Anopheles gambiae 1000 Genomes Project (Ag1000G) phase 3

Contributing studies

The *Anopheles gambiae* 1000 Genomes Consortium

January 14, 2021

Summary

The Ag1000G project is coordinated by a consortium of partners from a range of different research institutions and countries. This includes consortium members who are carrying out independent research studies in malaria endemic regions, and who have contributed mosquito specimens or mosquito DNA samples collected in the course of their own research. This document describes the studies that have contributed samples to phase 3 of the Ag1000G project, which includes wild-caught samples from 19 African countries. This document also provides information about the collection locations and methods, the people involved in the studies, and references to any published articles providing further information about the studies. Throughout this document we use species nomenclature following Coetzee et al. (2013). Unless otherwise stated, the DNA extraction method used for the collections described below was Qiagen DNeasy Blood and Tissue Kit (Qiagen Science, MD, USA).

Contents

Angola

Sample sets

`AG1000G-AO`

Study information

Adult mosquitoes were obtained by rearing larvae collected from breeding sites along the main roads connecting the municipalities of Kilamba-Kiaxi and Viana, Luanda province (-8.821, 13.291), in April/May 2009. These are peri-urban areas where malaria reaches hyperendemic levels. All specimens collected in the study area were typed as *An. coluzzii* (Fanello et al. 2002) although *An. melas* and *An. arabiensis* have also been recorded in the province (Cuamba et al. 2006; Calzetta et al. 2008). Specimens were stored on silica gel and DNA extraction was performed by a phenol-chloroform protocol described in Donnelly et al. (1999).

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Burkina Faso (1)

Sample sets

`AG1000G-BF-A, AG1000G-BF-B`

Study information

The Target Malaria project contributed samples from collections made in three villages separated by at most 30km: Bana (11.233, -4.472), Souroukoudinga (11.235, -4.535) and Pala (11.150, -4.235). These collections were made in July-August 2012, July and October 2014, and January, February and April 2015. The area is agricultural, with rice-growing areas near Bana and Souroukoudinga, and a large mango grove near Pala. Female mosquitoes were collected by human landing catch, pyrethrum spray collection or aspiration. Males were collected by swarm netting. Both *An. gambiae* and *An. coluzzii* (Wilkins et al. 2006) were collected. Specimens were stored in 80% ethanol and DNA was extracted using the DNeasy Tissue Kit (Qiagen) or using a simple CTAB method.

External resources

- `https://targetmalaria.org/`

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Acknowledgements

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Burkina Faso (2)

Sample sets

`AG1000G-BF-C`

Study information

Samples were contributed from collections of indoor resting adults made by spray catch from Monomtenga in central Burkina Faso (12.06, -1.17). These specimens were sorted morphologically to *An. gambiae s.l.*. Ovaries of half-gravid females were dissected and placed in numbered individual micro-tubes containing modified Carnoy's solution (1:3 glacial acetic acid: 100% ethanol). Carcasses were placed in correspondingly numbered micro-tubes over desiccant. Genomic DNA was isolated from individual mosquitoes using one of the following: DNeasy Extraction Kit (Qiagen, Valencia, CA), Puregene kit (Gentra Systems, Inc., Minneapolis, MN), DNAzol kit (Molecular Research Center, Inc., Cincinnati, OH.) or Easy-DNA kit (Invitrogen, Carlsbad, CA). *An. gambiae s.s.* and its molecular forms were identified using one of two rDNA-based PCR/RFLP assays, Fanello et al. (2002) or Santolamazza et al. (2004). Ovaries from specimens of the desired species were subject to polytene chromosome analysis.

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Cameroon (1)

Sample sets

AG1000G-CM-A

Study information

Pyrethrum spray collections were conducted in three villages in Cameroon during September and October 2009. These villages comprise a transect from forest (village of Mayos: 4.341, 13.558) to forest/savanna transition (village of Daiguene: 4.777, 13.844) to savanna (villages of Gado-Badzere and Zembe-Borongo: 5.747, 14.442) (Lobo et al. 2010). All contributed specimens were *An. gambiae s.s.* (Fanello et al. 2002). A proportion of specimens were karyotyped via scoring of polytene chromosomes (Coulibaly et al. 2007). Specimens were stored on silica gel, and DNA was extracted using a simple CTAB protocol and run over Qiagen columns.

