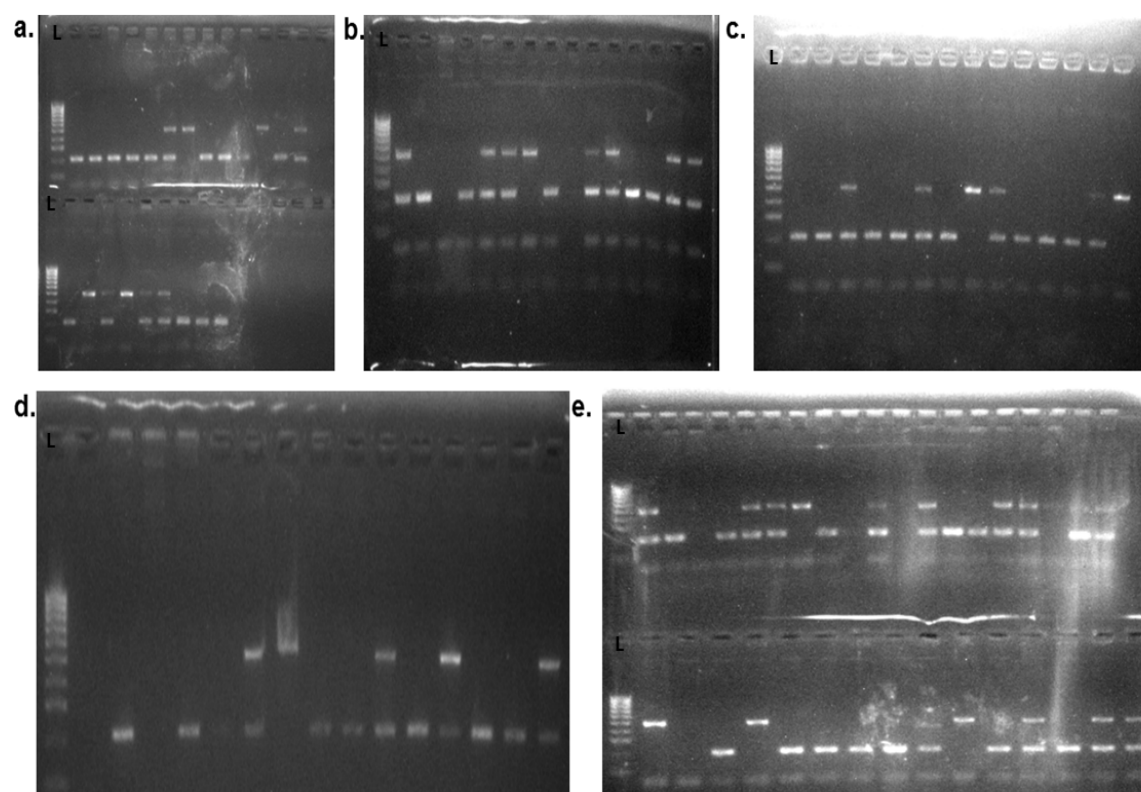
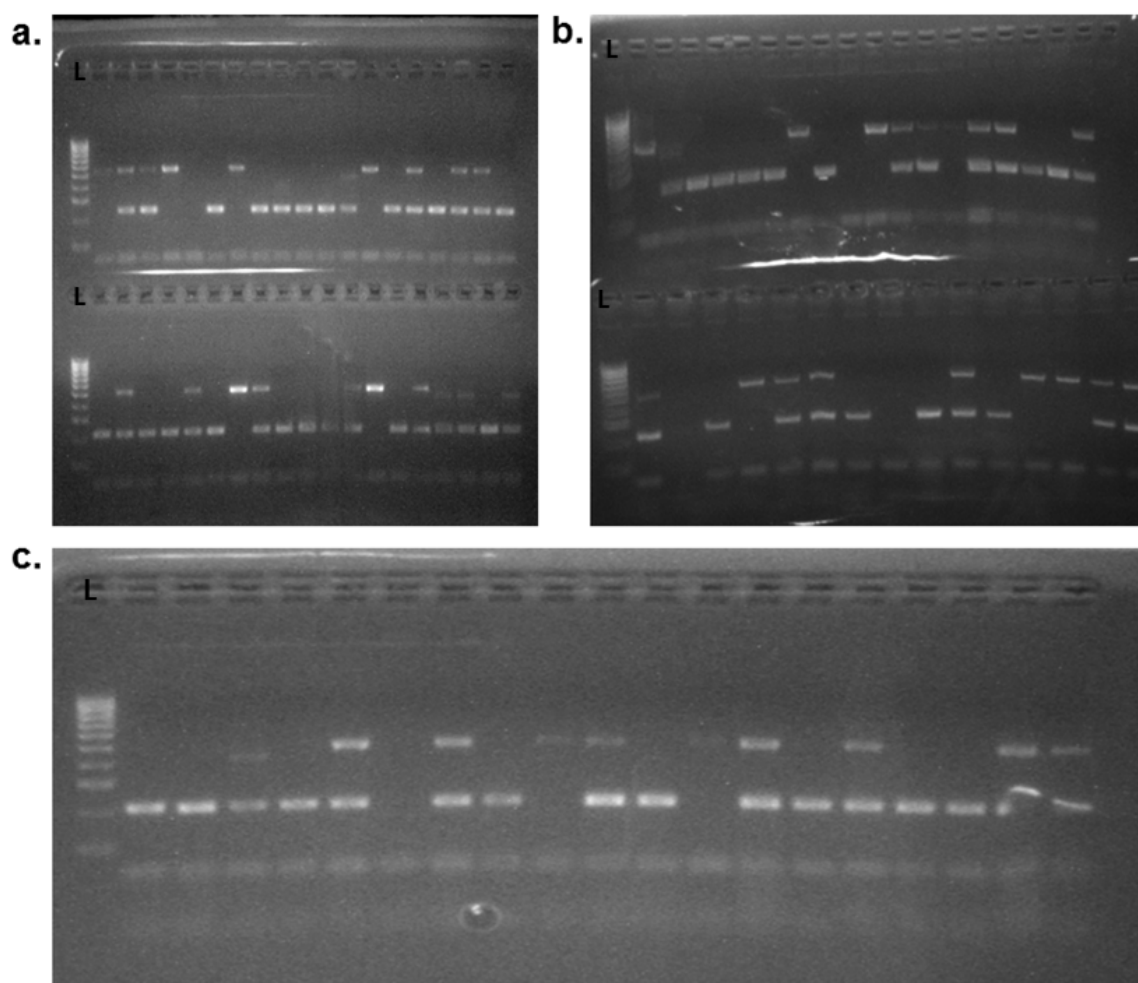


