Supplementary Material

Elevational divergence in the great tit complex revealed by major hemoglobin genes

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Figure S1 The posterior distributions of migration rates estimated by the coalescent isolation-with-migration model in IMa. The generally restrained gene flow in α^{A}-globin gene and upslope gene flow between HA and LA populations that is reversed to the downslope of reference loci are obviously exhibited (Figure 1).

Figure S2 Sampling design for Clinefit analyses in T1 and T2 parallel transects. (**a**) Locations of sampling sites along elevational transects; (**b**) Elevation profiles of sampling sites along each transect. The approximated ridge lines of transects contoured by straight lines between adjacent localities in Google Earth, the shadows represent for respective elevational gradients.

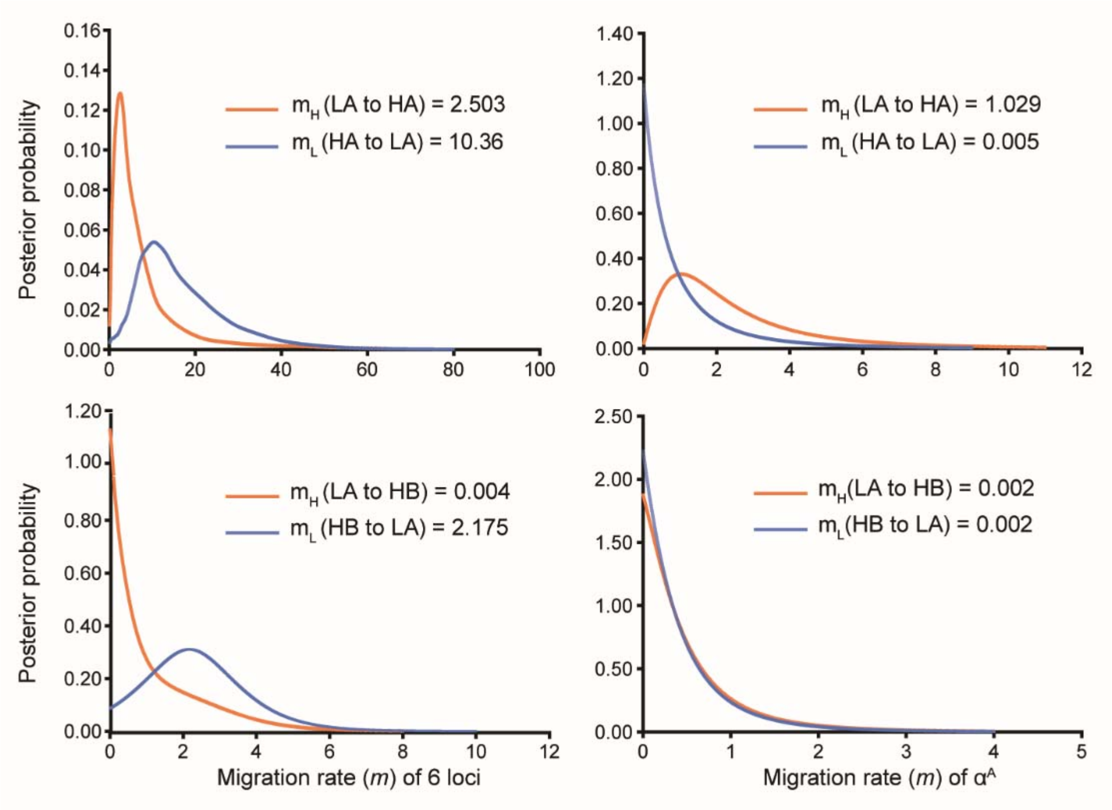
Figure S3 Allele frequencies of each amino acid substitutions of high-altitude alleles in global samples. (a) High-altitude replaced alleles in α^{A}-globin gene; **(b)** Flexible alleles of α^{A}-globin gene and β^{A}-globin gene.

Table S1 Specimen information and collection localities of the Great Tit complex.