Related publications

- NF Lobo et al. 2010. Breakpoint structure of the *Anopheles gambiae* 2Rb chromosomal inversion. *Malaria Journal.* **9**: 293

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Cameroon (2)

Sample sets

AG1000G-CM-B

Study information

These samples were collected as part of a study which took place in Cameroon in Central Africa. The country is commonly referred to as "miniature Africa", owing to the diversity of its climate, topography, landscape, and bio-ecological settings: arid savannas in the north gradually turn into rain forest in the south, along with highland areas, contribute to increase diversity of ecological settings. Anopheline mosquitoes were collected in 2005 from 64 locations covering a 1,500 km north-to-south transect that crossed all eco-geographical areas of Cameroon (Simard et al. 2009). Mosquito collection involved spraying aerosols of pyrethroid insecticides inside human dwellings, dead mosquitoes were retrieved from white sheets that were laid on the floor. Anopheline mosquitoes were identified using morphological identification keys (Gillies and De Meillon 1968; Gillies and Coetzee 1987). Ovaries from half-gravid *An. gambiae s.l.* females were dissected and stored in Carnoy's fixative solution (absolute ethanol:glacial acetic acid 3:1) for cytogenetic analyses. Carcasses were stored individually in tubes containing a desiccant and kept at -20°C until they were processed for molecular analysis. All half-gravid specimens collected in each village were identified to species and molecular forms using PCR-RFLP (Fanello et al. 2002).

Related publications

- F Simard et al. 2009. Ecological niche partitioning between *Anopheles gambiae* molecular forms in Cameroon: the ecological side of speciation. *BMC Ecology.* **9**: 17

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Cameroon (3)

Sample sets

AG1000G-CM-C

Study information

Samples were contributed from pyrethrum spray collections, larval sampling and human landing catches conducted in twenty locations during October 2013. These villages are scattered throughout the country and reflect a gradient of human-dominated environments, for example, forest (Manda: (5.726, 10.868) and Campo: (2.367, 9.817); forest/savanna transition (Tibati: (6.469, 12.629)); savanna (Lagdo: (9.049, 13.656)); suburban area (Nkolondom: (3.972, 11.516)) and urban areas (Douala: (4.055, 9.721) and Yaoundé: (3.880, 11.506). Contributed specimens were *An. gambiae* or *An. coluzzii* (Fanello et al. 2002). Population genomics studies indicated the presence of relatively differentiated subgroups within both species as well as clusters thriving in polluted breeding sites in large cities (Kamdem et al. 2017). Specimens were stored on silica gel. DNA was extracted using a Zymo research kit for adults, and a Qiagen kit for larvae.

Related publications

- C Kamdem et al. 2017. Pollutants and Insecticides Drive Local Adaptation in African Malaria Mosquitoes. *Molecular Biology and Evolution*. **34**: 1261–1275

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Central African Republic

Sample sets

`AG1000G-CF`

Study information

Collections were carried out in Bangui (4.367, 18.583), during December 1993, by indoor resting aspiration or pyrethrum spray catch.

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Bioko Island, Equatorial Guinea

Sample sets

`AG1000G-GQ`

Study information

Collections were performed during the rainy season in September 2002 by overnight CDC light traps in Sacriba of Bioko island (3.7, 8.7). Specimens were stored dry on silica gel before DNA extraction. Specimens contributed from this site were *An. gambiae* females, genotype determined by two assays (Scott et al. 1993; Santolamazza et al. 2004). All specimens had the $2L^{+a}/2L^{+a}$ karyotype as determined by the molecular PCR diagnostics (White et al. 2007). These mosquitoes represent a population that inhabited Bioko Island before a comprehensive malaria control intervention initiated in February 2004 (Sharp et al. 2007). After the intervention *An. gambiae* was declining, and more recently almost only *An. coluzzii* can be found (Overgaard et al. 2012).

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Côte d'Ivoire

Sample sets

`AG1000G-CI`

Study information

Samples were collected in Tiassale (5.898, -4.823), located in the evergreen forest zone of southern Côte d'Ivoire. The primary agricultural activity is rice cultivation in irrigated fields. High malaria transmission occurs during the rainy seasons, between May and November. Samples were collected as larvae from irrigated rice fields by dipping between May and September 2012. All larvae were reared to adults and females preserved over silica for DNA extraction. Specimens from this site were all *An. coluzzii*, determined by PCR assay (Santolamazza et al. 2004).

