Supplementary Material

Figure S1. Dot plots of sequence similarity between the globin gene clusters of *P. maniculatus* and *P. leucopus*. A) Pairwise comparisons of syntenic chromosomal regions containing the full complement of α-like globin genes, including the post-natally expressed *HBA* genes. The pattern of pairwise sequence matches provides evidence that the *HBA-T1* genes of both species are 1:1 orthologs, as are the pseudogenes at the 3' end of the clusters (*HBA-T3* in *P. maniculatus*, *HBA-T2* in *P. leucopus*). The fact that the majority of other *Peromyscus* species possess two duplicate *HBA* genes (see Fig. 3*A*) suggests that the ortholog of *P. maniculatus HBA-T2* was deleted in the common ancestor of *P. leucopus* and *P. gossypinus*. **B**) Pairwise comparisons of syntenic chromosomal regions containing the full complement of β-like globin genes, including the post-natally expressed *HBB* genes. The pattern of pairwise sequence matches provides clear evidence for 1:1 orthology of the *HBB* gene pair in *P. maniculatus* and *P. leucopus*, an inference that is confirmed by phylogenetic reconstructions of downstream flanking sequence (see Fig. 6*D*).

Figure S2 *Cytb* phylogeny showing that *P. maniculatus* specimens from northern California are distributed between two highly distinct clades. Branch-tip symbols are color-coded according to geographic region. Clade 1 contains specimens from geographically disparate high-altitude localities across the western US (see Fig. 2 for the full phylogeny).

Figure S3 Alignment of *HBA-T1* (A) and *HBA-T2* (B) amino acid sequences of *P. maniculatus* specimens from Humboldt Co., CA. The sequence logo above each alignment shows site-specific patterns of allele frequency variation in the *HBA-T1* and *HBA-T2* paralogs of *P. maniculatus* specimens from all other sampled localities. The sequence differences that distinguish the two *HBA* paralogs of the Humboldt mice are segregating as allelic variants in mice from other regions.

Figure S4 Maximum likelihood phylogenies depicting relationships among the *HBA* genes of *Peromyscus* species. Phylogeny reconstructions were based on four discrete data partitions: (**A**) 5'flanking region (upstream), (**B**) the complete coding sequence (cds), (**C**) intron 2, and (**D**) 3' flanking region (downstream). All four phylogenies show the characteristic pattern of concerted evolution where paralogs from the same species group together to the exclusion of positional orthologs in other species. Gene conversion among the *HBA* paralogs has been sufficiently extensive that phylogenetic reconstructions are not informative about orthologous relationships.

Figure S5 Alignment of *HBB-T1* (A) and *HBB-T2* (B) amino acid sequences of *P. maniculatus* specimens from San Luis Obispo Co., CA. The sequence logo above each alignment shows site-specific patterns of allele frequency variation in the *HBB-T1* and *HBB-T2* paralogs of *P. maniculatus* specimens from all other sampled localities. The fixed difference at β135 that distinguishes *HBB-T1* and *HBB-T2* of the San Luis Obispo mice is an allelic polymorphism in both *HBB* paralogs of mice from other regions.

Figure S6 Variation in the allosteric regulation of Hb-O_2 affinity within *P. maniculatus* and among different *Peromyscus* species. Sensitivity to allosteric effectors is indexed by the difference in log-transformed P_{50} values measured for stripped Hb samples in the presence and absence of Cl^- ions and DPG. Δlog-$P_{50(KCl\text{-}str)}$ measures Cl^- sensitivity, Δlog-$P_{50(DPG\text{-}str)}$ measures DPG sensitivity, and Δlog-$P_{50([KCl+DPG]\text{-}str)}$ measures sensitivity to both effectors together. *P. maniculatus* population samples are abbreviated as follows: A, Humboldt Co., CA; B, San Luis Obispo Co, CA; C, Mono Co., CA; D, Clear Creek Co., CO; and E, Yuma Co., CO.

Table S1 Specimens of *Peromyscus* used in the survey of polymorphism in the *HBA-T1*, *HBA-T2*, *HBB-T1*, and *HBB-T2* globin genes.