Table S2 Primers used in PCR of all genes in this study.

Table S3 Multi-locus summary statistics in nucleotide polymorphisms of the *P. minor* populations.

Table S4 Heterozygosity and genotype frequencies in α^{A}- and β^{A}-globin subunit of the *P. minor* by Hardy-Weinberg equilibrium test.



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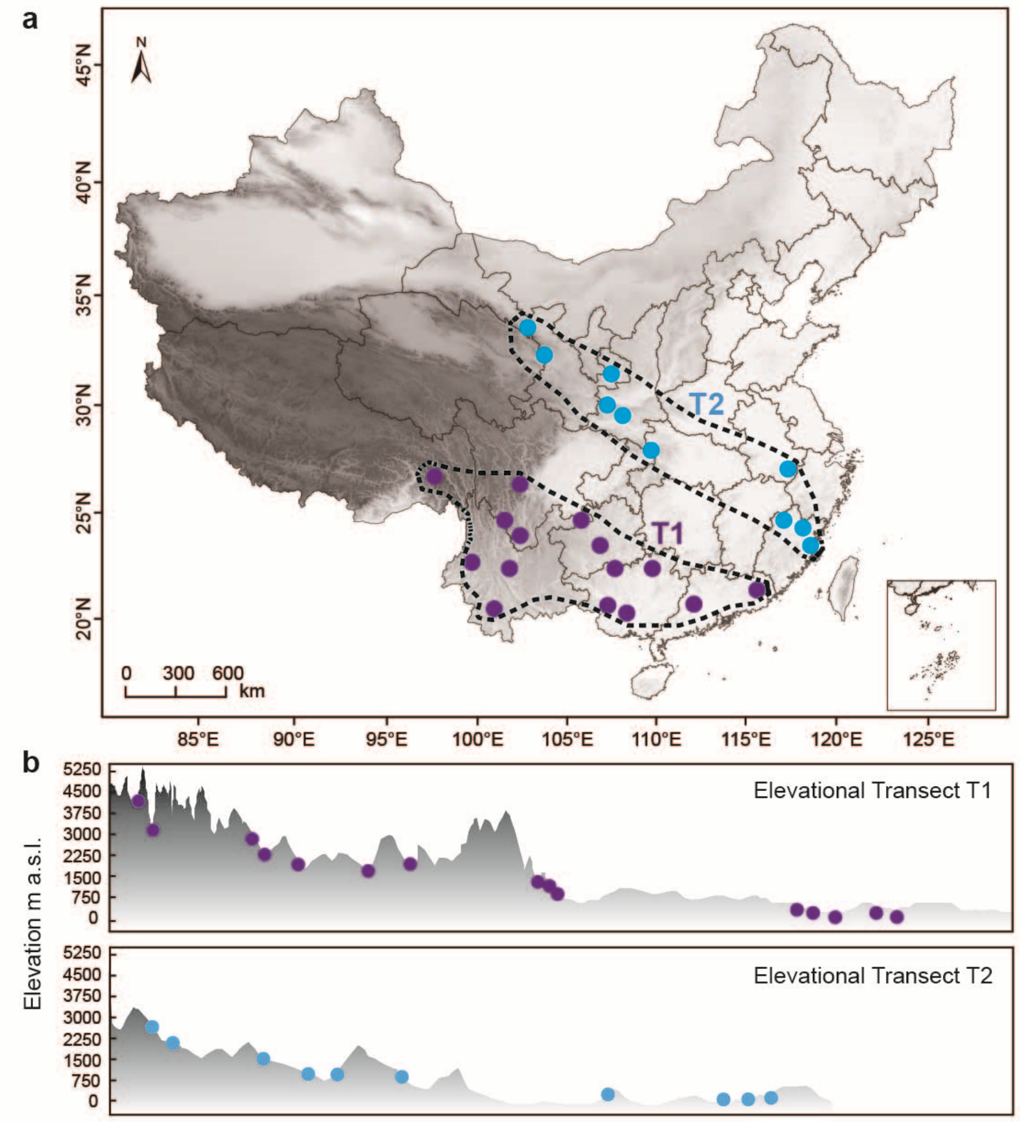