Related publications

- X Grau-Bové et al. 2020. Resistance to pirimiphos-methyl in West African *Anopheles* is spreading via duplication and introgression of the Ace1 locus. *bioRxiv*

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Ghana

Sample sets

`AG1000G-GH`

Study information

Samples were collected in Twifo Praso (5.609, -1.549), a peri-urban community located in semi-deciduous forest in the Central Region of Ghana. It is an extensive agricultural area characterised by small-scale (vegetable growing) and large-scale commercial farms such as oil palm and cocoa plantations. Mosquito samples were collected as larvae from puddles near farms between September and October, 2012. Madina (5.668, -0.219) is suburb of Accra within a coastal savanna zone of Ghana. It is an urban community characterised by myriad vegetable-growing areas. The vegetation consists of mainly grassland interspersed with dense short thickets often less than 5m high with a few trees. Specimens were sampled from puddles near roadsides and farms between October and December 2012. Takoradi (4.912, -1.774) is the capital city of the Western Region of Ghana. It is an urban community located in the coastal savanna zone. Mosquito samples were collected from puddles near road construction and farms between August and September 2012. Koforidua (6.094, -0.261) is the capital city of the Eastern Region of Southern Ghana and is located in semi-deciduous forest. It is an urban community characterized by numerous small-scale vegetable farms. Samples were collected from puddles near road construction sites and farms between August and September 2012. Larvae from all collection sites were reared to adults and females preserved over silica for DNA extraction. Both *An. gambiae* and *An. coluzzii* were collected from these sites, determined by PCR assay (Santolamazza et al. 2004).

Related publications

- J Essandoh et al. 2013. Acetylcholinesterase (Ace-1) target site mutation 119S is strongly diagnostic of carbamate and organophosphate resistance in *Anopheles gambiae ss* and *Anopheles coluzzii* across southern Ghana. *Malaria journal.* **12**: 1–10

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Democratic Republic of the Congo

Sample sets

`AG1000G-CD`

Study information

Samples were collected from Gbadolite (4.283, 21.017), a town located in the far north of the Democratic Republic of Congo (DRC) near the border with the Central African Republic, surrounded by forest. In common with much of DRC, malaria transmission rates are high, and the samples are *An. gambiae s.s.*, which is the dominant vector. Samples were collected as larvae from temporary pools within and around the town by dipping in early August 2015. All larvae were reared to adults and females preserved over silica for DNA extraction using Qiagen DNAEasy kits.

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Gabon

Sample sets

`AG1000G-GA-A`

Study information

Mosquitoes were collected by landing catches in the capital city Libreville (0.384, 9.455) in December 2000 (Pinto et al. 2006), an urban and polluted site. Malaria is endemic throughout the year. Specimens were stored in alcohol at -20°C. Co-occurrence of both *kdr* resistance alleles and absence of wild-type susceptible alleles have been reported in this population (Pinto et al. 2006). *An. coluzzii* and *An. melas* are also present in the region but at frequencies <1% (Mourou et al. 2012). Specimens were stored on silica gel and DNA extraction was performed by a phenol-chloroform protocol described in Donnelly et al. (1999).

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Guinea Bissau

Sample sets

`AG1000G-GW`

Study information

Guinea Bissau samples were collected from three sites in October 2010 by indoor CDC light traps. Safim (11.957, -15.649) and Antula (11.891, -15.582), from a south-western coastal region, characterised mainly by mixed flooded forests and croplands. Leibala is a neighbourhood of the eastern town of Gabu where shrubland and open deciduous forest predominate (12.272, -14.222). According to PCR-RFLP of the IGS (Fanello et al. 2002) and SINEX (Santolamazza et al. 2004) all samples were identified as *An. gambiae*. The *kdr* pyrethroid target site resistance mutation L995F occurs at high frequency in Leibala but at very low frequency in the western coastal region (Vicente et al. 2017). Malaria is meso-hyperendemic (Arez et al. 2003) and sporozoite rates are below 1% in the region. Specimens were stored on silica gel and DNA extraction was performed by a phenol-chloroform protocol described in Donnelly et al. (1999).

