High Genetic Differentiation between the M and S Molecular Forms of Anopheles gambiae in Africa

Caroline Esnault¹, Matthieu Boulesteix¹, Jean Bernard Duchemin², Alphonsine A. Koffi³, Fabrice Chandre⁴, Roch Dabiré⁵, Vincent Robert⁶,⁷, Frédéric Simard⁸,⁹, Frédéric Triplet¹⁰, Martin J. Donnelly¹¹, Didier Fontenille⁸, Christian Biémont¹*¹

¹Laboratoire de Biométrie et Biologie Evolutive (UMR 5558), CNRS, Université de Lyon, Université Lyon1, Villeurbanne, France, ²Centre de Recherche Médicale et Sanitaire (CERMES), Réseau International de l’Institut Pasteur, Niamey, Niger, ³Institut Pierre Richet, Institut National de Santé Publique, Abidjan, Côte d’Ivoire, ⁴Unité de Recherche 016, Institut de Recherche pour le Développement (IRD), CREC, Cotonou, Bénin, ⁵Unité de Recherche en Sciences de la Santé (IRSS), Bobo Dioulasso, Burkina Faso, ⁶Unité de Recherche 77, Institut de Recherche pour le Développement (IRD), Unité Scientifique du Museum 504, Museum National d’Histoire Naturelle, Paris, France, ⁷Institut Pasteur, Antananarivo, Madagascar, ⁸Laboratoire de Recherche sur le Paludisme, Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC), Yaoundé, Cameroun, ⁹Unité de Recherche 016, Institut de Recherche pour le Développement (IRD), Montpellier, France, ¹⁰Centre for Applied Entomology and Parasitology, School of Life Sciences, Keele University, Staffordshire, United Kingdom, ¹¹Vector Group, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, United Kingdom

Abstract

Background: Anopheles gambiae, a major vector of malaria, is widely distributed throughout sub-Saharan Africa. In an attempt to eliminate infective mosquitoes, researchers are trying to develop transgenic strains that are refractory to the Plasmodium parasite. Before any release of transgenic mosquitoes can be envisaged, we need an accurate picture of the differentiation between the two molecular forms of An. gambiae, termed M and S, which are of uncertain taxonomic status.

Methodology/Principal Findings: Insertion patterns of three transposable elements (TEs) were determined in populations from Benin, Burkina Faso, Cameroon, Ghana, Ivory Coast, Madagascar, Mali, Mozambique, Niger, and Tanzania, using Transposon Display, a TE-anchored strategy based on Amplified Fragment Length Polymorphism. The results reveal a clear differentiation between the M and S forms, whatever their geographical origin, suggesting an incipient speciation process.

Conclusions/Significance: Any attempt to control the transmission of malaria by An. gambiae using either conventional or novel technologies must take the M/S genetic differentiation into account. In addition, we localized three TE insertion sites that were present either in every individual or at a high frequency in the M molecular form. These sites were found to be located outside the chromosomal regions that are suspected of involvement in the speciation event between the two forms. This suggests that these chromosomal regions are either larger than previously thought, or there are additional differentiated genomic regions interspersed with undifferentiated regions.

Citation: Esnault C, Boulesteix M, Duchemin JB, Koffi AA, Chandre F, et al. (2008) High Genetic Differentiation between the M and S Molecular Forms of Anopheles gambiae in Africa. PLoS ONE 3(4): e1968. doi:10.1371/journal.pone.0001968

Editor: Philip Awadalla, University of Montreal, Canada

Received December 21, 2007; Accepted March 7, 2008; Published April 16, 2008

Copyright: © 2008 Esnault et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by the PAL+ program of the french Ministry of Research and CNRS.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: biemont@biomserv.univ-lyon1.fr