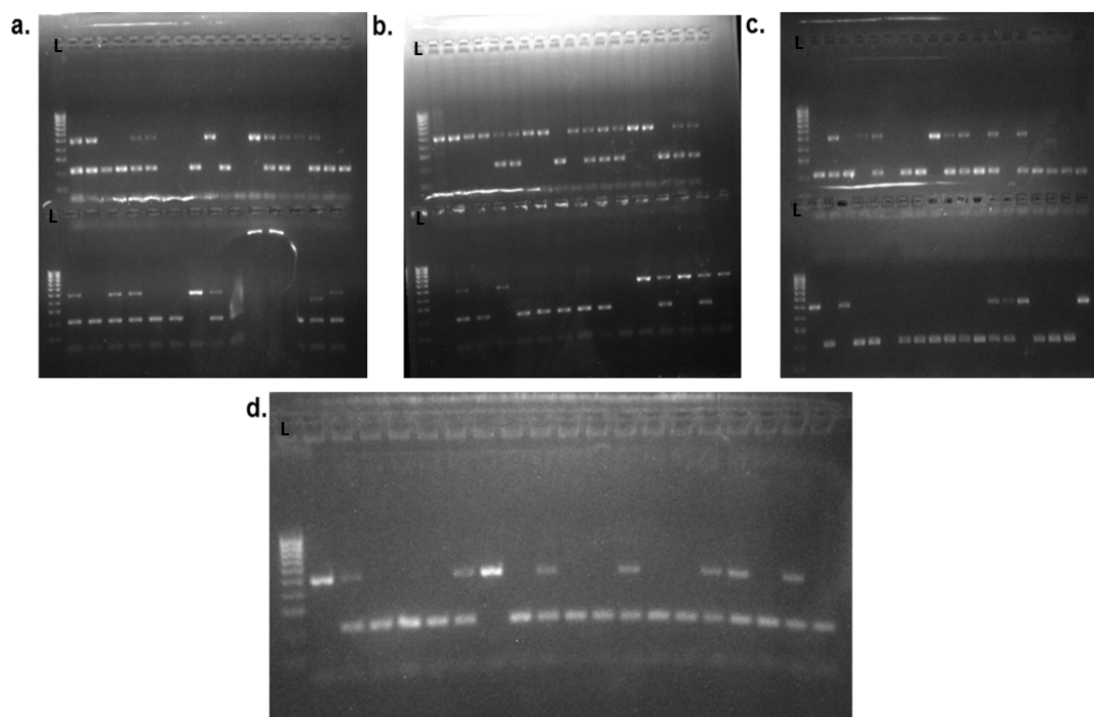
**Figure S1.** Map of the four sampling sites in northern Nigeria. Red star for BUK and Gamjin Bappa (Kano State), Auyo (Jigawa State) and Pantami (Gombe State).