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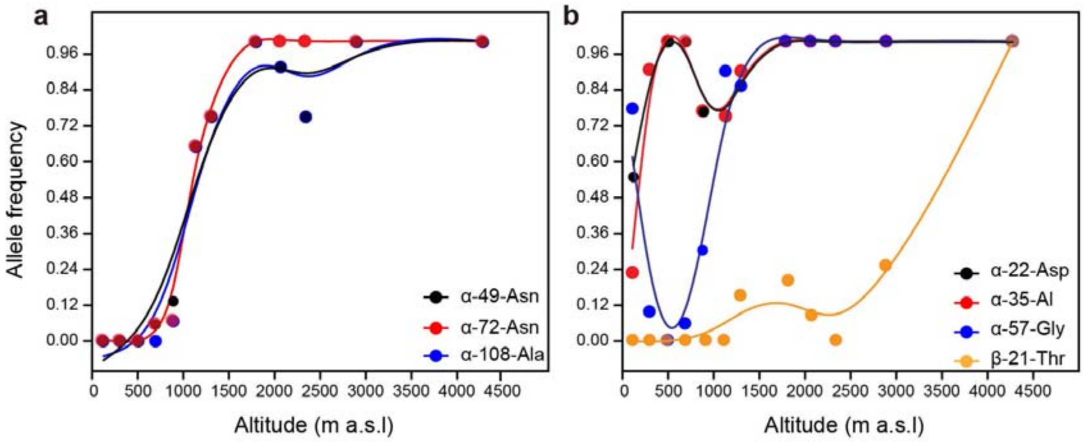

Figure S3 Allele frequencies of each amino acid substitutions of high-altitude alleles in samples of all clades (Figure 1). (a) High-altitude replaced alleles in α^{A}-globin gene; (b) Flexible alleles of α^{A}-globin gene and β^{A}-globin gene.

Table S1 Specimen information and collection localities of the Great Tit complex.

Species	**Locality**	**Elevation/m**	**Sample size**	**Clades**	**Type**	**Source**	**Dataset**
Parus minor	Jixi, Anhui, China	167	6	A	muscle/blood	IOZ	LA
Parus minor	Minhou, Fujian, China	19	1	A	muscle/blood	IOZ	LA
Parus minor	Fuzhou, Fujian, China	70	2	A	muscle/blood	IOZ	LA
Parus minor	Wuyi mountain, Fujian, China	153	2	A	muscle/blood	IOZ	LA
Parus minor	Zhaoqing, Guangdong, China	7	3	A	muscle/blood	IOZ	LA
Parus minor	Chaoan, Guangdong, China	11	1	A	muscle/blood	IOZ	LA
Parus minor	Guilin, Guangxi, China	169	3	A	muscle/blood	IOZ	LA
Parus minor	Nan'ning, Guangxi, China	117	1	A	muscle/blood	IOZ	LA
Parus minor	Fusui, Guangxi, China	97	3	A	muscle/blood	IOZ	LA
Parus minor	Lingui, Guangxi, China	210	1	A	muscle/blood	IOZ	LA
Parus minor	Su'nan, Gansu, China	2309	8	A	muscle/blood	IOZ	HA
Parus minor	Yongdeng, Gansu, China	2057	6	A	muscle/blood	IOZ	HA
Parus minor	Longdong, Gansu, China	1339	2	A	muscle/blood	IOZ	HA
Parus minor	Ningshan, Shaanxi, China	1380	1	A	muscle/blood	IOZ	HA
Parus minor	Foping, Shaanxi, China	1150	2	A	muscle/blood	IOZ	HA
Parus minor	San'guanmiao, Shaanxi, China	1540	1	A	muscle/blood	IOZ	HA