Introduction

Malaria causes the deaths of more than one million people each year, mostly in Africa (WHO/UNICEF World Malaria Report 2005). This disease and the relevant mortality are due to one of four Plasmodium species, which are transmitted by mosquitoes. Anopheles gambiae is the major vector in sub-Saharan Africa, which has the greatest disease burden. Various methods have been developed to control mosquitoes. However, the failure of traditional measures together with the spread of insecticide-resistance in natural vector populations [1,2], have spurred on attempts to find alternative, unconventional approaches. One of the most innovative strategies sets out to replace the entire wild populations of An. gambiae with genetically modified, Plasmodium-resistant individuals. This idea seems more plausible following the successful genetic transformation of some anopheline species, including An. gambiae, and the identification of putative target genes and gene drive mechanisms [3]. To ensure that the transgene spreads throughout the entire wild populations, however, we need to understand the population structure and level of gene flow of mosquito populations. This makes it very important to know whether the genetic differentiation between the two ‘molecular forms’ of An. gambiae, termed M and S, which are suspected of currently undergoing speciation [4–6], is a general phenomenon affecting all African populations. The distinction between the two forms was primarily based on sequence polymorphism in ribosomal DNA loci [7], which was subsequently confirmed by microsatellite data in Cameroon [8] and Mali [9], by the insertion patterns of various transposable elements (TEs) in Cameroon [10] and of the short interspersed nuclear elements (SINEs) MAGE and SINE200 in Burkina Faso, Central African Republic, Mali, and Kenya [11,12]. The kdr allele, which confers...
knock-down resistance to pyrethroid insecticides and dichlorodi-
phenyltrichloroethane (DDT), was mainly present in S individuals,
and so this too was segregated between the two molecular forms
[see 4, 12 for reviews]. Studies of the gene flow within and between
the two molecular forms revealed, however, complex patterns of
differentiation. Some analyses revealed greater differences be-
tween ecological zones [13], and between allopatric populations of
a given molecular form than between the M and S populations
[14], suggesting that the M and S speciation is not yet complete.
Some data suggest that islands of speciation are present within the
genomes of these two forms, mostly in the region near the
centromeres of the X and 2L chromosomes and in a region of the
2R chromosome, whereas genetic differentiation remains weak in
other regions of the genome [15–18]. This could explain why the
estimates of genetic differentiation between the M and S forms vary
depending on the type of markers used, and the location of the
markers in the genome [8,9,13,19]. To find out whether the
generic differentiation between the M and S molecular forms is
found throughout the geographical range of An. gambiae, we
studied the insertion polymorphism of three TEs. Because the
insertion sites of these TEs were scattered throughout the An.
gambiae genome, this study provides an overview of a large portion
of the mosquito genome. The insertion patterns of these TEs
reveal clear differentiation between the M and S forms, whatever
the geographical origin of the populations.

