Animal Biology

Geographic variation in skin structure in male Andrew's toad (*Bufo andrewsi*)

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Abstract

Variation in organ structure likely provides important clues on local adaptation and reflects the pressure target of natural selection. As one of the important organs, the skin plays a key role in adapting to complex environments by reducing water loss or increasing water absorption. Nevertheless, variation in the skin structure across different populations in a single species of anurans remains enigmatic. Here, we studied geographical variation in the skin structure of male Andrew's toads (*Bufo andrewsi*) across ten populations using histological methods. We quantified thickness of the skin, the epidermis, the loose layer, the compact layer, and of the epidermis, area of granular glands (GGs) and of ordinary mucous glands (OMGs), width of the calcified layer, and number of capillary vessels. We found that

the thickness of the skin, dermis and loose layer in dorsal skin increased with latitude whereas the area of granular glands decreased with altitude. Moreover, the width of the calcified layer in ventral skin decreased with latitude among populations. Our findings suggest that geographical variation in skin structure in male *B. andrewsi* is likely to reduce water loss or make water absorption occur faster in complex high-latitude environments, improving local adaptation.

Keywords

Anurans, *Bufo andrewsi*, geographical variation, local adaptation, skin structure

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Supplementary material

Table S1.

Descriptive information of the study sites and measures of mean (± SD) body size of male *Bufo andrewsi*.

Study sites	Latitude (° N)	Altitude (m)	Mean water vapor pressure (kPa)	Mean rain fall (mm)	Annual mean temperature (°C)	SVL (mm)	Body mass (g)
Yingxiu (YX)	31.03	888	1.48	67.67	16.04	78.83 ± 7.40	34.97 ± 4.28
Qilixia (QLX)	32.62	1351	1.16	70.25	9.89	82.73 ± 4.25	55.30 ± 10.71
Gengda (GD)	31.08	1524	1.18	63.67	10.88	65.21 ± 2.00	25.58 ± 3.12
Shiziba (SZB)	32.66	1651	1.11	70.83	9.20	80.27 ± 5.29	51.35 ± 4.29
Lamasi (LMS)	31.03	1961	1.03	62.00	8.62	68.06 ± 5.79	21.20 ± 4.58
Jiamuke (JMK)	27.58	2013	0.94	68.33	11.93	77.47 ± 6.38	50.24 ± 12.89
Qibie (QB)	27.57	2123	0.95	69.67	11.82	68.06 ± 2.20	33.95 ± 11.03
Yinchanggou(YCG)	30.97	2153	0.96	60.83	7.81	77.70 ± 5.18	26.24 ± 1.59
Pantiange (PTG)	27.33	2520	0.96	72.58	11.98	73.85 ± 5.11	39.00 ± 4.69
Sanchahe (SCH)	28.92	2554	0.81	69.08	8.80	60.99 ± 5.36	19.97 ± 0.72

Abbreviation: SVL = snout–vent length.

Table S2.

Description of the structure of dorsal skin of male *Bufo andrewsi* at the study sites.

Study sites	Thickness (μm)					Area (μm^2)		Calcified layer length (μm)	Capillary vessel number
	Total skin	Epidermis	Loose layer	Campact layer	Dermis	GGs	OMGs		
Yingxiu (YX)	373.4 ± 24.7	24.4 ± 5.3	201.9 ± 38.2	147.1 ± 28.6	349.0 ± 22.6	179580.7 ± 79896.1	6564.6 ± 2778.3	355.8 ± 104.8	12.0 ± 0.9
Qilixia (QLX)	263.5 ± 72.2	18.2 ± 2.1	153.1 ± 55.6	92.1 ± 20.8	245.3 ± 71.2	130734.5 ± 47353.3	2931.5 ± 1069.9	204.4 ± 52.0	12.0 ± 4.6
Gengda (GD)	287.7 ± 99.1	23.3 ± 3.7	146.2 ± 55.4	118.1 ± 53.1	264.3 ± 95.6	153185.7 ± 151970.9	4489.2 ± 1164.7	229.8 ± 114.0	16.5 ± 5.4
Shiziba (SZB)	330.1 ± 54.2	27.3 ± 7.9	178.8 ± 32.7	124.1 ± 30.5	302.8 ± 57.7	92386.8 ± 33774.9	3735.1 ± 1252.0	185.8 ± 74.0	18.4 ± 7.2
Lamasi (LMS)	227.3 ± 50.5	27.5 ± 9.6	119.6 ± 37.2	80.1 ± 15.4	199.8 ± 50.9	64143.4 ± 54790.2	3862.6 ± 852.4	201.5 ± 48.0	19.2 ± 7.7
Jiamuke (JMK)	214.0 ± 46.1	31.3 ± 5.0	87.6 ± 28.5	95.2 ± 20.8	182.8 ± 43.8	138420.8 ± 68813.1	6095.8 ± 1699.9	334.4 ± 132.4	16.5 ± 6.9
Qibie (QB)	189.3 ± 73.0	30.7 ± 7.6	74.9 ± 50.4	83.7 ± 21.8	158.5 ± 68.8	51905.9 ± 29476.9	5194.3 ± 2266.3	214.4 ± 39.5	21.5 ± 13.1
Yinchanggou (YCG)	300.3 ± 67.7	25.5 ± 6.5	174.7 ± 60.2	100.2 ± 20.9	274.8 ± 71.8	97345.4 ± 38126.9	4487.9 ± 1225.7	239.9 ± 95.5	15.5 ± 2.4
Pantiange (PTG)	227.0 ± 54.4	31.8 ± 6.5	90.4 ± 24.2	104.9 ± 48.3	195.2 ± 49.5	122840.2 ± 102022.5	3722.0 ± 1419.8	202.8 ± 46.1	19.5 ± 15.6
Sanchahe (SCH)	255.4 ± 72.4	22.4 ± 5.1	135.5 ± 53.2	97.6 ± 33.8	233.1 ± 68.7	94589.9 ± 64140.6	3728.7 ± 1132.2	135.2 ± 27.5	25.9 ± 1.5

Abbreviations: GG = granular gland; OMG = ordinary mucous gland.