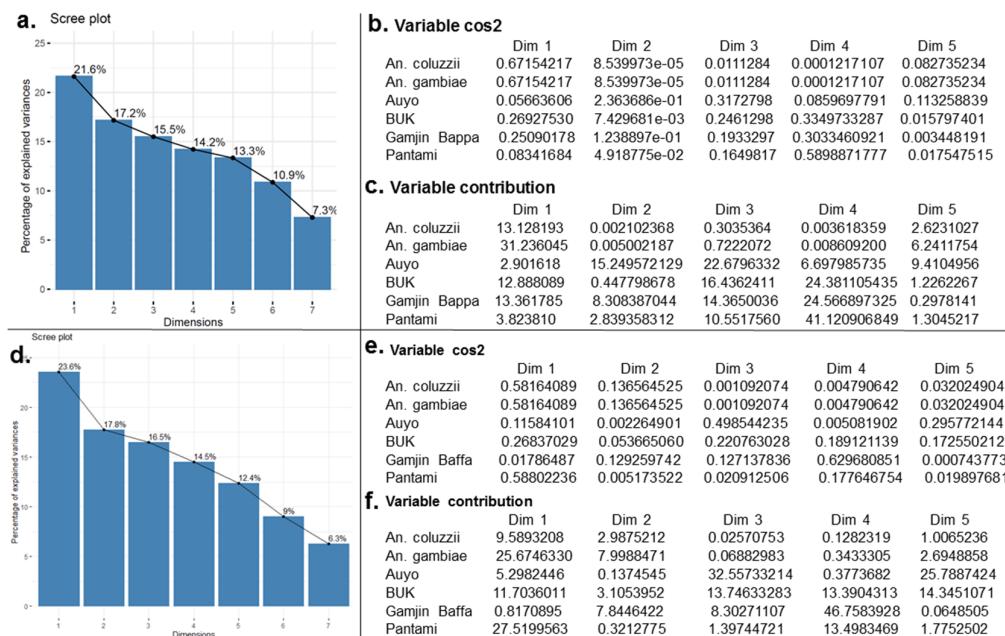
**Figure S2.** Agarose gel of 2La karyotyping using larvae from heat stress bioassay. **a.** top panel is for Auyo alive (lanes 1–13) while the lower panel is Auyo dead (lanes 1–9); **b.** BUK alive 1–13; **c.** BUK dead, 1–15; **d.** Gamjin Bappa alive (1–11) and dead (12 and 13); and **e.** Gamjin Bappa dead (1–3), Pantami alive (4–19) and Pantami dead (20–33). L is Bioline 100 bp DNA ladder ((100–1013 bp (40–200 ng/band))).