Species	Locality	Elevation/m	Sample size	Clades	Type	Source	Dataset
Parus minor	Bomi, Xizang, China	2828	1	B	muscle/blood	IOZ	HB
Parus minor	Mangkang, Xizang, China	4282	1	B	muscle/blood	IOZ	HB
Parus minor	Miiyi, Sichuan, China	1355	1	B	muscle/blood	IOZ	HB
Parus minor	Luding, Sichuan, China	1260	2	B	muscle/blood	IOZ	HB
Parus minor	Luding, Sichuan, China	1653	1	B	muscle/blood	IOZ	HB
Parus minor	Yanbian, Sichuan, China	2200	1	B	muscle/blood	IOZ	HB
Parus minor	Panzhihua, Sichuan, China	1900	2	B	muscle/blood	IOZ	HB
Parus minor	Xishuangbanna , Yunnan, China	1211	1	B	muscle/blood	IOZ	HB
Parus minor	Chuxiong , Yunnan, China	1872	1	B	muscle/blood	IOZ	HB
Parus minor	Lijiang , Yunnan, China	2916	4	B	muscle/blood	IOZ	HB
Parus minor	Lijiang , Yunnan, China	1305	2	B	muscle/blood	IOZ	HB
Parus minor	Lijiang ,Yunnan, China	2410	2	B	muscle/blood	IOZ	HB
Parus minor	Gaoligong , Yunnan, China	2406	2	B	muscle/blood	IOZ	HB
Parus minor	Gaoligong, Yunnan, China	2031	2	B	muscle/blood	IOZ	HB
Parus minor	Lijiang ,Yunnan, China	1305	1	B	muscle/blood	IOZ	Network
Parus minor	Chishui, Guizhou, China	947	2	A-B	muscle/blood	IOZ	Network/ClineFit
Parus minor	Guiding, Guizhou, China	1118	6	A-B	muscle/blood	IOZ	Network/ClineFit

Species	**Locality**	**Elevation/m**	**Sample size**	**Clades**	**Type**	**Source**	**Dataset**
Parus minor	Libo, Guizhou, China	820	3	A-B	muscle/blood	IOZ	Network/ClineFit
Parus minor	Shennongjia, Hubei, China	870	2	A	muscle/blood	IOZ	Network/ClineFit
Parus minor	Shangzhi, Heilongjiang, China	380	6	A	muscle/blood	IOZ	Network
Parus minor	Baishan, Jilin, China	808	1	A	muscle/blood	IOZ	Network
Parus minor	Zuojia, Jilin, China	200	4	A	muscle/blood	IOZ	Network
Parus minor	Langxi, Anhui, China	13	1	A	muscle/blood	IOZ	Network
Parus minor	Jixi, Anhui, China	167	1	A	muscle/blood	IOZ	Network
Parus minor	Qingyang, Gansu, China	1036	1	A	muscle/blood	IOZ	Network
Parus minor	Foping, Shaanxi, China	1000	2	A	muscle/blood	IOZ	Network
Parus minor	Ningshan, Shaanxi, China	1000	2	A	muscle/blood	IOZ	Network
Parus minor	Ningqiang, Shaanxi, China	1050	1	A	muscle/blood	IOZ	Network
Parus minor	Jiedong, Guangdong, China	11	1	A	muscle/blood	IOZ	Network
Parus minor	Guilin, Guangxi, China	169	1	A	muscle/blood	IOZ	Network
Parus major	Yili, Xinjiang, China	589	2	D-E	muscle/blood	IOZ	Network
Parus major	Fuhai, Xinjiang, China	498	2	D-E	muscle/blood	IOZ	Network
Parus major	Buerjin, Xinjiang, China	664	5	D-E	muscle/blood	IOZ	Network
Parus major	Wulumuqi, Xinjiang, China	838	2	D-E	muscle/blood	IOZ	Network

