Guelbeogo *et al. Malaria Journal* 2014, **1** :65
http://www.malariajournal.com/content/13/1/65

MALARIA JOURNAL

Behavioural divergence of sympatric *Anopheles funestus* populations in Burkina Faso

Wamdaogo M Guelbeogo[1*], N'Fale Sagnon[1], Fang Liu[2], Nora J Besansky[3*] and Carlo Costantini[4]

Background: In Burkina Faso, two chromosomal forms of the malaria vector *Anopheles funestus*, Folonzo and Kiribina, are distinguished by contrasting frequencies of shared polymorphic chromosomal inversions. Sympatric and synchronous populations of Folonzo and Kiribina mate assortatively, as indicated by a significant deficit of heterokaryotypes, and genetic associations among inversions on independently segregating chromosome arms. The present study aimed to assess, by intensive longitudinal sampling, whether sympatric Folonzo and Kiribina populations are characterized by behavioural differences in key malaria vectorial parameters.

Methods: The study was conducted in two adjacent villages near Ouagadougou, in the dry savanna of central Burkina Faso. Mosquito adult resting behaviour of both forms was compared based on parallel indoor/outdoor collections across six breeding seasons; 8,235 fully karyotyped samples of half-gravid females were analysed in total. Additionally, indoor/outdoor human biting behaviour, host selection, and *Plasmodium falciparum* sporozoite rate was assessed and compared between chromosomal forms.

Results: The Kiribina form was numerically predominant in the area. However, the Folonzo form was significantly over-represented in indoor resting collections and showed stronger post-prandial endophily, while Kiribina predominated outdoors. Neither form was statistically distinguishable in human biting behaviour, and both were more likely to seek human blood meals indoors than outside. The human blood index and sporozoite rate were comparably high in both chromosomal forms in indoor collections (>89% and >8%, respectively).

Conclusions: Both Kiribina and Folonzo chromosomal forms are formidable malaria vectors in Burkina Faso. However, the significantly greater tendency for the Kiribina form to rest outdoors despite its pronounced anthropophily suggests that uniform exposure of the overall *An. funestus* population to indoor-based vector control tools cannot be expected; Kiribina is more likely to evade indoor interventions and escape unharmed outdoors, reducing the efficacy of malaria control. Accordingly, more efficient methods to detect Kiribina and Folonzo, and a more complete understanding of their distribution and behaviour in Africa are advocated.

Keywords: *Anopheles funestus*, Anthropophily, Behavioural divergence, Chromosomal forms, Exophily, Folonzo, Kiribina, Malaria vector, West Africa

, *Anopheles gambiae*, *Anopheles coluzzii*, *Anopheles arabiensis* *Anopheles funestus*,

[1,2]. (*An. gambiae*) , (. .,) [3,4]. *Anopheles funestus* [5,] , . ,

* Correspondence: guelbeogo.cnrfp@fasonet.bf; nbesansk@nd.edu
[1]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou 01 BP 2208, Burkina Faso
[3]Eck Institute for Global Health, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA
Full list of author information is available at the end of the article

An. funestus [-11]. *An. funestus sensu stricto,* ([,12]). *Anopheles funestus s.s.* 1 [13,14]. [15-20]. D [1]. 3 [1 ,1]. [1 ,1]. 3 3 , *An. funestus* [1]. *An. gambiae* [21], [22]. D 3 [23,24].

An. coluzzii *An. gambiae* ([25,2]). *Plasmodium falciparum* [23,24,2]. [1]. ,235 .

Study area

35 (12 11′54 1 23′43) (12 11′3 1 23′11) 1 [1 ,24]. *An. funestus* ( ), (-), , (-). D [22].

Chromosomal form identification

[2] 1.5 (. , 3.1), *An. funestus* D D - P [24]. −20 . P *An. funestus* [2]

et al.
[13]. , 2%
et al.
[1].
, 0. %.