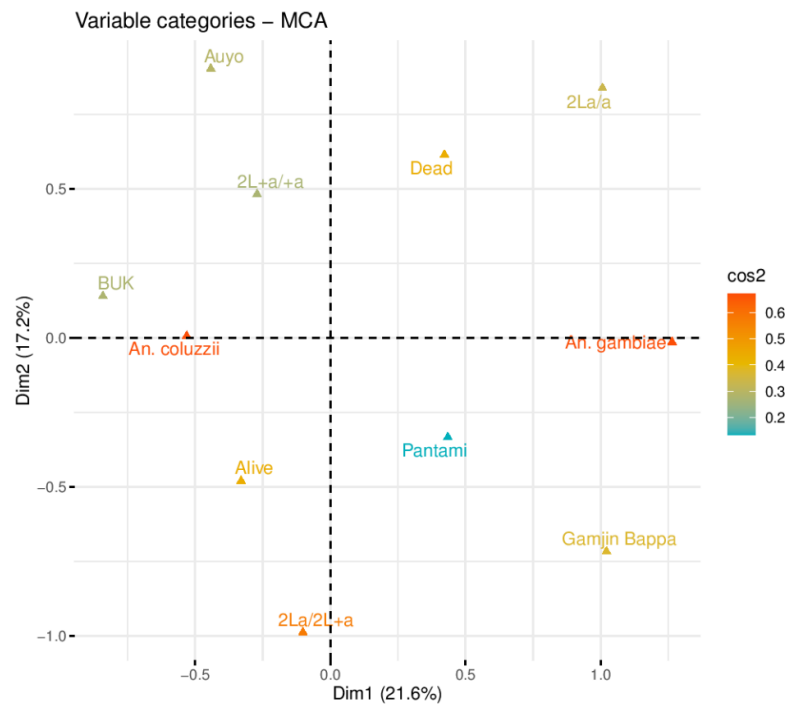
**Figure S3.** Agarose gel of 2La karyotyping using larvae from permethrin bioassay. **a.** BUK alive (lanes 1–9), BUK dead (10–20), Gamjin Bappa alive (21–26) and Gamjin Bappa dead (27–37); **b.** Pantami alive (1–15) and Pantami dead (16–30); **c.** Auyo alive 1–11 and Auyo dead 12–17. Number 18 not identified from species identification. L is Bioline 100 bp DNA ladder ((100–1013 bp (40–200 ng/band))).



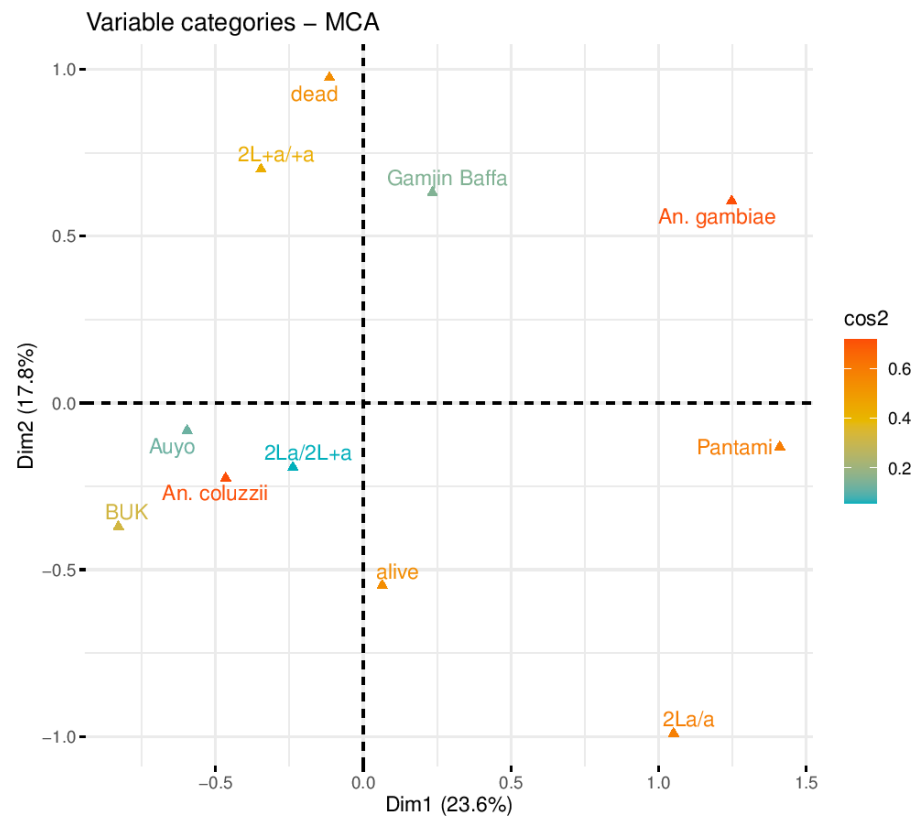
**Figure S4.** Agarose gel of 2La karyotyping using adult females from permethrin bioassay. **a.** Auyo alive (lanes 1–16), Auyo dead (17–28), BUK alive (29–31); **b.** BUK alive (1–19), BUK dead (20–29), Pantami alive (30); **c.** Pantami alive (1–16), Pantami dead (17–26), Gamjin Bappa dead (27–35); and **d.** Gamjin Bappa alive (1–19). L is Bioline 100 bp DNA ladder ((100–1013 bp (40–200 ng/band))).