Species	**Locality**	**Elevation/m**	**Sample size**	**Clades**	**Type**	**Source**	**Dataset**
Parus major	E'ergu'na, Inner Mongolia, China	680	8	D	muscle/blood	IOZ	Network
Parus major	Teshig, Mongolia	955	5	D	muscle/blood	IOZ	Network
Parus major	Teshig, Mongolia	756	4	D	muscle/blood	IOZ	Network
Parus major	Zvolen, Slovakia	284	8	D	muscle/blood	IOZ	Network
Parus major	Cherga, Altai Region, Russia	457	3	D	muscle/blood	IOZ	Network
Parus major	Kamlak village, Altai Region, Russia	279	6	D	muscle/blood	IOZ	Network
Parus major	Bialowieza nat. park, Poland	159	2	D	muscle/blood	IOZ	Network
Parus major	Sjællandsodde, Denmark	15	1	D	muscle/blood	IOZ	Network
Parus major	Sønderborg, Denmark	23	1	D	muscle/blood	IOZ	Network
Parus major	Lesbos, Greece	158	1	D	muscle/blood	IOZ	Network
Parus major	Tajikistan	1911	1	E	muscle/blood	IOZ	Network
Parus major	Condard village, Varzob region, Tajikistan	1195	2	E	muscle/blood	IOZ	Network
Parus major	Khodzhaobigarm village, Varzob region, Tajikistan	2066	3	E	muscle/blood	IOZ	Network

Species	**Locality**	**Elevation/m**	**Sample size**	**Clades**	**Type**	**Source**	**Dataset**
Parus major	Sebiston village, Dangara region, Tajikistan	1345	1	E	muscle/blood	IOZ	Network
Parus cinereus	Mysore, India	749	1	C	muscle/blood	IOZ	Network

Table S2 Primers and submissions of all genes in this study.

Locus	Primer name	Sequence (5'-3')	Annealing temp/°C	GeneBank Accession	Reference of primers
Unlinked mitochondrial loci					
CR	H636	GAGATGAGGAGTATTCAACCGAC	53	(Zhao et al. 2012)	(Kvist et al. 2003)
	L16700	ATCATAAATTCTCGCCGGGACTCT			
ND2	H6313	CTCTTATTTAAGGCTTTGAAGGC	53	MF322444 ~ MF322446	(Sorenson et al. 1999)
	L5219	CCCATACCCCGAAAATGATG			
Unlinked autosomal loci					
Fib	Fib5	CGCCATACAGAGTATACTGTGACAT	54	MF322459 ~ MF322504	(Kimball et al. 2009)
	Fib6	GCCATCCTGGCGATTCTGAA			
TGFB2	TGFB2.5F	GAAGCGTGCTCTAGATGCTG	58	MF322447 ~ MF322458	(Kimball et al. 2009)
	TGFB2.6R	AGGCAGCAATTATCCTGCAC			
Myo	Myo2	GCCACCAAGCACAAGATCCC	52	MF322320 ~ MF322443	(Kimball et al. 2009)
	Myo3F	TTCAGCAAGGACCTTGATAATGACTT			
ODC	OD6	GACTCCAAAGCAGTTTGTCGTCTCAGTGT	56	MF322196 ~ MF322319	(Friesen et al. 1999)
	OD8R	ATTGGTGGTGGCTTCCCTGGCTCTGAAGA			
Haemoglobin genes					
α^A	A11F	AGGTGACATCATGGTGCTGTC	56	MF321902 ~ MF322069	This study
	A668R	TAACGGTACTTGGCAGTCAG			
	A12F	GAGGTGACATCATGGTGCTGT	56		This study
	A689R	ACACGTCCTAACGGTACTTGGC			
β^A	B76F	TGCCCAGCGTGCCATCCTTCG	64	MF322070 ~ MF322195	This study
	B1767R	GCGGCTCTTCAGTGGTACTTGC			
	B21F	GGAGAAGCAGCTCATCACCG	60		This study
	B1263R	TGGTATTTGCGGGCCAGAG			