Results and Discussion

Twenty-one An. gambiae populations from ten African countries
were studied: two populations from Benin, two from Burkina Faso,
two from Cameroon, three from Ghana, five from Ivory Coast,
one from Madagascar, one from Mali, one from Mozambique,
three from Niger, and one from Tanzania (see Fig. 1, which also
indicates the number of mosquitoes of the M and S molecular
groups in each population). The M and S forms were distinguished
based on their rDNA sequence polymorphism. The non-
Long Terminal Repeat (LTR) retrotransposon Avaα8, the LTR
retrotransposon Ozymandias, and the DNA transposon Crusoe were
taken into consideration [10]. Individual TE insertion profiles
were obtained by the Transposon Display method [20,21]. This
 technique is very similar to the Sequence-Specific Amplification
 Polymorphism, except that the PCR amplifies a DNA sequence
defined by one primer anchoring to a conserved region of the TE,
and another primer anchoring to an adapter attached to flanking
sites generated by enzymatic restriction digestion. The presence
and absence of TE insertions can thus be scored in individuals
(Fig. 2). We therefore compared the TE insertion profiles of
individuals from all the populations by estimating the inter-
population differentiation indices, Pfst. This parameter is analogous
to Fst, and can be used to analyze the presence/absence data
[22] obtained by Transposon Display. The mean Pfst values
between the M and S populations (0.57 ± 0.07 for Avaα8,
0.19 ± 0.04 for Ozymandias, 0.23 ± 0.05 for Crusoe), are higher than
the Fst between M populations (0.12 ± 0.06, 0.07 ± 0.04, and
0.11 ± 0.07, for Avaα8, Ozymandias, and Crusoe, respectively) or S
populations (0.10 ± 0.06, 0.12 ± 0.09, and 0.12 ± 0.07, for Avaα8,
Ozymandias, and Crusoe, respectively) (see Table 1 for the between-
population Pfst and the associated P-values). A graphical
representation using a Principal Coordinate Analysis (PCoA)
(Fig. 3–5) clearly distinguishes between individuals of these two
forms, whatever the TE and the population considered, and shows
that individuals of a given molecular form cluster together. This
similarity of the results for all three TEs is reinforced by significant
Pearson correlation coefficient values between the Pfst values
obtained for the three TEs (r = 0.72 for Avaα8 vs Ozymandias;
0.70 for Avaα8 vs Crusoe; r = 0.77 for Ozymandias vs Crusoe; P-
values < 1 x 10^-6). These data thus clearly reveal a high degree of
differentiation between the M and S molecular forms in all 21
populations studied, with some “specific” TE insertion sites being
present at high frequency in one or other form (Table 1). Among
20 such sites, 6 were found in all individuals (4 on M and 2 on S),
whereas 14 were present at high frequency in one form or the
other (see Fig 6). To check for possible differentiation between
populations of the M or S forms, we did Principal Coordinate
Analysis (PCoA) on either the M or the S populations. No
differentiation between populations of either the M or S form was
detected, which suggests the absence of specific insertion sites
for groups of populations apart from the M and S forms. This
indicates that most insertion sites were widespread and highly
polymorphic between populations.

To localize the specific TE insertions on the chromosome arms
of An. gambiae, we extracted the corresponding 32P labeled bands
from polyacrylamide gels, and sequenced the DNA to make sure
that the bands corresponded to the expected TEs and to obtain the
sequences flanking the TEs. Among the 20 specific insertion sites
that were attempted to be sequenced (13 on M and 7 on S), 7 were
not isolated, 7 were isolated but were found to be integrated within
repeated sequences or transposable elements and could not be
localized, 3 were located in the “unannotated” chromosome. This
suggests that some of these insertions were embedded within the
heterochromatin or were inserted within nests of TEs, which could
themselves be heterochromatic. The localization of some of the
TEs specific to one of the molecular forms within the
eeterochromatin, raises the important possibility that drastic
differences in the composition of heterochromatin may exist
between populations, and the question of the influence of
heterochromatin on genetic differentiation and speciation pro-
cesses, once again highlighting the need for more intensive
research on this particular genomic region [23]. Three of the
specific insertions were however unambiguously localized on
chromosomes. They consisted of two Crusoe (Crusoe-1, Crusoe-2)
and one Ozymandias (Ozym-1) insertions specific to the populations
of the M molecular form. These insertions were localized in the
division 21 of the 2L chromosome (outside the known inversions),
and division 16 of the 2R chromosome (outside the 2Rd inversion,
at 800 kb from the inversion breakpoint) for Crusoe, and division 33
of the 3R chromosome for Ozymandias. These locations are outside
the genomic regions previously identified as being genetically
differentiated in the M and S forms [4,8,9,16,17,19] (see Fig. 7),
suggesting either that the chromosomal regions involved in this
differentiation are more extensive than expected, or that there are
additional differentiated regions interspersed with undifferentiated
regions. More detailed analyses of these regions are necessary. It
has been shown that differential population adaptation can be
determined from a subset of genes while gene flow still exists
between the species under speciation [24,25]. The “islands of
speciation” that define the M and S forms may thus be
extending gradually, reducing gene flow and fixing some TE
insertions close to the selected islands. Because the three
localized insertions were outside the known inversions and not
in the “islands of speciation”, these sites could result simply
from genetic drift that has occurred after the separation of the
M and S forms. If so, the fixed sites and the sites at high
frequency would correspond to the sites of high occupancy
frequency in the original founders, and the polymorphic
insertion sites (sites with low occupancy frequency) would
correspond to more recent transposition events, as observed in
colonizing species [26]. This kind of insertion site frequency
pattern is compatible with the idea that *An. gambiae* has speciated or differentiated relatively recently [27]. According to the hypothesis of founder effects, the presence of fixed TE insertion sites in each molecular form could suggest that gene flow is more restricted than it has usually been thought to be, which would be consistent with the virtual absence of hybrids in nature [8, although an unusual frequency of hybrids was found in a population from Guinea Bissau; J. Pinto, personal communication]. However, among the 6 fixed sites that we sequenced, only one (*Crusoe-1*) was localized on the chromosome arms, the others were either on the unknown chromosome, or clearly embedded within heterochromatin or other transposable elements. In addition, *Crusoe-1*, which is fixed in the M form, reaches a frequency of 0.35 in the S form, suggesting it had been a site of high frequency in the initial population from which the M and S forms both derive. The two other localized sites, *Ozym-1* and *Crusoe-2*, which were outside the known inversions and the “islands of speciation”, were present at an intermediate frequency in the M populations (Fig 6), but at very low frequency in the S form. All these data are in agreement with founder events [26, 28] and then global expansion in Africa.