**Figure S5.** Relative contribution of variables on the factor map. **a., b.** and **c.** are Scree plot of the 7 dimensions, cos2 and variables contributions, respectively for dimensions 1–5, from heat stress bioassay. Highest values reflect highest contribution to the dimension (variability); **d., e.** and **f.** are Scree plot of the 7 dimensions, cos2 and variables contributions, respectively for dimensions 1–5, from adult bioassays with permethrin.



**Figure S6.** cos2 of variables with *An. coluzzii* and *An. gambiae* s.s. (*An. gambiae* in the plot) exhibiting the highest inertia/contribution.



**Figure S7.** cos2 of the variables with *An. coluzzii* and *An. gambiae* s.s. (*An. gambiae* in the plot) exhibiting the highest inertia/contribution.

Table S1. Primers used for qRT-PCR.

Gene Name	Gene ID	Primer Name	Sequence (5'-3')	Size (bp)	Efficiency
<i>hsp90</i>	AGAP006959	qhsp90_AGAP006959-F qhsp90_AGAP006959-R	AAGCAGATCGTGAACAAGCA GCCTTCATGATGCGCTCC	227	0.898
<i>hsp90_beta</i>	AGAP001424	qhsp90_AGAP001424-F qhsp90_AGAP001424-R	GCTGTGTGCGGTGGTAATTA CTTGATGGCTTCCTCTTCGC	213	0.946
<i>hsp83</i>	AGAP006958	qhsp83-F qhsp83-R	CCTTCGCATTTCAGGCTGAG GACTCCAGCTTCGAGGGATC	160	0.987
<i>hsp70 1/8</i>	AGAP004944	qhsp70 1/8-F qhsp70 1/8-R	GAGATGCTGCCAAGAACCAG GGCCGTTTCCTTCATCTTGG	239	0.898
<i>hsp90 ATPase</i>	AGAP010514	qhsp_AGAP010514-F qhsp_AGAP010514-R	GGACATGGTGACCGCATTC CCGGAATCATCGTTTGGGTC	239	0.892
<i>hsp70</i>	AGAP004581	qhsp70-F qhsp70-R	CGCAAGTTCAAGAAGGACGT TCGAGCGGAACAGATCAGAG	199	0.974
<i>TPS 1/2</i>	AGAP008227	qTPS1/2-F qTPS1/2-R	CAGGAGGCACAAACCAAGAG AGTTGGTGTGTTGTCCGTTG	237	0.932
<i>IR21a</i>	AGAP008511	qIR21a-F qIR21a-R	GTTGGAGATGCGGCTGTTG AGAATCACCGCTACCCAGAC	300	0.971
<i>IR25a</i>	AGAP010272	qIR25a-F qIR25a-R	GCAATTATAGCGATCATGGGAC AAGTGTGGCGGTCGATTTTC	245	0.817
<i>Elongation factor Tu</i>	AGAP005128	EF-F EF-R	GGCAAGAGGCATAACGATCAATGCG GTCCATCTGCGACGCTCCGG	130	0.979
<i>RPS7</i>	AGAP010592	RPS7-F RPS7-R	CCACCATCGAACACAAAGTTGA TGCTGCAAACTTCGGCTATTC	98	0.897

Table S2. Summary of the 2La inversion distribution in larvae and adult *Anopheles* mosquitoes.

Site / Phenotype	Karyotype			Total
Heat stress assay (44 °C for 30 min)				
Auyo				
	2La/a	2La/+a	2L+a/+a	
Alive	2	2	9	13
Dead	2	3	4	9
BUK				
Alive	1	8*	5*	14
Dead	2	2*	9*	13
Gamjin Bappa 44 °C				
Alive	1	4	7	12
Dead	3	4	0	7
Pantami				
Alive	2	9	5	16
Dead	3	4	7	14
Larval bioassay (100 mg/mL Permethrin)				
Auyo				
Alive	2	4	5	11
Dead	0	3	3	6
BUK				
Alive	3	2	4	9
Dead	1	4	6	11