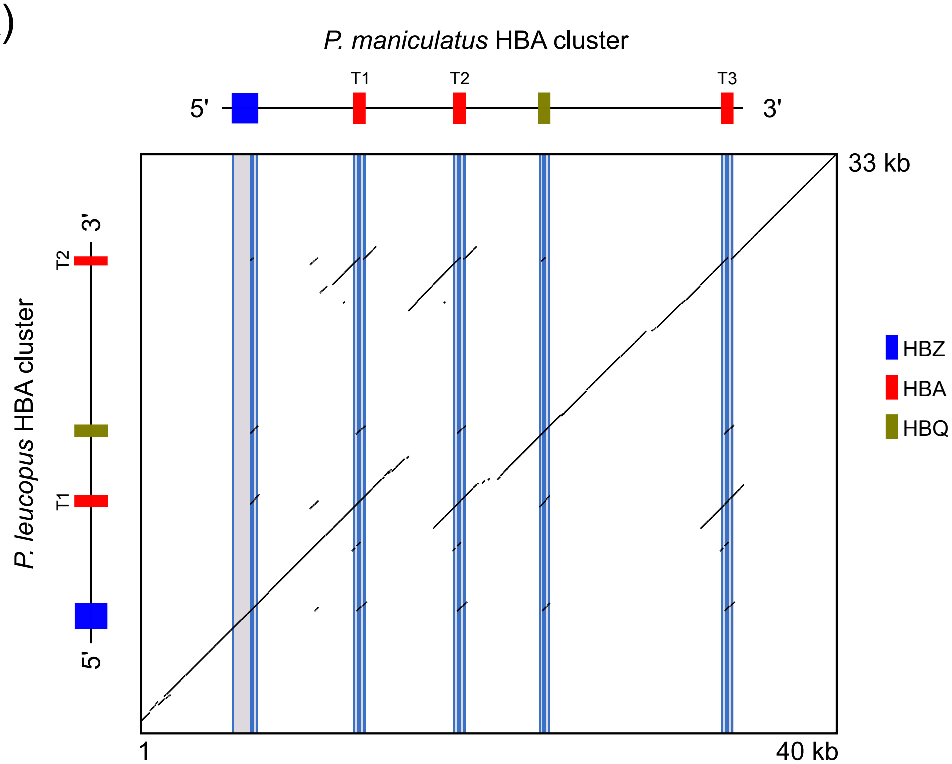
Table S2 Interparalog divergence between the tandemly linked *HBA-T1* and *HBA-T2* genes of *P. maniculatus* and *P. keeni*.

Table S3 Interparalog divergence between the tandemly linked *HBB-T1* and *HBB-T2* genes of *P. maniculatus*, *P. keeni*, and *P. leucopus*.

Table S4 Estimates of genetic variances for functional properties of naturally occurring Hb variants in *P. maniculatus*.