Resting behaviour

( )
[30].
/
2005–200 ( ).
( ) (P)

Human biting behaviour

( ).
(21.00–05.00),
2002- 2003.
,
An. funestus ,
,

Blood meal identification and *Plasmodium falciparum* detection

2005–
200 200 –200

(, ,)
( )
P. falciparum
(P) (+)

Data analysis

( )

P. falciparum P .
([31]),
,
5%
( ). *P*-
P 2 2
, P <0.05

Ethical approval

An. funestus
/
,235 1.
,
,
50% ,
,
2 2
(1).
An. funestus
,

1 *Anopheles funestus*

Season	Sample	Total	Folonzo	Kiribina	OR	0.95 CI	*P*
1999-2000	ISC/indoor	1154	377	777	1.94	1.29-2.93	0.001
	PIT/outdoor	155	31	124			
2000-2001	ISC/indoor	1164	208	956	1.58	0.55-4.54	NS
	PIT/outdoor	33	4	29			
2001-2002	ISC/indoor	2733	659	2074	0.95	0.49-1.84	NS
	PIT/outdoor	48	12	36			
2002-2003	ISC/indoor	485	301	184	3.03	1.70-5.37	<0.0001
	PIT/outdoor	57	20	37			
2005-2006	ISC/indoor	99	63	36	2.33	0.49-11.02	NS
	PIT/outdoor	7	3	4			
2006-2007	ISC/indoor	932	343	589	11.87	8.93-15.76	<0.0001
	PIT/outdoor	1368	64	1304			
Pooled	ISC/indoor	6567	1951	4616	4.84	4.02-5.82	<0.0001
	PIT/outdoor	1668	134	1534			

OR, odds ratio of indoor/outdoor resting by Folonzo *versus* Kiribina; CI, confidence interval; ISC, insecticide spray-sheet catch; PIT, Muirhead-Thomson pit-shelter [30].

(2). 2002–2003 1,000 2002–3, *versus* (3). *versus* 2005–200 200 –200 . 5. .3%, (4).

2 - - *Anopheles funestus* (200 –200)

Sample*	Total	Folonzo	Kiribina	OR	0.95 CI	*P*
ISC/indoor	38	9	29	5.64	2.43-13.10	0.0003
PIT/outdoor	518	27	491			

OR, odds ratio of post-prandial indoor resting by Folonzo *versus* Kiribina for outdoor-feeding *An. funestus* (2005–2007 season); CI, confidence interval; PIT, Muirhead-Thomson pit-shelter [30]; ISC, insecticide spray-sheet catch.
*Includes only *An. funestus* having blood fed exclusively on cattle, based on blood meal identification.

(P <0.00), 30 52 , 10 % 1.44), 2005–200 *P. falciparum* (.5- . % 5). 32 (3%)

An. funestus *et al.* [1] (%),

Anopheles funestus **(2002 –200)**

Month	Sample	Total	Folonzo	Kiribina	OR	0.95 CI	*P*
October	HLC/indoor	363	263	100	0.87	0.54-1.40	NS
	HLC/outdoor	117	88	29			
November	HLC/indoor	218	151	67	1.18	0.74-1.87	NS
	HLC/outdoor	131	86	45			
December	HLC/indoor	70	42	28	0.62	0.30-1.30	NS
	HLC/outdoor	58	41	17			
January	HLC/indoor	80	45	35	1.63	0.73-3.65	NS
	HLC/outdoor	34	15	19			
Pooled	HLC/indoor	731	501	230	1.04	0.79-1.37	NS
	HLC/outdoor	340	230	110			

OR, odds ratio of indoor/outdoor human biting by Folonzo *versus* Kiribina; CI, confidence interval; HLC, human landing catch.