The wide distribution of *An. gambiae* suggests the possibility of population adaptations to local climatic conditions, resulting in local differentiation between populations of a same molecular form, as has indeed been observed for M populations in Cameroon and Mali, in addition to the M and S molecular form differentiation [29]. Both the M and S forms exist in Western Africa, while only the S form has been found in Eastern Africa, which implies that the S form has greater climatic adaptability or migratory capacities than the M form. Although these two forms may coexist in the same area, they appear to be in the process of incipient speciation throughout Africa. No differences have been observed in *Plasmodium* infection rates between sympatric M and S forms in Cameroon [6]. Therefore, any attempt to construct a genetically-modified, *Plasmodium*-resistant mosquito, with the intention of replacing natural, infected populations, or any other strategy of controlling *An. gambiae*, will have to take this incipient speciation between the M and S molecular forms of the mosquito into account.

**Materials and Methods**

**Mosquitoes**

A total of 446 *An. gambiae* mosquitoes (257 M and 189 S) were sampled from 21 sites in Africa: Ladji (6°21’ N, 2°27’ E), Lema (7°46’ N, 2°14’ E) in Benin, Vallée du Kou (VK7, 11°24’ N, 4°24’ W), Soum (12°35’ N, 2°17’ E) in Burkina Faso, Simbok (3°49’ N, 11°28’ E), Ipono (2°22’ N, 9°49’ E) in Cameroon, Bonia (10°52’ N, 1°07’ W), M’be (7°14’ N, 5°32’ W), Nieky (5°52’ N, 4°49’ W), Mampong (5°24’ N, 4°16’ W), Yaokoffikro (7°11’ N, 5°1’ W) in Ivory Coast, Banizoumbou (13°32’ N, 2°40’ E), Kosseye (13°31’ N, 2°1’ E), Zindarou (13°26’ N, 2°55’ E) in Niger, Beforona (18°58’ S, 48°15’ E) in Madagascar, Bankoumana (12°50’ N, 5°47’ W) in Mali, Furvela (23°43’ S, 35°18’ E) in Mozambique, and Zenet (5°16’ S, 38°36’ E) in Tanzania. The species and molecular form of the specimens were identified using Fanello *et al.*’s protocol [30].