Related publications

- JL Vicente et al. 2017. Massive introgression drives species radiation at the range limit of *Anopheles gambiae*. *Scientific Reports*. **7**:

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Guinea and Mali (1)

Sample sets

`AG1000G-GN-A, AG1000G-GN-B`

Study information

Collections were made from four different study sites around the border between Guinea and Mali. From Mali; Takan (11.47, -8.33) and Toumani Oulena (10.83, -7.81) are both small villages in the Yanfolila district of southern Mali and represent the Sudanian savannah ecological zone. Takan is arid savannah, while Toumani Oulena is humid savannah. In Guinea Conakry, mosquitoes were sampled from Koraboh (9.28, -10.03), a small village in the Kissidougou district in the Faranah region representing a semi-forest site with intermediate ecology, a mix of savannah and forest, and in Koundara (8.48, -9.53), a small village in the Macenta district in the Nzerekore region representing deep forest ecology. All reported collections occurred in October and November 2012. At each site, mosquitoes were collected using three different methods: human-landing capture, indoor manual aspirator or pyrethroid spray catch, and larval capture - where the first and second instar larvae were raised to adult in a field insectary under standard insectary conditions prior to DNA isolation from the adults, and the third and fourth instar larvae were preserved directly for DNA isolation, without rearing in the insectary. The two distinct methods of larval collection were used to control for possible genetic bias inherent in lab rearing of captured larvae. Across sites, all types of larval sites were sampled, including both temporary and permanent sites. Human-landing captures were performed both inside dwellings and outside (>10 m from dwelling) at night between 18:00 and 06:30. The indoor aspirator or spray catches were done in the morning between 06:00 and 12:00. Adult specimens or third and fourth instar larvae were preserved immediately in 80% ethanol until later DNA extraction. First and second instar larvae were raised to adults in nearby field insectaries and upon emergence were preserved in 80% ethanol. DNA was extracted from mosquitoes using DNAzol by the provided protocol (Invitrogen, CA, USA).

Related publications

- B Coulibaly et al. 2016. Malaria vector populations across ecological zones in Guinea Conakry and Mali, West Africa. *Malaria Journal*. **15**:

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Mali (2)

Sample sets

`AG1000G-ML-A`

Study information

Collections were made in four villages in the Koulikoro region; Tieneguebougou (12.810, -8.080) approximately 20 km north of Bamako, and Kababougou (12.890, -8.150), Ouassorola (12.900, -8.160), Sogolombougou (12.880, -8.140), approximately 30 km north of Bamako. The collections were made in August 2014 by human landing catch and pyrethrum spray catch. Both *An. gambiae* and *An. coluzzii* (Fanello et al. 2002) were collected. Specimens were stored in 80% ethanol.

External resources

- `https://targetmalaria.org/`

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Mali (3)

Sample sets

AG1000G-ML-B

Study information

Collections of indoor resting adults were made by spray catch from seven villages in the southern part of Mali in August-September 2004: Banambani (12.800, -8.050), Bancoumana (12.200, -8.200), Douna (13.210, -5.900), Fanzana (13.200, -6.130), Kela (11.880, -8.450), Moribobougou (12.690, -7.870) and N'Gabakoro (12.680, -7.840). Specimens were sorted morphologically to *An. gambiae s.l.*. Ovaries of half-gravid females were dissected and placed in numbered individual micro-tubes containing modified Carnoy's solution (1:3 glacial acetic acid: 100% ethanol). Carcasses were placed in correspondingly numbered micro-tubes over desiccant. Genomic DNA was isolated from individual mosquitoes using one of the following: DNeasy Extraction Kit (Qiagen, Valencia, CA), Puregene kit (Gentra Systems, Inc., Minneapolis, MN), DNAzol kit (Molecular Research Center, Inc., Cincinnati, OH.) or Easy-DNA kit (Invitrogen, Carlsbad, CA). *An. gambiae s.s.* and its molecular forms were identified using one of two rDNA-based PCR/RFLP assays (Fanello et al. 2002; Santolamazza et al. 2004). Ovaries from specimens of the desired species were subject to polytene chromosome analysis.

Related publications

- MB Coulibaly et al. 2007. PCR-based karyotyping of *Anopheles gambiae* inversion 2Rj identifies the BAMAKO chromosomal form. *Malaria Journal*

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Kenya

Sample sets

`AG1000G-KE`

Study information

Kenyan specimens were obtained from villages located in Kilifi County near the Kenyan coast between 2000 and 2014. All *Anopheles* mosquito sampling was conducted indoors using CDC light traps which were hung at 6pm and collected at 6am the following morning during the rainy season in September. Specimens were stored in 80% ethanol. All specimens contributed to the project were identified as *An. gambiae* using the species complex diagnostic assay of (Scott et al. 1993) *An. gambiae*, *An. funestus*, *An. arabiensis*, *An. merus* were present at sampling locations Sporozoite rates for the area during previous studies were 1.47% (Midega et al. 2012).