P. falciparum (%) *An. funestus* *versus* *An. funestus*, *An. funestus* *An. funestus* [34]. *An. funestus* [34], [1 ,35-3]. [34]. *An.* *gambiae* *An. arabiensis* [3,32]. *An. funestus.* [33]. *An. funestus*

Anopheles funestus **(200 –200)**

Sample	Blood meal	Folonzo	Kiribina	OR	0.95 CI	*P*
ISC/indoors	Human + Mixed	191 + 21 (212)	232 + 11 (243)	2.81	1.30-6.07	0.006
	Bovine	9	29			
	Total	221	272			
	HBI	95.9%	89.3%			
PIT/outdoors	Human + Mixed	2 + 1 (3)	13 + 25 (38)	1.44	0.42-4.95	NS*
	Bovine	27	491			
	Total	30	529			
	HBI	10.0%	7.2%			

*NS by Fisher Exact test. Chi-square not calculated due to an expected cell frequency below 5.
OR, odds ratio of human/bovine blood meal by Folonzo *versus* Kiribina; CI, confidence interval; ISC, insecticide spray-sheet catch; PIT, Muirhead-Thomson pit-shelter [30]; Mixed, blood meal of human and bovine origin; HBI, human blood index.

Plasmodium falciparum
Anopheles funestus
(200 –200)

Sample	CSP	Folonzo	Kiribina	OR	0.95 CI	*P*
ISC/indoor	+	27	45	0.97	0.59-1.59	NS
	-	291	469			
	Total	318	514			
	%CSP+	8.5%	8.8%			
PIT/outdoor	+	0	32	0.00	0.00-2.14	NS*
	-	61	1035			
	Total	61	1067			
	%CSP+	0.0%	3.0%			

*NS by Fisher Exact test.
CSP, circumsporozoite protein; OR, odds ratio of sporozoite infection in Folonzo *versus* Kiribina; CI, confidence interval; ISC, insecticide spray-sheet catch; PIT, Muirhead-Thomson pit-shelter [30].

[52]. ... ([35,50]). ... A ... *An. funestus* ...

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
CC, NFS and NB conceived the study. WG performed the field collections. NFS provided logistical support throughout the study. WG performed the karyotyping. WG, CC and FL analysed results. WG, CC and NB wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements
We thank the inhabitants of Koubri and Kuiti for their collaboration, and the entomological team of CNRFP for their efforts during the course of this study. M A Yameogo, O Grushko and D Boccolini were instrumental in polytene chromosome analysis. This research was financially supported by grant R01 AI48842 from the US National Institutes of Health to NJB; re-entry grant A 41413 to WG from WHO/TDR; and WHO MIM/TDR grant 50090 to NFS.

Author details
[1]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou 01 BP 2208, Burkina Faso. [2]Department of Applied and Computational Mathematics and Statistics, University of Notre Dame, Notre Dame, IN 46556, USA. [3]Eck Institute for Global Health, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA. [4]Institut de Recherche pour le Développement (IRD), Unité Mixte de Recherche MIVEGEC (UM1, UM2, CNRS 5290, IRD 224), Centre IRD de Montpellier 956, Avenue Agropolis BP 64501, Montpellier Cedex 5 34394, France.