**Figure 1. Geographic origin of the African *An. gambiae* populations.** Sample sizes of the M and S molecular forms in each population are indicated in gray and black, respectively. Az = Azureti, Ban = Bankoumana, Baniz = Banizoumbou, Bef = Beforona, Bon = Bonia, Fur = Furvela, Ipo = Ipono, Kos = Kosseye, Lad = Ladji, Lem = Lema, Mam = Mampong, M’be = M’be, Nia = Niamoue, Nie = Nieky, Odu = Odumasy, Sim = Simbok, Soum = Soum, VK7 = Vallée du Kou, Yao = Yaokoffikro, Zen = Zenet, Zin = Zindarou. The populations were from B = Benin, BF = Burkina Faso, C = Cameroon, G = Ghana, IC = Ivory Coast, MG = Madagascar, ML = Mali, MZ = Mozambique, N = Niger, T = Tanzania.

doi:10.1371/journal.pone.0001968.g001
Total genomic DNA was isolated from individual mosquitoes using a standard phenol-chloroform extraction procedure after proteinase K digestion. The Transposon Display was performed using a modified version of the protocol used by Zampicinini et al. [31], as follows: 50 to 100 ng of genomic DNA was digested with 10 units of HhaI for 6 hours at 37°C; during the first round of amplification, 3 mM of MgCl₂ and 0.625 Units of Taq Polymerase were used; during the second amplification run, 0.2 mM of adaptor primer, 0.05 mM of nested TE-specific primer with HEX fluorescent labeling, 2.5 mM of MgCl₂ and 0.625 Units of Taq Polymerase were used. The last steps of the nested-amplification cycles lasted 45 sec, instead of 1 min. The sequences of adaptors and primers are shown in Table S2. Negative controls were performed using the adaptor-primer or the element specific-primer alone.

The PCR products were diluted 5-fold, and 1 μl of the dilution was loaded onto a MegaBace 1000 capillary sequencer (Amersham BioSciences) with an ET900-ROX standard size marker (Amersham BioSciences). Raw data were analyzed by GeneticProfiler software (Amersham BioSciences). To confirm whether the amplified DNAs were identical to the expected TE product, 6–8 fragments were cloned using the Topo TA cloning kit (Invitrogen), following the Manufacturer’s instructions, and sent to GeinoScreen for sequencing. All analyzed fragments corresponded to the expected TE.

**Data analysis**

Each band on the capillary gels was automatically ascribed a molecular weight according to the DNA ladder, which was loaded on each capillary. We assumed that the DNA bands with the same molecular weight shared the same TE insertion. The individual TE insertion patterns obtained from the Transposon Display were thus recorded as a binary matrix of 0 and 1 denoting the absence or presence of a given peak on the capillary gel, respectively. The between-population genetic divergence, Φst, was calculated for each pair of population samples for the three transposable elements considered separately. This Φst, which allows for the
dominant nature of TE, is an analogue of the fixation index of inter-population differentiation, \( F_{ST} \) [22,32]. Because the inter-population index values calculated from samples consisting of less than 5 individuals were not reliable, these values were not included in the calculation of the mean \( W_{st} \) values between populations.

Graphical representations of the proximities between individuals were obtained using a Principal Coordinate Analysis (PCoA), using the R package ade4 [33]. All individuals were included in these analyses, because those from small samples were not expected to bias the results, as they were not assigned a priori to any specific population. For each population, we then drew the ellipses centered on the gravity center of each scatterplot, with the size of the two first axes equal to 1.5 times the standard deviation of the coordinates of the projections on the axes. MANOVA between molecular forms was performed using JMP Version 7 software (SAS Institute Inc.), and the variance components were tested for significance by nonparametric randomization tests with the null hypothesis of no population structure.

The detection of population differentiation by the PCoA is based on the sites that are either fixed or at high frequency in a form and not in the other. Hence sites with very high insertion polymorphism play no role in the differentiation.