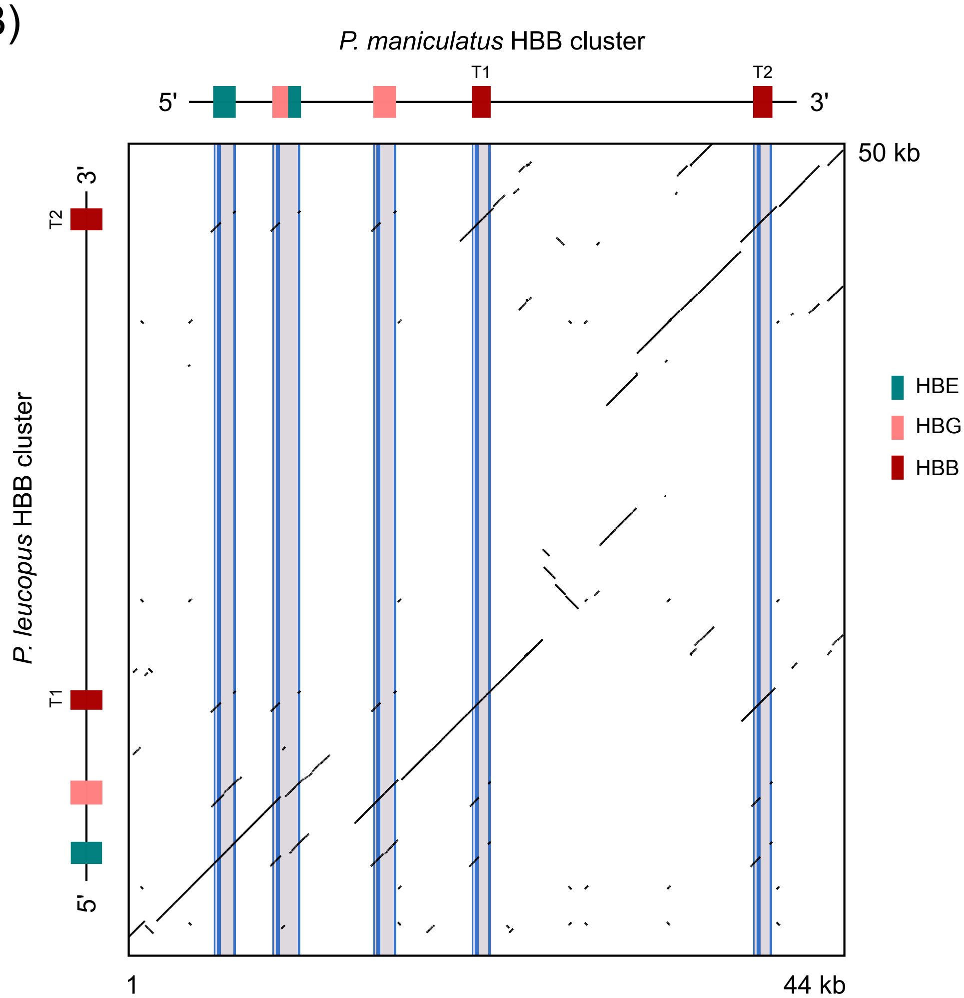
Table S5 Trait-specific genetic variances for 21 amino acid polymorphisms in the *HBA* and *HBB* genes of *P. maniculatus*.

A)