Related publications

- JT Midega et al. 2012. Wind direction and proximity to larval sites determines malaria risk in Kilifi District in Kenya. *Nature Communications*. **3**:

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Malawi

Sample sets

`AG1000G-MW`

Study information

Specimens were obtained from villages within the catchment of the Majete Malaria Project, Chikhwawa District, Malawi (-15.933, 34.755) (McCann et al. 2017). Mosquitoes were collected indoors and outdoors by Suna light trap from April through August 2015. Chickhwawa District is an area with perennial and intense malaria transmission (Bennett et al. 2013). All specimens were *An. arabiensis* (Fanello et al. 2002) Specimens were stored over silica and DNA was extracted using the Qiagen plate protocol.

Related publications

- RS McCann et al. 2017. Assessment of the effect of larval source management and house improvement on malaria transmission when added to standard malaria control strategies in southern Malawi: study protocol for a cluster-randomised controlled trial. *BMC Infectious Diseases*. **17**:

External resources

- `https://mesamalaria.org/mesa-track/majete-integrated-malaria-control-project-mmp`

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Mayotte Island

Sample sets

`AG1000G-FR`

Study information

Collections were taken from multiple sites on the island of Mayotte. Samples were collected as larvae during March-April 2011 in temporary pools by dipping. Sites included Mtsanga Charifou (-12.991, 45.156) and Combani (-12.779, 45.143). Larvae were stored in 80% ethanol prior to DNA extraction. All specimens contributed were *An. gambiae s.s.* (Santolamazza et al. 2004) with the standard $2L^{+a}/2L^{+a}$ or inverted $2L^{a}/2L^{a}$ karyotype as determined by the molecular PCR diagnostics (White et al. 2007). Samples were identified as males or females by the sequencing read coverage of the X chromosome using LookSeq (Manske and Kwiatkowski 2009).

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Mozambique

Sample sets

`AG1000G-MZ`

Study information

Mosquito samples were collected in Furvela (-23.716, 35.299), Mozambique, by CDC light traps between December 2003 and April 2004. Specimens were stored on silica gel and DNA was extracted according to Collins et al. (1987). Contributed specimens consisted of *An. gambiae* individuals identified according to Fanello et al. (2002). Furvela is a rural village located in Inhambane Province, where malaria is transmitted mainly by *An. gambiae* and *An. funestus* (Charlwood et al. 2014). *An. arabiensis* and *An. merus* are also found at low frequency. Sporozoite rates around 4% have been reported in *An. gambiae* from Furvela (Charlwood et al. 2014).

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Tanzania

Sample sets

AG1000G-TZ

Study information

Tanzanian samples were collected from four distinct locations. Moshi samples came from lower Mabogini (-3.400, 37.350), rice fields near lower Moshi on the southern slope of Mount Kilimanjaro, a region shown to have increasing resistance to pyrethroids (Matowo et al. 2014). Mosquitoes were collected as larvae, during the rice growing season in August-September 2012, raised to adults and females bioassayed in WHO tubes for one hour with 0.05% lambda cyhalothrin (Organization 2016). Alive and dead mosquitoes were preserved over silica. In Tanzanian samples screened in Kabula et al. (Kabula et al. 2012), Moshi was the most pyrethroid resistant population, they were found to be completely DDT susceptible, only in one out of 642 mosquitoes assayed by Matowo et al. (2014) was found to carry a *kdr* resistance mutation (Vgsc-995F).

Tarime collections took place in the village of Komaswa (-1.417, 34.183) about 410 km north west of Moshi, during August 2012. Mosquito larvae were collected, raised to adults and females bioassayed with a range of insecticides in WHO tubes for one hour (Organization 2016), finding almost complete multi-insecticide susceptibility: permethrin (100% mortality), lambda cyhalothrin (97%), fenitrothion (100%), DDT (100%) and bendiocarb (100%) (Matiya et al. 2019).