Locus	Primer name	Sequence (5'-3')	Annealing temp/°C	GeneBank Accession	Reference of primers
	B665F	GGAGCAAGAGCAAAGTCTGG	sequencing primers		This study
	B815R	GGGACAAACAGTGGGGTT			

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Table S3 Multi-locus summary statistics in nucleotide polymorphisms of the *P. minor* populations

Gene	**Population**	**L/bp**	**N**	S	h	H_{d}	π	R	**Tajima's** D	**Fu's** F_S
α^{A}	All	660	132	17	20	0.872	0.0059	10.3	0.669	-2.846
(Chr 14)	HB	660	46	5	6	0.574	0.0013	46.3	-0.674	-1.707
	HA	660	40	11	9	0.812	0.0039	10.5	-0.020	-0.639
	LA	660	46	9	13	0.909	0.0035	63.8	0.388	-4.071
β^{A}	All	453	108	14	14	0.555	0.0019	35.3	**-1.820***	**-10.156***
(Chr 1B)	HB	453	34	8	8	0.752	0.0033	79	-0.682	-2.066
	HA	453	28	6	6	0.439	0.0012	153	**-1.847***	-3.628
	LA	453	46	7	8	0.418	0.0011	> 1.e+4	**-1.838***	-6.396
TGFB2	All	334	132	27	32	0.940	0.0087	7.2	-1.246	**-20.175***
(Chr 3)	HB	334	46	10	18	0.926	0.0064	> 1.e+4	-0.242	-11.610
	HA	334	40	21	15	0.881	0.0143	2.6	-0.106	-2.257
	LA	334	46	15	24	0.952	0.0115	110	0.392	-13.805
Fib	All	281	132	14	17	0.756	0.0041	> 1.e+4	**-1.447***	**-11.191***

Gene	**Population**	**L/bp**	**N**	S	h	H_{d}	π	R	**Tajima's** D	**Fu's** F_S
(Chr 4)	HB	281	46	7	9	0.786	0.0049	89.4	-0.392	-2.874
	HA	281	40	4	5	0.658	0.0029	> 1.e+4	-0.350	-0.918
	LA	281	46	10	11	0.753	0.0041	> 1.e+4	**-1.431***	-6.127
Myo	All	556	128	17	22	0.844	0.0027	> 1.e+4	**-1.541***	-16.126
(Chr 1)	HB	556	44	12	15	0.872	0.0032	> 1.e+4	-1.081	-8.794
	HA	556	38	6	7	0.799	0.0024	> 1.e+4	-0.207	-1.418
	LA	556	46	11	12	0.757	0.0020	> 1.e+4	**-1.652***	-7.907
ODC	All	574	128	21	23	0.785	0.0030	26	**-1.557***	-15.156
(Chr 3)	HB	574	44	8	9	0.608	0.0024	2.1	-0.664	-2.826
	HA	574	40	10	9	0.671	0.0025	7.2	-1.154	-2.901
	LA	574	44	13	13	0.778	0.0027	37.5	**-1.473***	-6.984
CR	All	539	66	50	29	0.909	0.0230	2	0.482	-2.654
(mtDNA) CR	HB	539	23	30	18	0.964	0.0117	8.3	-0.958	**-8.068***

Gene	Population	L/bp	N	S	h	H_d	π	R	Tajima's D	Fu's F_S
(mtDNA)	HA	539	20	8	7	0.768	0.0029	10.6	-1.050	-2.001
	LA	539	23	27	7	0.735	0.0128	0.001	-0.234	3.495
ND2	All	737	66	32	14	0.621	0.0128	0.001	1.287	3.785
(mtDNA)	HB	737	23	13	10	0.870	0.0024	61.8	**-1.727***	**-4.599***
	HA	737	20	1	2	0.100	0.0001	> 1.e+4	-1.164	-0.879
	LA	737	23	3	3	0.170	0.0004	0.001	-1.731	-1.305

Notes: Chr, position in Zebra finch chromosomes {Alev *et al*. 2009}; mtDNA, mitochondrial DNA; L, length; N, number of chromosomes; S, number of segregating sites; h, number of haplotypes; H_d, haplotype diversity; π, nucleotide diversity; Tajima's D, statistics of Tajima's D and Fu's F_S, statistics of Fu's F_S test (Bold* is deviated from expected neutral equilibrium at $P < 0.05$). θ_w/bp, population mutation rate per site based on the number of segregating sites and recombination rate per gene {Watterson 1975}. R, recombination rate per gene.