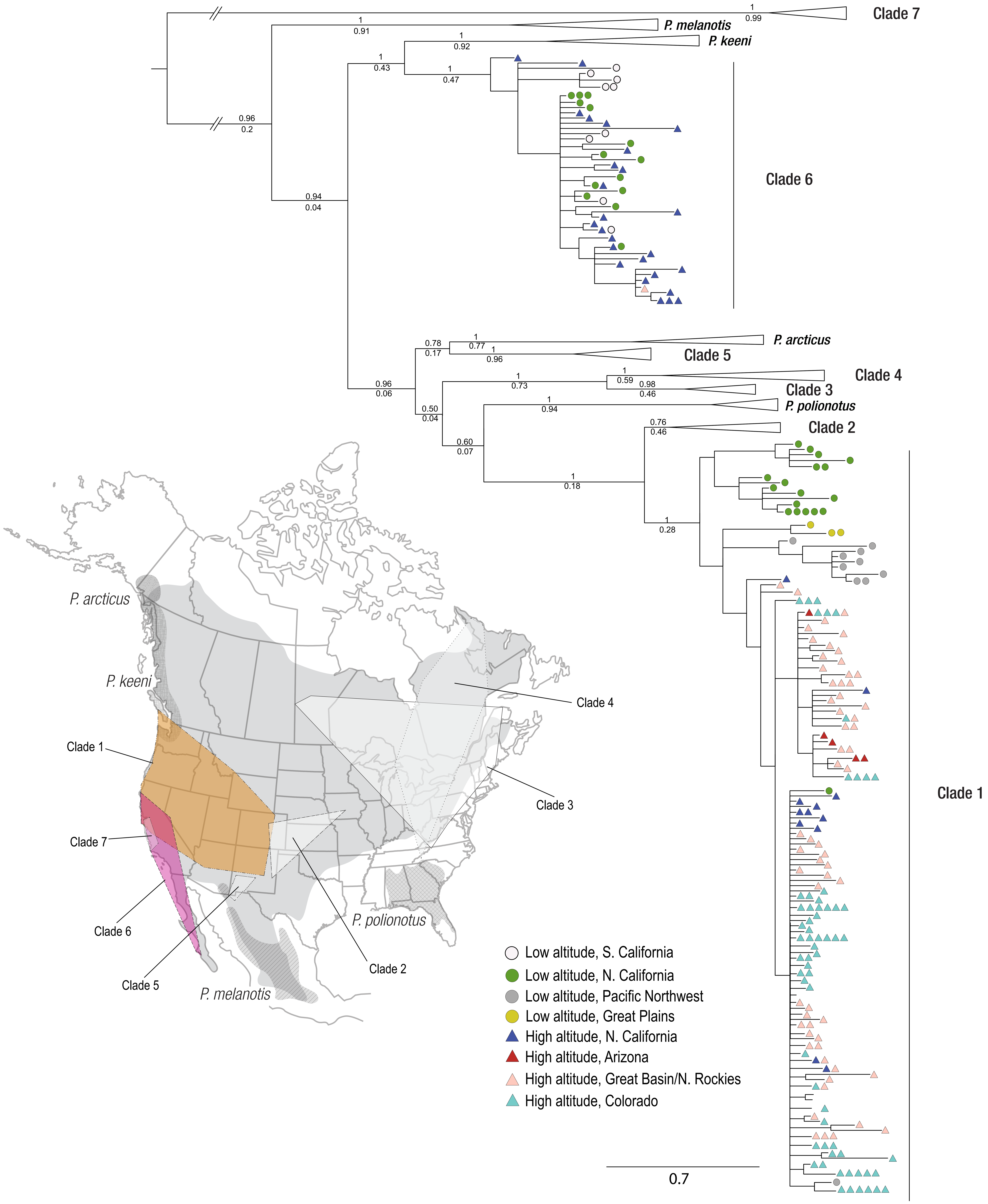


P. maniculatus HBA cluster
T1
T2
T3
5'
3'
33 kb