Muheza samples were collected from Zeneti village (-5.217, 38.650), northeast Tanzania. Malaria is intense and perennial with transmission peaking after the rainy season in May and June (Kabula et al. 2012). Mosquitoes were sampled between November 2012 and May 2013. Indoor resting collections were used to obtain live females for deltamethrin susceptibility testing and pyrethrum spray catches were used for mosquitoes that were collected for blood meal analysis. Collections were conducted between 06:00 and 09:00 from randomly selected houses. Live mosquitoes collected for susceptibility testing were provided with 10% glucose solution and transported to the field insectary. Mosquitoes were sorted and morphologically identified to species, carcasses were stored individually over desiccant for laboratory processing.

Muleba (1.750, 31.667), the final collection region, is in the North-western part of Tanzania. The district is known to be a malaria epidemic prone area with unstable transmission of varying seasonality. The highest peak of malaria transmission is usually reached between May-July and November-January, which results from proceeding rain seasons. There have been malaria vector control efforts since 2007 when indoor residual spraying using lambda cyhalothrin was introduced. Insecticide resistance in this district is coupled with high frequency of *kdr* pyrethroid target site mutations in the *An. gambiae s.s.* population (Kabula et al. (2012), Protopopoff et al. (2013)). Sampling was conducted over six months, which include both dry and rainy season and covers 6 villages selected to represent all major ecological systems in the district.

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The Gambia (1)

Sample sets

`AG1000G-GM-A`

Study information

Indoor resting female mosquitoes were collected by pyrethrum spray catch from four hamlets around Njabakunda (13.55, -15.90), North Bank Region, The Gambia between August and October 2011. The four hamlets were Maria Samba Nyado, Sare Illo Buya, Kerr Birom Kardo, and Kerr Sama Kuma; all are within 1 km of each other. This is an area of unusually high hybridization rates between *An. gambiae s.s.* and *An. coluzzii* (Caputo et al. (2008), Nwakanma et al. (2013)). Njabakunda village is approximately 30km to the west of Farafenni town and 4km away from the Gambia River. The vegetation is a mix of open savannah woodland and farmland. With apparent high gene-flow in the region, it is problematic to assign species to these samples.

Related publications

- DC Nwakanma et al. 2013. Breakdown in the Process of Incipient Speciation in *Anopheles gambiae*. *Genetics*. **193**: 1221–1231

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The Gambia (2)

Sample sets

AG1000G-GM-B

Study information

Specimens were collected along the Gambia River from the western coastal region of The Gambia (Caputo et al. 2008), in August 2006. *An. gambiae* and *An. coluzzii* specimens were identified to species following the PCR-RFLP protocol (Fanello et al. 2002) using DNA extracted from the mosquito leg. Only *An. coluzzii* specimens were collected from villages of Tankular (13.417, -16.033) and Kalataba (13.550, -15.617). *An. gambiae* and *An. coluzzii* specimens were found in sympatry and collected from villages of Yallal Tankonjala (13.550, -15.700), Sare Samba Sowe (13.583, -15.900) and Hamdalai (13.567, -16.0167). PCR-RFLP protocol also revealed the presence of mosquitoes with hybrid *An. gambiae*/*An. coluzzii* genotype in Yallal Tankonjala and Sare Samba Sowe. Collections of indoor daytime-resting half gravid mosquitoes were carried out mainly in human dwellings and, in few cases, in animal shelters. Collections were carried out by pyrethroid and/or paper-cup mouth aspirators from 12 AM to sunset, and kept in vials with desiccant. Ovaries were dissected, maintained into Carnoy fixative (three parts pure ethanol:one part glacial acetic acid) and stored at -20C before polytene chromosome preparations (Caputo et al. 2008) Chromosome scoring was carried out under a phase-contrast optical microscope. Paracentric inversion karyotypes were scored according to the nomenclature and conventions of Coluzzi et al. (1979) and Touré et al. (1998).

Related publications

- B Caputo et al. 2008. *Anopheles gambiae* complex along The Gambia river, with particular reference to the molecular forms of *An. gambiae s.s. Malaria Journal.* **7**: 182

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The Gambia (3)

Sample sets

AG1000G-GM-C

Study information

Adult mosquitoes were collected at Wali Kunda in the rural, central river region of The Gambia (13.567, -14.917). The area is 180 km from the sea, on the south bank of the River Gambia, in flat Sudan savannah with a small fishing village (and a research field station) as well as rice fields and swamplands. The dominant *Anopheles* vector species in this region is *An. coluzzii* (Opondo et al. 2016). Mosquitoes were captured using human landing collections both inside and outside huts for 19 days in October and November 2012. Mosquitoes were stored in RNAlater or dried over silica gel and stored at -20C.