Received: 21 January 2014 Accepted: 20 February 2014
Published: 24 February 2014

References

1. Sinka ME, Bangs MJ, Manguin S, Rubio-Palis Y, Chareonviriyaphap T, Coetzee M, Mbogo CM, Hemingway J, Patil AP, Temperley WH, Gething PW, Kabaria CW, Burkot TR, Harbach RE, Hay SI: **A global map of dominant malaria vectors.** *Parasit Vectors* 2012, **5:**69.
2. Gillies MT, De Meillon B: *The Anophelinae of Africa South of the Sahara.* 2nd edition. Johannesburg: South African Institute for Medical Research; 1968.
3. Coluzzi M, Sabatini A, Petrarca V, Di Deco MA: **Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex.** *Trans R Soc Trop Med Hyg* 1979, **73:**483–497.
4. White BJ, Collins FH, Besansky NJ: **Evolution of *Anopheles gambiae* in relation to humans and malaria.** *Annu Rev Ecol Evol Syst* 2011, **42:**111–132.
5. Garros C, Harbach RE, Manguin S: **Systematics and biogeographical implications of the phylogenetic relationships between members of the Funestus and Minimus groups of *Anopheles* (Diptera: Culicidae).** *J Med Entomol* 2005, **42:**7–18.
6. Harbach RE: **The classification of genus *Anopheles* (Diptera: Culicidae): a working hypothesis of phylogenetic relationships.** *Bull Entomol Res* 2004, **94:**537–553.
7. Coetzee M, Koekemoer LL: **Molecular systematics and insecticide resistance in the major African malaria vector *Anopheles funestus*.** *Annu Rev Entomol* 2013, **58:**393–412.
8. Spillings BL, Brooke BD, Koekemoer LL, Chiphwanya J, Coetzee M, Hunt RH: **A new species concealed by *Anopheles funestus* Giles, a major malaria vector in Africa.** *Am J Trop Med Hyg* 2009, **81:**510–515.
9. Hackett BJ, Gimnig J, Guelbeogo W, Costantini C, Koekemoer LL, Coetzee M, Collins FH, Besansky NJ: **Ribosomal DNA internal transcribed spacer (ITS2) sequences differentiate *Anopheles funestus* and *An. rivulorum*, and uncover a cryptic taxon.** *Insect Mol Biol* 2000, **9:**369–374.
10. Cohuet A, Simard F, Toto J-C, Kengne P, Coetzee M, Fontenille D: **Species identification within the *Anopheles funestus* group of malaria vectors in Cameroon and evidence for a new species.** *Am J Trop Med Hyg* 2003, **69:**200–205.
11. Koekemoer LL, Misiani EA, Hunt RH, Kent RJ, Norris DE, Coetzee M: **Cryptic species within *Anopheles longipalpis* from southern Africa and phylogenetic comparison with members of the *An. funestus* group.** *Bull Entomol Res* 2009, **99:**41–49.
12. Sinka ME, Bangs MJ, Manguin S, Coetzee M, Mbogo CM, Hemingway J, Patil AP, Temperley WH, Gething PW, Kabaria CW, Okara RM, Van Boeckel T, Godfray HC, Harbach RE, Hay SI: **The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic precis.** *Parasit Vectors* 2010, **3:**117.
13. Sharakhov I, Braginets O, Grushko O, Cohuet A, Guelbeogo WM, Boccolini D, Weill M, Costantini C, Sagnon N, Fontenille D, Yan G, Besansky NJ: **A microsatellite map of the African human malaria vector *Anopheles funestus*.** *J Hered* 2004, **95:**29–34.
14. Green CA, Hunt RH: **Interpretation of variation in ovarian polytene chromosomes of Anopheles funestus Giles, A. parensis Gillies, and A. aruni?** *Genetica* 1980, **51:**187–195.
15. Lochouarn L, Dia I, Boccolini D, Coluzzi M, Fontenille D: **Bionomical and cytogenetic heterogeneities of *Anopheles funestus* in Senegal.** *Trans R Soc Trop Med Hyg* 1998, **92:**607–612.
16. Costantini C, Sagnon NF, Ilboudo-Sanogo E, Coluzzi M, Boccolini D: **Chromosomal and bionomic heterogeneities suggest incipient speciation in *Anopheles funestus* from Burkina Faso.** *Parassitologia* 1999, **41:**595–611.
17. Guelbeogo WM, Grushko O, Boccolini D, Ouedraogo PA, Besansky NJ, Sagnon NF, Costantini C: **Chromosomal evidence of incipient speciation in the Afrotropical malaria mosquito *Anopheles funestus*.** *Med Vet Entomol* 2005, **19:**458–469.
18. Michel AP, Ingrasci MJ, Schemerhorn BJ, Kern M, Le Goff G, Coetzee M, Elissa N, Fontenille D, Vulule J, Lehmann T, Sagnon N, Costantini C, Besansky NJ: **Rangewide population genetic structure of the African malaria vector *Anopheles funestus*.** *Mol Ecol* 2005, **14:**4235–4248.
19. Koekemoer LL, Kamau L, Garros C, Manguin S, Hunt RH, Coetzee M: **Impact of the Rift Valley on restriction fragment length polymorphism typing of the major African malaria vector *Anopheles funestus* (Diptera: Culicidae).** *J Med Entomol* 2006, **43:**1178–1184.
20. Choi KS, Koekemoer LL, Coetzee M: **Population genetic structure of the major malaria vector Anopheles funestus s.s. and allied species in southern Africa.** *Parasit Vectors* 2012, **5:**283.
21. Coluzzi M, Petrarca V, DiDeco MA: **Chromosomal inversion intergradation and incipient speciation in *Anopheles gambiae*.** *Boll Zool* 1985, **52:**45–63.
22. Guelbeogo WM, Sagnon N, Grushko O, Yameogo MA, Boccolini D, Besansky NJ, Costantini C: **Seasonal distribution of *Anopheles funestus* chromosomal forms from Burkina Faso.** *Malar J* 2009, **8:**239.
23. Michel AP, Grushko O, Guelbeogo WM, Lobo NF, Sagnon N, Costantini C, Besansky NJ: **Divergence with genetionAPi C:**