1
40 kb
P. leucopus HBA cluster
HBZ
HBA
HBQ

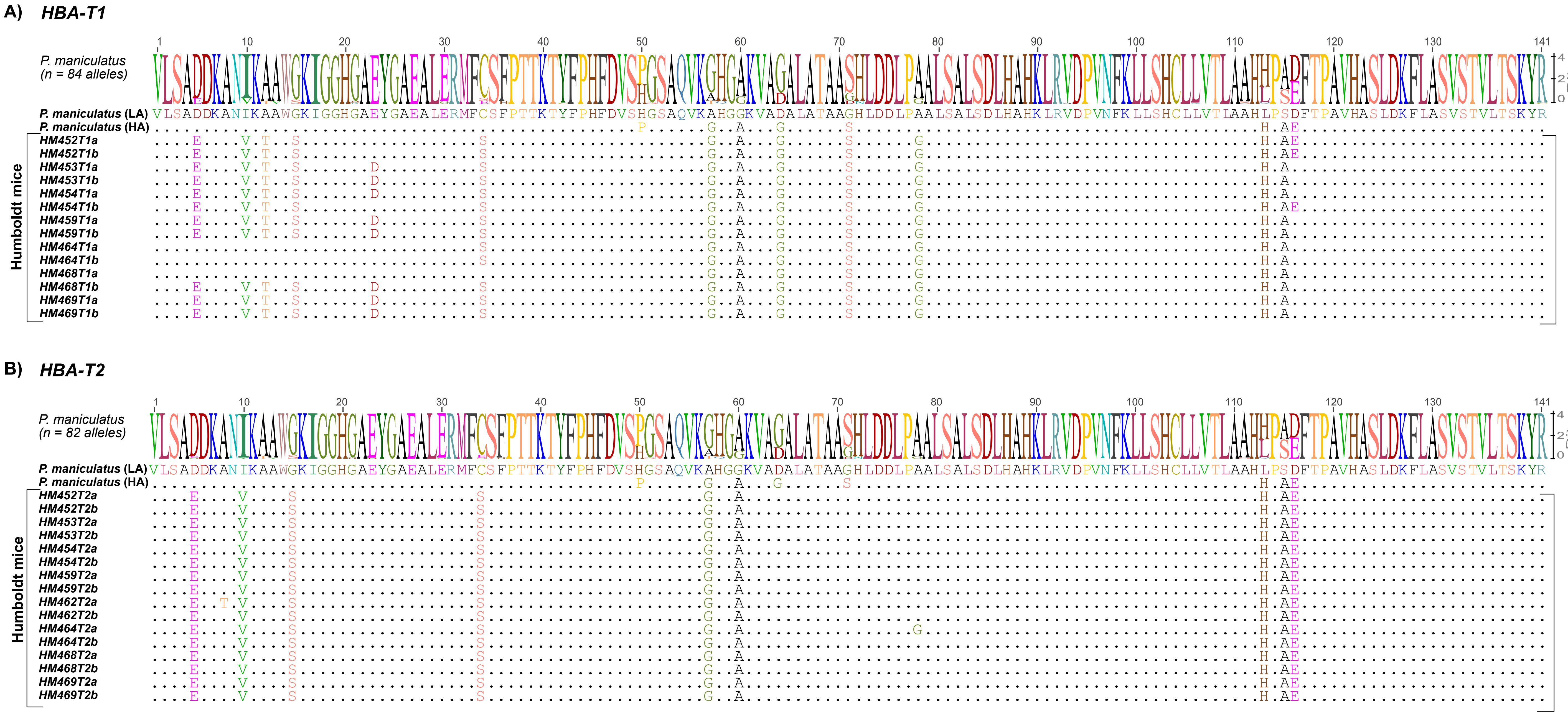
B)