Gamjin Bappa				
Alive	1	2	3	6
Dead	1	5	5	11
Pantami				
Alive	4	5	6	15
Dead	3	8	5	16
Adult bioassay (0.75 % Permethrin)				
Auyo				
Alive	3 <sup>†</sup>	9	3 <sup>†</sup>	15
Dead	1 <sup>†</sup>	5	6 <sup>†</sup>	12
BUK				
Alive	4 <sup>†</sup>	9	9 <sup>†</sup>	22
Dead	0 <sup>†</sup>	2	8 <sup>†</sup>	10
Gamjin Bappa				
Alive	5 <sup>†</sup>	5	9 <sup>†</sup>	19
Dead	0 <sup>†</sup>	4	5 <sup>†</sup>	9
Pantami				
Alive	9 <sup>†</sup>	4	2 <sup>†</sup>	15
Dead	3 <sup>†</sup>	4	3 <sup>†</sup>	10

\* statistically significant between alive and dead for 2La/+<sup>a</sup> vs 2L<sup>+</sup>a/+<sup>a</sup> for BUK, OR = 7.2 (1.08 – 4.79),  $\chi^2 = 4.68$ ,  $p = 0.03$ .

<sup>†</sup> statistically significant between alive and dead for 2La/a vs 2L<sup>+</sup>a/+<sup>a</sup> for all sites, OR = 5.02 (1.48 – 6.93),  $\chi^2 = 7.45$ ,  $p = 0.01$ .

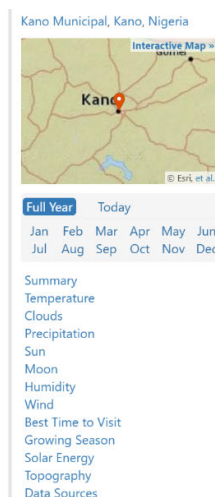
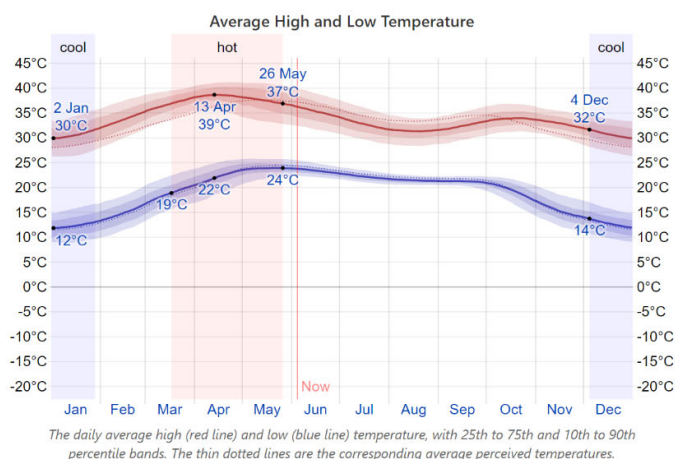
### Supplementary Information: Ambient temperature

Monthly averages of ambient temperature for the 4 sampling sites based on a statistical analysis of historical hourly weather reports and model reconstructions from 1 January 1980 to 31 December 2016. The weather-related data were taken entirely from NASA's MERRA-2 satellite-era reanalysis. This reanalysis combines a variety of wide-area measurements in a state-of-the-art global meteorological model to reconstruct the hourly history of weather throughout the world on a 50-kilometer grid (<https://weatherpark.com/> accessed on 6 June 2021).

#### Temperature

The hot season lasts for 2.3 months, from 17 March to 26 May, with an average daily high temperature above 37°C. The hottest day of the year is 13 April, with an average high of 39°C and low of 22°C.

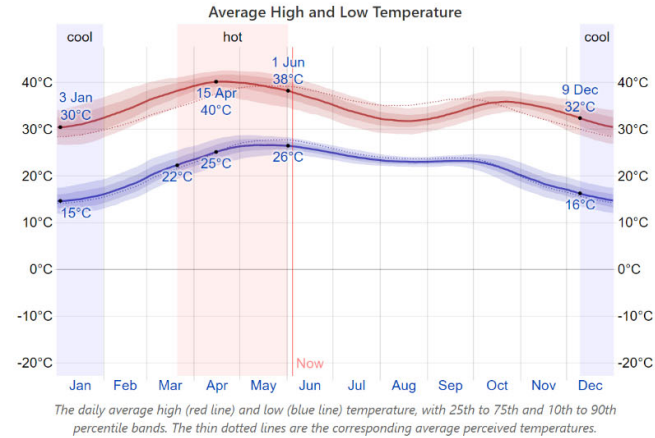
The cool season lasts for 1.7 months, from 4 December to 28 January, with an average daily high temperature below 32°C. The coldest day of the year is 2 January, with an average low of 12°C and high of 30°C.