Identification of transposable element insertion sites

Fragments obtained from the Transposon Display were separated on a 6% denaturing polyacrylamide gel. Samples were diluted with one volume of loading dye (95% formamide, 0.05% xylene cyanol FF, and 0.05% bromophenol blue), heat denatured at 95°C for 5 min, and immediately cooled on ice. Polyacrylamide gel was pre-run at 75 W for 30 min. Six \( \mu l \) of each sample were run at 75 W for 4 h in 1xTBE. We used radioactive \( ^{32}P \) labeling; the gel was transferred to Whatman 3 MM paper, and vacuum dried at 65°C for 1 h; dried gels were exposed to X-ray films overnight or for 48 h, depending on the signal intensity [34]. The fragments of interest were cut from the gels, the DNA was eluted from the bands at 100°C for 15 min and resuspended in 150 \( \mu l \) of sterile water. The fragments were amplified according to the second amplification run of the Transposon Display protocol, and cloned using the Topo TA cloning kit (Invitrogen). About 5 clones for each fragment were sequenced by GenoScreen. The genomic localizations of the sequenced DNAs were determined by interrogation of the \textit{Anopheles gambiae} genome database (Ensembl AgamP3 assembly, release 46.3i). Among the 14 sequenced fragments, only three presented a flanking sequence localized in only one site on the chromosome arm. These three fragments corresponded to two insertions of the DNA transposon \textit{Crusoe} and to one insertion of the LTR retrotransposon \textit{Ozymandias}. Their specificity to the M form was confirmed by PCR. Amplifications were performed following the second amplification run of Transposon Display, using primers \textit{Crusoe}-1F 5'-CCTATT-
Figure 6. Frequencies of the 20 insertion sites sequenced in the M (red squares), and S (green squares) molecular forms. Insertion sites localized on the chromosomes: Crusoe-1-2, Ozym-1. Insertion sites localized on the unknown chromosome: Aara8-2-3-5. Insertion sites integrated within other transposable elements or repeated sequences: Aara8-1-4-6, Ozym-2-5, Crusoe-3-4. Insertion sites not isolated: Aara8-7, Ozym-3-4-6-7, Crusoe-5-6.
doi:10.1371/journal.pone.0001968.g006

Table 1. Number of insertion sites and average site numbers±SE of Aara8, Ozymandias, and Crusoe for the populations of the S and M molecular forms.

|                   | Number of insertion sites | Mean insertion sites number±SE |
|-------------------|---------------------------|-------------------------------|
|                   | Specific to M molecular form | Specific to S molecular form | Common to both forms | Total number of loci with an insertion | M molecular form | S molecular form |
| Aara8             | 4                         | 3                             | 3                     | 110                                     | 6.58±1.71       | 6.69±1.43       |
| Ozymandias        | 5                         | 2                             | 12                    | 245                                     | 19.41±5.78      | 12.99±3.73      |
| Crusoe            | 4                         | 2                             | 7                     | 169                                     | 10.33±3.01      | 11.67±3.84      |

doi:10.1371/journal.pone.0001968.t001

Figure 7. Position on chromosomes 2 and 3 of the three TE insertions specific to the M molecular form (in box). The loci Ag2H325, Ag2H417, Ag2H769, Ag3H555, Ag3H170, Ag3H750 from [8], kdr from [4], and Ion channel and LIM from [17], have been shown to differentiate the two M and S forms in previous studies. The chromosomal inversions of the An. gambiae genome are indicated below the chromosome arms. The GPRor39, GPRor38, and UNK1 loci, indicated by asterisks, have been shown to discriminate between the two forms only in Cameroon [16].
doi:10.1371/journal.pone.0001968.g007
GATTTGTCCGACACTG-3, Crusoe-1 R 5'-TCACTTACGG-TTGAAACAG-3', Crusoe-2 F 5'- CCTATTGATTGTCGACACTG-3', Crusoe-2 R 5'-TTTACCTGGC TTTGGAAT-3' and Ozym-1 F 5'-TGCTATAGCACTGCAGACA-3', Ozym-1 R 5'-CTGAAAGTGTGTCGTCGACC-3' for the Crusoe-1, Crusoe-2 and Ozym-1 insertions, respectively.