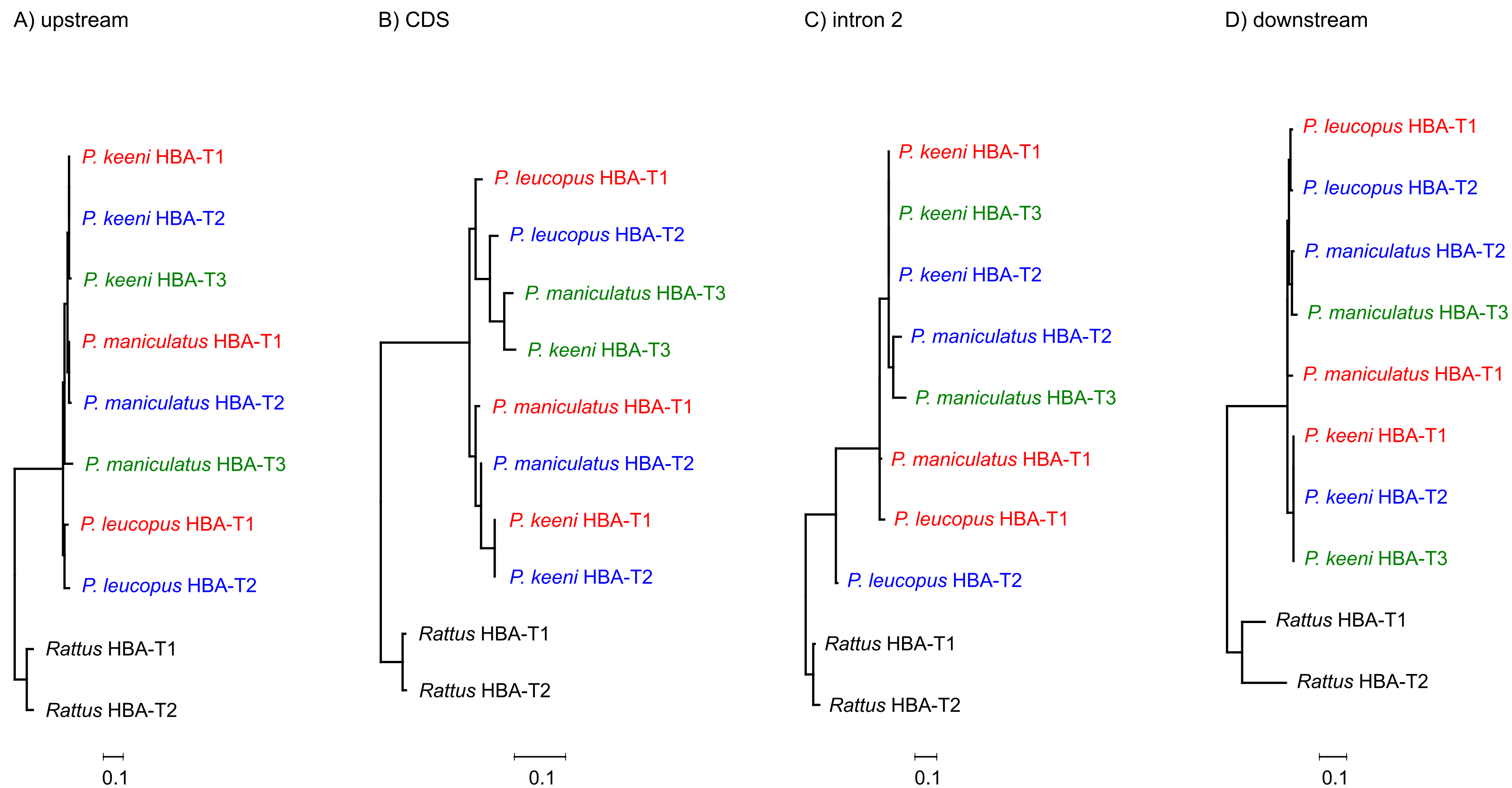
P. maniculatus HBB cluster
T1
T2
5'
3'
50 kb
1
44 kb
P. leucopus HBB cluster
HBE
HBG
HBB