26. della Torre A, Fanello C, Akogbeto M, Dossou-yovo J, Favia G, Petrarca V, Coluzzi M: **Molecular evidence of incipient speciation within *Anopheles gambiae s.s.* in West Africa.** *Insect Mol Biol* 2001, **10:**9–18.
27. Dia I, Sagnon N, Guelbeogo MW, Diallo M: **Bionomics of sympatric chromosomal forms of *Anopheles funestus* (Diptera: Culicidae).** *J Vector Ecol* 2011, **36:**343–347.
28. Gillies MT, Coetzee M: *A Supplement to the Anophelinae of Africa South of the Sahara.* Johannesburg: The South African Institute for Medical Research; 1987.
29. Della Torre A: **Polytene chromosome preparation from anopheline mosquitoes**. In *Molecular Biology of Disease Vectors: A Methods Manual.* Edited by Crampton JM, Beard CB, Louis C. London: Chapman & Hall; 1997:329–336.
30. Service MW: *Mosquito Ecology. Field Sampling Methods.* 2nd edition. London: Elsevier Applied Science Publishers, Ltd; 1993.
31. Bland JM, Altman DG: **Statistics notes. The odds ratio.** *Br Med J* 2000, **320:**1468.
32. Powell JR, Petrarca V, della Torre A, Caccone A, Coluzzi M: **Population structure, speciation, and introgression in the *Anopheles gambiae* complex.** *Parassitologia* 1999, **41:**101–113.
33. Molineaux L, Grammicia G: *The Garki Project. Research on the Epidemiology and Control of Malaria in the Sudan Savanna of West Africa.* Geneva, Switzerland: World Health Organization; 1980.
34. Zahar AR: *Vector Bionomics in the Epidemiology and Control of Malaria.* Geneva, Switzerland: World Health Organization; 1985.
35. Ayala D, Fontaine MC, Cohuet A, Fontenille D, Vitalis R, Simard F: **Chromosomal inversions, natural selection and adaptation in the malaria vector *Anopheles funestus*.** *Mol Biol Evol* 2011, **28:**745–758.
36. Cohuet A, Dia I, Simard F, Raymond M, Rousset F, Antonio-Nkondjio C, Awono-Ambene PH, Wondji CS, Fontenille D: **Gene flow between chromosomal forms of the malaria vector *Anopheles funestus* in Cameroon, Central Africa, and its relevance in malaria fighting.** *Genetics* 2005, **169:**301–311.
37. Dia I, Boccolini D, Antonio-Nkondjio C, Costantini C, Fontenille D: **Chromosomal inversion polymorphism of *Anopheles funestus* from forest villages of South Cameroon.** *Parassitologia* 2000, **42:**227–229.
38. Moiroux N, Gomez MB, Pennetier C, Elanga E, Djenontin A, Chandre F, Djegbe I, Guis H, Corbel V: **Changes in *Anopheles funestus* biting behavior following universal coverage of long-lasting insecticidal nets in Benin.** *J Inf Dis* 2012, **206:**1622–1629.
39. Dia I, Lochouarn L, Boccolini D, Costantini C, Fontenille D: **Spatial and temporal variations of the chromosomal inversion polymorphism of *Anopheles funestus* in Senegal.** *Parasite* 2000, **7:**179–184.
40. Kamau L, Hunt R, Coetzee M: **Analysis of the population structure of *Anopheles funestus* (Diptera: Culicidae) from western and coastal Kenya using paracentric chromosomal inversion frequencies.** *J Med Entomol* 2002, **39:**78–83.