Table S3.

Description of the structure of ventral skin of male *Bufo andrewsi* at the study sites

Study site	Thickness (μm)					Area (μm^2)		Calcified layer length (μm)	Capillary vessel number
	Total skin	Epidermis	Loose layer	Compact layer	Dermis	GGs	OMGs		
Yingxiu (YX)	240.1 ± 55.3	35.3 ± 9.8	116.5 ± 44.1	88.3 ± 16.8	204.7 ± 51.4	38750.3 ± 18316.8	5691.7 ± 804.2	169.0 ± 74.9	16.6 ± 8.7
Qilixia (QLX)	189.0 ± 43.5	32.7 ± 9.5	90.3 ± 30.2	67.0 ± 16.7	157.3 ± 44.0	42784.7 ± 10713.5	5148.6 ± 2399.1	99.3 ± 34.7	15.6 ± 3.3
Gengda (GD)	200.2 ± 48.2	26.3 ± 6.5	106.7 ± 23.6	67.2 ± 35.8	173.9 ± 45.7	38317.4 ± 25600.7	4789.4 ± 2088.3	82.7 ± 25.6	15.4 ± 9.9
Shiziba (SZB)	204.7 ± 54.3	38.5 ± 8.2	95.0 ± 20.0	71.2 ± 41.3	166.2 ± 57.4	37188.4 ± 8812.6	4553.7 ± 770.7	152.8 ± 24.9	16.1 ± 5.1
Lamasi (LMS)	225.4 ± 93.8	29.8 ± 10.0	114.1 ± 67.0	81.4 ± 20.8	195.6 ± 87.3	41496.4 ± 25561.9	4476.9 ± 696.4	101.4 ± 27.8	18.6 ± 6.9
Jiamuke (JMK)	171.7 ± 16.8	42.1 ± 15.1	57.7 ± 4.6	72.0 ± 11.5	129.7 ± 10.7	25348.0 ± 10007.0	5950.8 ± 2193.9	126.8 ± 56.2	23.7 ± 6.3
Qibie (QB)	202.0 ± 41.3	34.3 ± 5.2	81.2 ± 35.7	86.5 ± 13.4	167.6 ± 44.5	37048.6 ± 9394.9	5428.8 ± 798.5	233.7 ± 104.4	24.9 ± 6.0
Yinchanggou (YCG)	177.6 ± 27.4	30.7 ± 6.9	81.5 ± 23.1	65.4 ± 14.1	146.9 ± 32.1	40612.1 ± 14950.7	5341.6 ± 447.9	129.8 ± 36.9	16.3 ± 4.4
Pantiange (PTG)	180.9 ± 21.0	37.6 ± 8.2	66.2 ± 20.2	77.0 ± 16.9	143.2 ± 17.1	18913.5 ± 7426.3	3740.0 ± 282.8	148.8 ± 89.1	18.2 ± 7.6
Sanchahe (SCH)	212.1 ± 14.5	25.2 ± 7.1	108.1 ± 16.9	78.7 ± 5.0	186.8 ± 17.5	37990.4 ± 14616.2	4494.6 ± 512.4	101.5 ± 36.9	31.0 ± 8.1

Table S4.

Relationships between skin structure in dorsal skin and water vapor pressure, rainfall and annual temperature among populations.

Variables	Mean water vapor pressure (kPa)		Mean rainfall (mm)		Annual mean temperature (°C)	
	r^2	p	r^2	p	r^2	p
Skin thickness	0.228	<0.01	0.015	0.397	<0.01	0.959
Epidermis thickness	0.086	0.039	0.071	0.623	0.048	0.126
Dermis thickness	0.250	<0.01	0.019	0.342	0.0016	0.781
Loose layer thickness	0.246	<0.01	0.034	0.202	0.032	0.210
Compact layer thickness	0.130	0.010	<0.01	0.996	0.053	0.108
GG area	0.058	0.094	<0.01	0.977	0.065	0.075
OMG area	<0.01	0.856	0.066	0.072	0.063	0.079
Calcified layer width	0.055	0.106	0.030	0.232	0.145	<0.01
Capillary vessel number	0.166	<0.01	0.045	0.756	0.058	0.097

Table S5.

Relationships between skin structure in ventral skin and water vapor pressure, rainfall and annual temperature among populations.

Variables	Mean water vapor pressure (kPa)		Mean rainfall (mm)		Annual mean temperature (° C)	
			r^2	p	r^2	p
Skin thickness	0.027	0.264	0.008	0.548	<0.01	0.929
Epidermis thickness	0.001	0.820	0.076	0.055	0.078	0.051
Dermis thickness	0.040	0.169	0.014	0.413	<0.01	0.761
Loose layer thickness	0.112	0.019	0.019	0.344	0.030	0.236
Compact layer thickness	0.017	0.371	<0.01	0.916	0.032	0.220
GG area	0.039	0.177	0.039	0.211	0.079	0.053
OMG area	<0.01	0.846	0.070	0.063	<0.01	0.938
Calcified layer width	0.002	0.784	0.023	0.330	0.032	0.247
Capillary vessel number	0.251	<0.01	<0.01	0.594	<0.01	0.943

Figure S1. The exact regions of the dorsal and ventral skin that were extracted from the specimens.