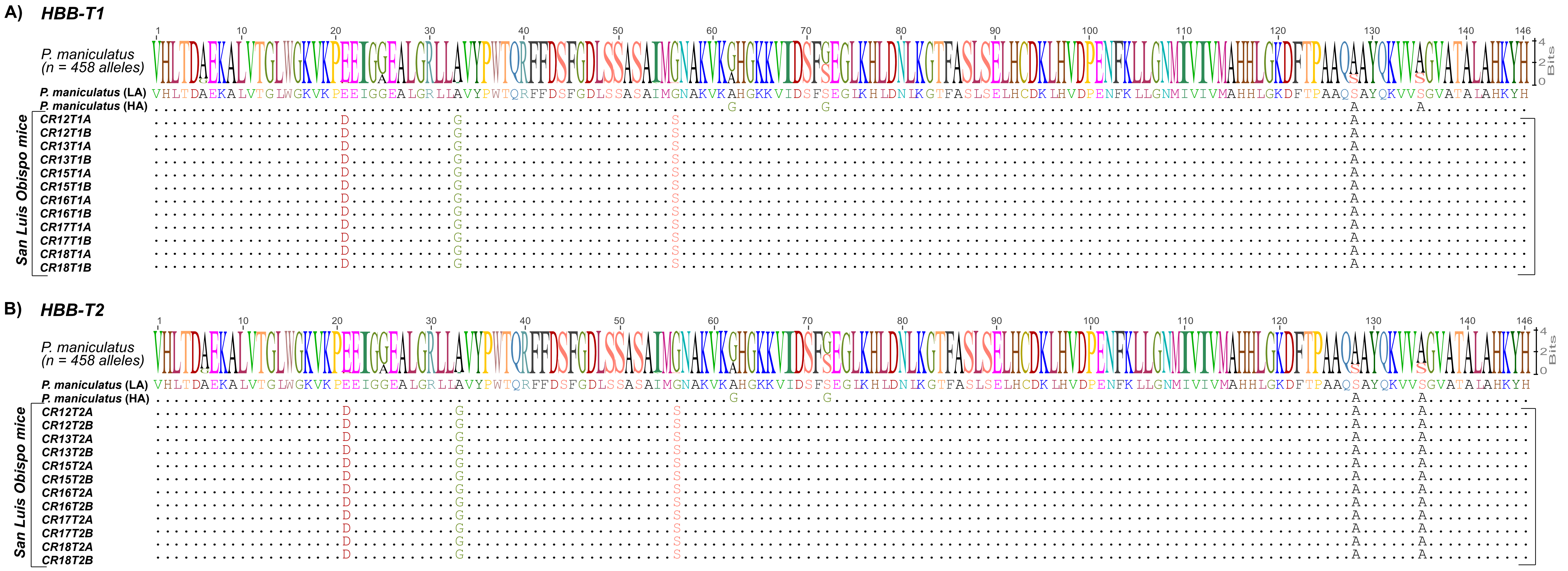


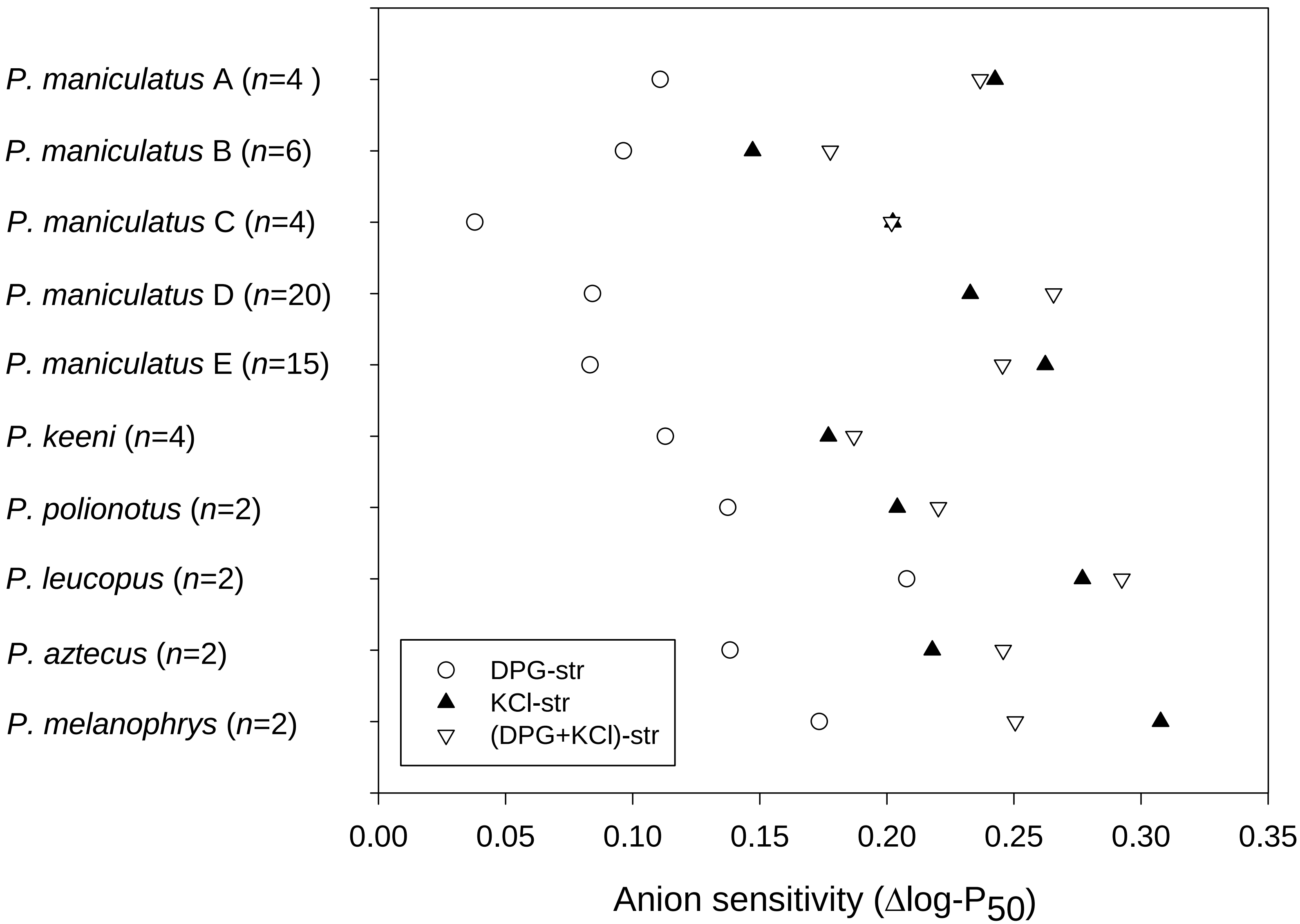
Clade 7
P. melanotis
P. keeni
Clade 6
P. arcticus
Clade 5
Clade 4
Clade 3
P. polionotus
Clade 2
Clade 1
P. arcticus
P. keeni
Clade 1
Clade 7
Clade 6
Clade 5
P. melanotis
P. polionotus
Clade 2
Clade 3
Clade 4
Low altitude, S. California
Low altitude, N. California
Low altitude, Pacific Northwest
Low altitude, Great Plains
High altitude, N. California
High altitude, Arizona
High altitude, Great Basin/N. Rockies
High altitude, Colorado
0.7