41. Holt RA, Subramanian GM, Halpern A, Sutton GG, Charlab R, Nusskern DR, Wincker P, Clark AG, Ribeiro JM, Wides R, Salzberg SL, Loftus B, Yandell M, Majoros WH, Rusch DB, Lai Z, Kraft CL, Abril JF, Anthouard V, Arensburger P, Atkinson PW, Baden H, de Berardinis V, Baldwin D, Benes V, Biedler J, Blass C, Bolanos R, Boscus D, Barnstead M, *et al*: **The genome sequence of the malaria mosquito *Anopheles gambiae*.** *Science* 2002, **298:**129–149.
42. Neafsey DE, Christophides GK, Collins FH, Emrich SJ, Fontaine MC, Gelbart W, Hahn MW, Howell PI, Kafatos FC, Lawson D, Muskavitch MA, Waterhouse RM, Williams LJ, Besansky NJ: **The evolution of the Anopheles 16 genomes project.** *G3* 2013, **3:**1191–1194.
43. Subbarao SK: *Anopheles Species Complexes in South and South-East Asia. SEARO Technical Publication No. 57.* New Delhi: World Health Organization, Regional Office for South-East Asia; 2007.
44. Toure YT, Petrarca V, Traore SF, Coulibaly A, Maiga HM, Sankare O, Sow M, DiDeco MA, Coluzzi M: **The distribution and inversion polymorphism of chromosomally recognized taxa of the *Anopheles gambiae* complex in Mali, West Africa.** *Parassitologia* 1998, **40:**477–511.
45. Coluzzi M, Sabatini A, Della Torre A, Di Deco MA, Petrarca V: **A polytene chromosome analysis of the *Anopheles gambiae* species complex.** *Science* 2002, **298:**1415–1418.
46. Kitzmiller JB, Frizzi G, Baker RH: **Evolution and speciation within the *Maculipennis* Complex of the genus *Anopheles*.** In *Genetics of Insect Vectors of Disease.* Edited by Wright JW, Pal R. Amsterdam: Elsevier; 1967.
47. Hoffmann AA, Rieseberg LH: **Revisiting the impact of inversions in evolution: from population genetic markers to drivers of adaptive shifts and speciation?** *Ann Rev Ecol Evol Syst* 2008, **39:**21–42.
48. Kirkpatrick M, Barton N: **Chromosome inversions, local adaptation and speciation.** *Genetics* 2006, **173:**419–434.
49. Kirkpatrick M: **How and why chromosome inversions evolve.** *PLoS Biol* 2010, **8**(9):e1000501. doi:10.1371/journal.pbio.1000501.
50. Ayala D, Guerrero RF, Kirkpatrick M: **Reproductive isolation and local adaptation quantified for a chromosome inversion in a malaria mosquito.** *Evolution* 2013, **67:**946–958.
51. Cheng C, White BJ, Kamdem C, Mockaitis K, Costantini C, Hahn MW, Besansky NJ: **Ecological genomics of *Anopheles gambiae* along a latitudinal cline: a population-resequencing approach.** *Genetics* 2012, **190:**1417–1432.
52. Coluzzi M: **Spatial distribution of chromosomal inversions and speciation in anopheline mosquitoes**. In *Mechanisms of Speciation.* Edited by Barigozzi C. New York: Alan R. Liss, Inc; 1982:143–153.

doi:10.1186/1475-2875-13-65
Cite this article as: Guelbeogo *et al.*: Behavioural divergence of sympatric *Anopheles funestus* populations in Burkina Faso. *Malaria Journal* 2014 **13**:65.