Supporting Information

Table S1

Found at: doi:10.1371/journal.pone.0001968.s001 (1.58 MB DOC)

Table S2

Found at: doi:10.1371/journal.pone.0001968.s002 (0.03 MB DOC)

References

1. Chandra F, Manguin S, Berenguers C, Dossou Yovo J, Darriet F, et al. (1999) Current distribution of a pyrethroid resistance gene (ldh) in Anopheles gambiae complex from west Africa and further evidence for reproductive isolation of the Moipti form. Trop Med Int Health 4: 311–322.
2. Amorosa LF Jr., Corbellini G, Coluzzi M (2005) Lessons learned from malaria: Italy’s past and sub-Saharan’s future. Health Place 11: 67–73.
3. Boete C (2006) Genetically Modified Mosquitoes for Malaria Control. Austin, Texas, USA: Landes Biosciences. pp 174.
4. della Torre A, Costantini C, Besansky NJ, Caccone A, Petrarca V, et al. (2002) Emphasis on chromosomal and molecular forms of Anopheles gambiae s.s. Insect Mol Biol 12: 241–243.
5. Favia G, Lanfrancotti A, Spuno L, Siden-Kiamis L, Louis C (2003) Molecular characterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of Anopheles gambiae s.s. Insect Mol Biol 10: 19–23.
6. Wondji CS, Nanfack P, Petracca V, E Fully, J, Santolamazza F, et al. (2005) Species and populations of the Anopheles gambiae complex in Cameroon with special emphasis on chromosomal and molecular forms of Anopheles gambiae s.s. J Med Entomol 42: 990–1005.
7. Favia G, Lanfrancotti A, Spuno L, Siden-Kiamis L, Louis C (2003) Molecular characterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of Anopheles gambiae s.s. Insect Mol Biol 10: 227–233.
8. Wondji CS, Nanfack P, Petracca V, E Fully, J, Santolamazza F, et al. (2005) Species and populations of the Anopheles gambiae complex in Cameroon with special emphasis on chromosomal and molecular forms of Anopheles gambiae s.s. J Med Entomol 42: 990–1005.
9. Favia G, Lanfrancotti A, Spuno L, Siden-Kiamis L, Louis C (2003) Molecular characterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of Anopheles gambiae s.s. Insect Mol Biol 10: 227–233.
10. Roulesteix M, Simard F, Antonio-Nkondjo C, Awaono-Ambene HP, Fontenelle 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.
11. Barnes MJ, Lobo NF, Goulbourn 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.
12. della Torre A, Tu Z, Petracca V (2005) On the distribution and genetic differentiation of Anopheles gambiae s.s. molecular forms. Insect Biochem Mol Biol 35: 755–769.
13. Ayawo AE, Wretem D, Wilson MD, Donnelly MJ (2007) Ecological zones rather than molecular forms predict genetic differentiation in the malaria vector Anopheles gambiae s.s. in Ghana. Genetics 175: 751–761.
14. Lehmann T, Licht M, Elissa N, Marka BT, Chimimba JM, et al. (2003) Population Structure of Anopheles gambiae in Africa. J Hered 94: 131–147.
15. Stump AD, Fitzpatrick MC, Lobo NF, Traore S, Sagnon N, et al. (2005) Centromere-proximal differentiation and speciation in Anopheles gambiae. Proc Natl Acad Sci U S A 102: 15930–15935.
16. Turner TL, Hahn MW, Nuzhdin SV (2005) Genomic islands of speciation in Drosophila sex madi. Proc Natl Acad Sci U S A 102: 15930–15935.
17. Turner TL, Hahn MW (2007) Locus- and population-specific selection and differentiation between incipient species of Anopheles gambiae. Mol Ecol Evol 24: 2132–2138.
18. Slotman MA, Reimer LJ, Thiemann T, Dolo G, Fondo E, et al. (2006) Reduced recombination rate and genetic differentiation between the M and S forms of Anopheles gambiae s.s. Genetics 174: 2081–2093.