Temperature

The hot season lasts for 2.4 months, from 21 March to 1 June, with an average daily high temperature above 38°C. The hottest day of the year is 15 April, with an average high of 40°C and low of 25°C.

The cool season lasts for 1.7 months, from 9 December to 31 January, with an average daily high temperature below 32°C. The coldest day of the year is 3 January, with an average low of 15°C and high of 30°C.



Auyo, Jigawa, Nigeria

Interactive Map

Full Year Today

Jan	Feb	Mar	Apr	May	Jun
Jul	Aug	Sep	Oct	Nov	Dec

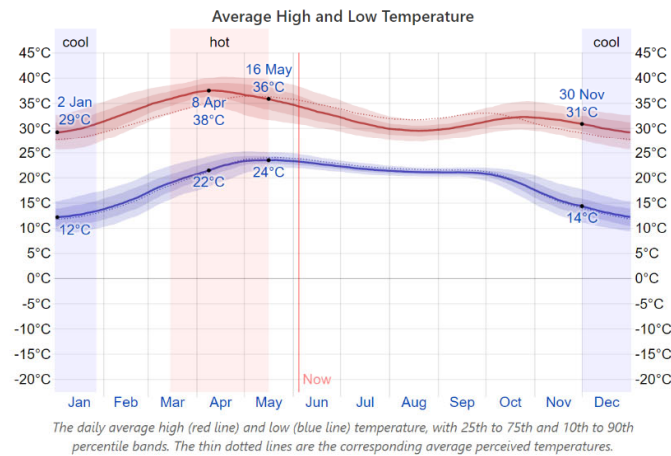
Summary

- Temperature
- Clouds
- Precipitation
- Sun
- Moon
- Humidity
- Wind
- Best Time to Visit
- Growing Season
- Solar Energy
- Topography
- Data Sources

Temperature

The hot season lasts for 2.0 months, from 15 March to 16 May, with an average daily high temperature above 36°C. The hottest day of the year is 8 April, with an average high of 38°C and low of 22°C.

The cool season lasts for 1.9 months, from 30 November to 27 January, with an average daily high temperature below 31°C. The coldest day of the year is 2 January, with an average low of 12°C and high of 29°C.



Karaye

Karaye, Kano, Nigeria

Interactive Map

Full Year Today

Jan	Feb	Mar	Apr	May	Jun
Jul	Aug	Sep	Oct	Nov	Dec

Summary

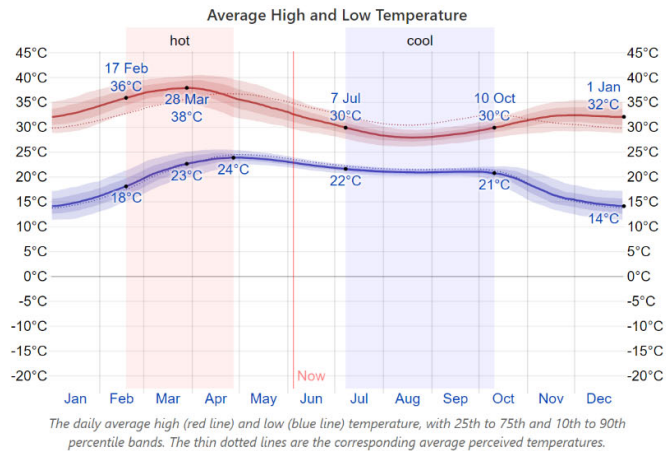
- Temperature
- Clouds
- Precipitation
- Sun
- Moon
- Humidity
- Wind
- Best Time to Visit
- Growing Season
- Solar Energy
- Topography
- Data Sources



Temperature

The hot season lasts for 2.3 months, from 17 February to 27 April, with an average daily high temperature above 36°C. The hottest day of the year is 28 March, with an average high of 38°C and low of 23°C.

The cool season lasts for 3.1 months, from 7 July to 10 October, with an average daily high temperature below 30°C. The coldest day of the year is 1 January, with an average low of 14°C and high of 32°C.



Gombe

Gombe, Gombe, Nigeria



Full Year											
Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec

- Summary
- Temperature
- Clouds
- Precipitation
- Sun
- Moon
- Humidity
- Wind
- Best Time to Visit
- Growing Season
- Solar Energy
- Topography
- Data Sources

Files S1–S3 are provided separately as Excel files.