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Uganda

Sample sets

`AG1000G-UG`

Study information

Specimens were obtained from two locations in Uganda: Nagongera, 30km to the North of Lake Victoria near the border with Kenya, and Kihihi, in the very South-West of the country. In Nagongera, Tororo District (0.77, 34.026), mosquitoes were collected by CDC light trap, resting and window trap collections, during October 2012. This is an area of intense perennial malaria transmission (Kilama et al. 2014). Additional details of vector population bionomics may be found in (Okello et al. 2006; Ramphul et al. 2009; Mawejje et al. 2013; Weetman et al. 2014). Specimens were stored in 80% ethanol and DNA was extracted using the Qiagen plate protocol. In Kihihi subcounty, Kanungu District (-0.751, 29.701), resting mosquitoes were collected during October and November 2012. Kihihi is located in an upland area with seasonal malaria transmission (Kilama et al. 2014). Specimens were stored in 80% ethanol and DNA was extracted using the Qiagen plate protocol. All specimens from both collections were *An. gambiae* (Fanello et al. 2002).

Related publications

- M Kilama et al. 2014. Estimating the annual entomological inoculation rate for *Plasmodium falciparum* transmitted by *Anopheles gambiae s.l.* using three sampling methods in three sites in Uganda. *Malaria Journal.* **13**:

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Colony crosses

Sample sets

`AG1000G-X`

Study information

15 crosses were contributed to Ag1000G phase 3, 11 of which were previously released in Ag1000G phase 2. Crosses were generated using parents from eight different colonies: G3 (MRA-112); Kisumu (MRA-762); Pimperena (canonical representative of *An. gambiae* species; MRA-861); Ghana (recent colony of *An. coluzzii* from Okyereko, southern Ghana (Edi et al. 2014); Mali-NIH (canonical representative of *An. coluzzii* species; Niono, MRA-860); (P)Akron (Benin, MRA-913); Nagongera (Tororo, Uganda); and Tiassalé (southern Côte d'Ivoire (Edi et al. 2014). The cross family labels, *e.g.* "29-2", are identifiers used for each of the crosses within the contributor project and have no special meaning.

Anopheles gambiae is a swarm-mater and crosses were therefore undertaken in mixed groups involving 4-10 females from a single colony with 1-4 males from each from a different colony in plastic cups covered with netting with 10% sugar water provided ad libitum. Females were fed on human blood. After 3 days, males were removed and individually preserved in 95% ethanol. Gravid or half-gravid females were then removed and placed in 1.5ml Eppendorf tubes. Females that did not appear gravid were given a second blood meal before placing in Eppendorf tubes for egg laying. Following egg deposition, females were removed and stored in tubes containing ethanol for subsequent DNA extraction. Eggs were floated in clear plastic trays (15x10x5cm) and, following hatching, larvae were raised on finely-ground fish food (Tetramin). Trays were checked daily and pupae were placed individually into small, labelled centrifuge tubes. Offspring were removed on eclosion and stored in individual tubes containing ethanol. DNA was extracted from parents and offspring using the Qiagen DNeasy kit.

A preliminary assessment of the father of each cross was obtained by genotyping seven microsatellite loci in the mother, potential fathers and five or six offspring. Where possible, the colony of origin of each father was established using individual clustering of the mothers and fathers in BAPS version 5.2, with cluster identity mapped to colony of origin via the mothers (for which the colony was known) (The Anopheles gambiae 1000 Genomes Consortium 2017). The four crosses that are novel to phase 3: B5, K2, K4 and K6, required further analysis to ascertain the true father of each cross, given mother and offspring. For each cross for which the father was in doubt, the list of potential parental pairs was determined. For each of these pairs Mendelian error was computed for every sample of the progeny and the median value (among samples) was plotted. In these four crosses (B5, K2, K4 and K6) one pair yielded median Mendelian errors significantly lower for every autosome than all other pairs, identifying the parsimonious parents. Two of the novel crosses, K4 and K6, were found to be fathered by the same male, `AC0398`.

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Further information

For further information about the Ag1000G project, please visit `https://www.malariagen.net/ag1000g`. For further information about the Ag1000G phase 3 SNP data release, please visit `www.malariagen.net/data/ag1000g-phase3-snp`. If you have any questions regarding the data release, please start a new discussion at `https://github.com/malariagen/vector-public-data/discussions`.

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