. J Vector Ecol 27: 102–106.

26. della Torre A, Fanello C, Akiogbe M, Dossou-Goye J, Favia G, et al. (2001) Molecular evidence of incipient specialization within Anopheles gambiae s.s. in West Africa. Insect Mol Biol 10: 9–10.

27. Moreno M, Salgueiro P, Vicente J, Cano J, Berzosa P, et al. (2007) Genetic population structure of Anopheles gambiae in Equatorial Guinea. Malaria J 6: 137.

28. Reimer LJ, Tripet F, Slotman M, Spielman A, Fonjul E, et al. (2005) An unusual distribution of the kdr gene among populations of Anopheles gambiae on the island of Bioko, Equatorial Guinea. Insect Mol Biol 14: 683–688.

29. Pinto J, Donnelly MJ, Sousa CA, Malac-Vacas J, Gil V, et al. (2003) An island within an island: genetic differentiation of Anopheles gambiae in São Tomé, West Africa, and its relevance to malaria vector control. Hereditas 141: 407–414.

30. Marshall J, Pinto J, Chaobrod JD, Gente G, Santolamazza F, et al. (2008) Exploring the origin and degree of genetic isolation of Anopheles gambiae from the islands of São Tomé and Príncipe, potential sites for testing transgenic-based vector control. Evol Appl 1: 631–644.

31. WHO (2011) World Malaria Report.

32. Lee P-W, Liu C-T, Rampao HS, do Rosario V, Shaio M-F (2010) Preliminary analysis of malaria on the Island of Príncipe. Malaria J 9: 26.

33. Teklehaimanot HD, Teklehaimanot A, Kiszewski A, Rampaa HS, Sachs JD (2009) Malaria in São Tomé and Príncipe: on the brink of elimination after three years of effective antimalarial measures. Am J Trop Med Hyg 80: 133–140.

34. Kleinenschmidt I, Sharp B, Benavente LE, Schwabe C, Torres M, et al. (2006) Reduction in infection with Plasmodium falciparum one year after the introduction of malaria control interventions on Bioko Island, Equatorial Guinea. Am J Trop Med Hyg 74: 972–978.

35. Pardo G, Desalvo MA, Molina L, Castoñio E, Lvanga M, et al. (2006) Impact of different strategies to control Plasmodium infection and anaemia on the island of Bioko (Equatorial Guinea). Malaria J 5: 10.

36. Lanzaro G, Nuzhdin S, Tripet F (2006) Tools for monitoring the genetic structure and stability of mosquito populations. Bridging laboratory and field research for genetic control of disease vectors. 157–164.

37. Donnelly MJ, Cuamba N, Charlwood JD, Collins FH, Townson H (1999) Genetic differentiation among populations of Plasmodium falciparum on the island of Bioko, Equatorial Guinea. Insect Mol Biol 8: 185–193.

38. Favia G, Lanfrancotti A, Spinosi L, Siden-Kiasmos I, Louis C (2001) Molecular characterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of Anopheles gambiae s.s. Insect Mol Biol 10: 19–23.

Supporting Information

Table S1 Distribution of Herves sites detected over the six sampled populations. (DOCX)

Acknowledgments

We thank Barbara Ngudiankama and Kristina Pilitt (University of Maryland, USA) for valuable technical advice. We also acknowledge Dr. Conceição Ferreira (Centro Nacional de Endemias) for logistic support to mosquito collections carried out in São Tomé and Príncipe.

Author Contributions

Conceived and designed the experiments: DOB JP PS. Performed the experiments: PS. Analyzed the data: PS DOB. Contributed reagents/materials/analysis tools: DOB FS JP MM. Wrote the paper: PS JP.
39. Wright SI, Le QH, Schoen DJ, Bureau TE (2001) Population dynamics of an ac-like transposable element in self- and cross-pollinating Arabidopsis. Genetics 158: 1279–1288.

40. O'Brochta DA, Subramanian R, Orsetti J, Peckham E, Nolan N, et al. (2006) hAT element population genetics in Anopheles gambiae s.l. in Mozambique. Genetic 127: 185–198.

41. Beaumont MA, Balding DJ (2004) Identifying adaptive genetic divergence among populations from genome scans. Mol Ecol 13: 969–980.

42. Beaumont MA, Nicholi RA (1996) Evaluating Loci for Use in the Genetic Analysis of Population Structure. Proc R Soc B-Biol Sci 263: 1619–1626.

43. Antao T, Beaumont MA (2011) Mecheza: A Workbench to Detect Selection Using Dominant Markers. Bioinformatics.

44. Holsinger KE, Lewis PO (2005) Hickory: A Package for Analysis of Population Genetic Data v1. Storrs: Department of Ecology and Evolutionary Biology, University of Connecticut.

45. Weir B, Cockerham C (1984) Estimating F-statistics for the analysis of population structure. Evolution 38: 1358–1370.

46. Rouset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145: 1219–1228.

47. Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479–491.

48. Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Ecol Bioinform Online 1: 47–50.

49. Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (1996) GENETIX 4.05, computer program, logiciel sous Windows TM pour la génétique des populations.

50. Pinto J, Donnelly MJ, Sousa CA, Gil V, Ferreira C, et al. (2002) Genetic structure of Anopheles gambiae (Diptera: Culicidae) in São Tomé and Príncipe (West Africa): implications for malaria control. Mol Ecol 11: 2183–2187.

51. Ribeiro H, Ramos HC, Pires CA (1990) Sobre os vectores da malaria em São Tomé e Príncipe. Garcia de Orta Série Zoologia 15: 153–152.

52. Molina R, Bento A, Roche J, Blanca F, Amelia C, et al. (1993) Baseline entomological data for a pilot malaria control program in Equatorial Guinea. J Med Entomol 30: 622–624.

53. Jones PJ (1994) Biodiversity in the Gulf of Guinea: an overview. Biodiversity & Conservation 3: 772–794.

54. Mateu E, Comas D, Calafell F, Pérez-Leszañ A, Abade A, et al. (1997) A tale of two islands: population history and mitochondrial DNA sequence variation of Bioko and São Tomé, Gulf of Guinea. Annals of Human Genetics 61: 507–518.

55. Lehmann T, Hasley WA, Grebert H, Collins PH (1998) The effective population size of Anopheles gambiae in Kenya: implications for population structure. Mol Biol Evol 15: 264–276.

56. Simard F, Lehmann T, Lemasson J, Diatta M, Fontenelle D (2000) Persistence of Anopheles arabiensis during the severe dry season conditions in Senegal: an indirect approach using microsatellite loci. Insect Mol Biol 9: 467–479.