References

Alev C, Shinmyozu K, McIntyre B, Sheng G, 2009, Genomic organization of zebra finch alpha and beta globin genes and their expression in primitive and definitive blood in comparison with globins in chicken. *Dev Genes and Evol* 219(7): 353-360.

Watterson G, 1975. On the number of segregating sites in genetical models without recombination. *Theor Popul Biol* 7(2):256-276.

Table S4 Heterozygosity and genotype frequencies in α^{A}- and β^{A}-globin subunit of the *P. minor* by Hardy-Weinberg equilibrium test.

α^{A}-globin genotypes	**Population**						**HB**	**HA**	**LA**
	Haplotypic H_o/H_e						0.435*/ 0.578	0.750 / 0.812	0.783*/ 0.909
	Genotypic H_o/H_e						*NA*	0.500 / 0.509	0.435 / 0.587
	22	**35**	**49**	**57**	**72**	**108**			
1	Asp	Ala	Asn	Gly	Asn	Ala	100% (23)	45.0% (9)	—
	·	·	·	·	·	·			
2	·	·	·	·	·	·	—	30.0% (6)	—
	·	·	Ser	·	·	Val			
3	·	·	·	·	·	·	—	10.0% (2)	—
	·	·	Ser	Ala	His	Val			
4	·	·	·	·	·	·	—	5.0% (1)	—
	Glu	Thr	Ser	·	His	Val			
5	·	·	Ser	·	·	Val	—	5.0% (1)	—
	·	·	Ser	·	·	Val			
6	·	·	Ser	Ala	His	Val	—	5.0% (1)	8.7% (2)
	Glu	Thr	Ser	·	His	Val			
7	·	·	Ser	Ala	His	Val	—	—	4.3% (1)
	·	Thr	Ser	·	His	Val			
8	Glu	Thr	Ser	·	His	Val	—	—	30.4% (7)
	Glu	Thr	Ser	·	His	Val			
9	·	Thr	Ser	·	His	Val	—	—	26.1% (6)
	·	Thr	Ser	·	His	Val			
10	Glu	Thr	Ser	·	His	Val	—	—	26.1% (6)

α^{A}-globin genotypes	**Population**						**HB**	**HA**	**LA**
	Haplotypic H_o/H_e						0.435*/ 0.578	0.750 / 0.812	0.783*/ 0.909
	Genotypic H_o/H_e						*NA*	0.500 / 0.509	0.435 / 0.587
	22	**35**	**49**	**57**	**72**	**108**			
	·	Thr	Ser	·	His	Val			
11	·	·	Ser	Ala	His	Val	—	—	4.3% (1)
	·	Thr	Ser	·	His	Val			
β^{A}-globin genotypes	**Population**						**HB**	**HA**	**LA**
	Haplotypic H_o/H_e						0.412*/ 0.752	0.286 / 0.439	0.304 / 0.351
	Genotypic H_o/H_e						0.000*/ 0.371	0.071 / 0.071	—
	21								
1	Thr						23.5% (4)	—	—
	·								
2	Ala						—	7.1% (1)	—
	·								
3	Ala						76.5% (13)	92.9% (13)	100% (23)
	Ala								

H_o, observed heterozygosity (* $P < 0.05$); H_e, expected heterozygosity; the replacements mainly exist in highland populations are shown in bold; The genotypic frequencies in observed genotypes are listed following the Hardy-Weinberg equilibrium frequencies and the number of individuals are shown in parenthesis.