19. Gentile G, Slotman M, Kremaier V, Powell JR, Caccione A (2001) Attempts to molecularly distinguish cryptic taxa in Anopheles gambiae s.s. Insect Mol Biol 10: 25–32.
20. Van den Breek D, Maes T, Sauer M, Zethof J, De Keukelaere P, et al. (1998) Transposon Display identifies individual transposable elements in high copy number lines. Plant J 13: 121–129.
21. Casa AM, Brouwer C, Nagel A, Wang L, Zhang Q, et al. (2006) The MITE family heartbreaker [HBw]: molecular markers in maize. Proc Natl Acad Sci U S A 97: 10083–10089.
22. Peakall R, Smouse PE, Huff DR (1995) Evolutionary implications of allozyme and RAPD variation in diploid populations of dioecious buffalograss Buchloe dactyloides. Mol Ecol 4: 135–147.
23. Johnson L (2007) Transposon silencing: The extraordinary epigenetics of a transposon trap. Heredity. doi:10.1038/sj.hdy.6801064.
24. Macpherson JM, Gonzalez J, Witten DM, Davis JC, Rosenberg NA, et al. (2008) Nonadaptive explanations for signatures of partial selective sweeps in Drosophila pseudoobscura and close relatives. Mol Biol Evol 19: 472–488.
25. Wu G-L, Ting C-T (2004) Genes and speciation. Nature Rev Genet 5(2): 114–122.
26. Garcia Guerrero MP, Fontdevila A (2007) The evolutionary history of Drosophila buzzatii. XXXVI. Molecular structural analysis of Osutide retrotransposon insertions in colonizing populations unveils drift effects in founder events. Genetics 175: 301–310.
27. Mukabayire O, Cariati J, Wang X, Toure´ YT, Coluzzi M, Besansky NJ (2001) Patterns of DNA sequence variation in chromosomally recognized taxa of Anopheles gambiae s. s. evidence from rDNA and single-copy loci. Insect Mol Biol 10: 33–46.
28. Macpherson JM, Gonzalez J, Witten DM, Davis JC, Rosenberg NA, et al. (2008) Nonadaptive explanations for signatures of partial selective sweeps in Drosophila. Mol Biol Evol (in press).
29. Slotman MA, Tripet F, Cornel AF, Meares CR, Lee Y, et al. (2007) Evidence for subdivision within the M molecular form of Anopheles gambiae. Mol Ecol 16: 639–649.
30. Fanello C, Santolamazza F, della Torre A (2002) Simultaneous identification of species and molecular forms of the Anopheles gambiae complex by PCR-RFLP. Med Vet Entomol 16: 461–464.
31. Zanupinini G, Blinov A, Cervella P, Guryev V, Sella G (2004) Insertional polymorphism of a non-LTR mobile element (NLRCth1) in European populations of Chlamydomas ruinaus (Diptera, Chironomidae) as detected by transposon insertion display. Genome 47: 1134–1163.
32. Excouderc L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479–491.
33. Chessel D, Dufour AB, Thioulouse J (2004) The ade4 package - I: One-table methods. R news 4: 5–10.
34. Melayah D, Bonnivard E, Chalhoub B, Audeon C, Grandbastien MA (2001) The mobility of the tobacco Tc1 retrotransposon correlates with its transcriptional activation by fungal factors. Plant J 26: 159–168.

Acknowledgments

We thank Abdoulaye Diabate for his help with collecting wild mosquitoes, Emmanuelle Lerat and Cristina Vieira for their helpful comments, the technical DTAMB platform at Lyon for providing access to the MegaBACE™ capillary sequencer, Corinne Mhiri for her help with sequencing of some specific TE insertions.

Author Contributions

Conceived and designed the experiments: DF CB MD CE MB. Performed the experiments: CE. Analyzed the data: CB CE. Contributed reagents/materials/analysis tools: DF VR FS JD RD MD MB AK FC FT. Wrote the paper: CB CE.