A)
HBA-T1
P. maniculatus
(n = 84 alleles)
P. maniculatus (LA)
VLSADDKANIKAAWGKIGGHGAEYGAEALERMFCSFPTTKTYFPHFDVSHGSAQVKAHGGKVADALATAAGHLDDLPAALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPSDFTPAVHASLDKFLASVSTVLTSKYR
P. maniculatus (HA)
Humboldt mice
HM452T1a
HM452T1b
HM453T1a
HM453T1b
HM454T1a
HM454T1b
HM459T1a
HM459T1b
HM464T1a
HM464T1b
HM468T1a
HM468T1b
HM469T1a
HM469T1b
Bits
B)
HBA-T2
P. maniculatus
(n = 82 alleles)
P. maniculatus (LA)
VLSADDKANIKAAWGKIGGHGAEYGAEALERMFCSFPTTKTYFPHFDVSHGSAQVKAHGGKVADALATAAGHLDDLPAALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPSDFTPAVHASLDKFLASVSTVLTSKYR
P. maniculatus (HA)
Humboldt mice
HM452T2a
HM452T2b
HM453T2a
HM453T2b
HM454T2a
HM454T2b
HM459T2a
HM459T2b
HM462T2a
HM462T2b
HM464T2a
HM464T2b
HM468T2a
HM468T2b
HM469T2a
HM469T2b
Bits


A) upstream
P. keeni HBA-T1
P. keeni HBA-T2
P. keeni HBA-T3
P. maniculatus HBA-T1
P. maniculatus HBA-T2
P. maniculatus HBA-T3
P. leucopus HBA-T1
P. leucopus HBA-T2
Rattus HBA-T1
Rattus HBA-T2
0.1
B) CDS
P. leucopus HBA-T1
P. leucopus HBA-T2
P. maniculatus HBA-T3
P. keeni HBA-T3
P. maniculatus HBA-T1
P. maniculatus HBA-T2
P. keeni HBA-T1
P. keeni HBA-T2
Rattus HBA-T1
Rattus HBA-T2
0.1
C) intron 2
P. keeni HBA-T1
P. keeni HBA-T3
P. keeni HBA-T2
P. maniculatus HBA-T2
P. maniculatus HBA-T3
P. maniculatus HBA-T1
P. leucopus HBA-T1
P. leucopus HBA-T2
Rattus HBA-T1
Rattus HBA-T2
0.1
D) downstream
P. leucopus HBA-T1
P. leucopus HBA-T2
P. maniculatus HBA-T2
P. maniculatus HBA-T3
P. maniculatus HBA-T1
P. keeni HBA-T1
P. keeni HBA-T2
P. keeni HBA-T3
Rattus HBA-T1
Rattus HBA-T2
0.1


A) HBB-T1
P. maniculatus
(n = 458 alleles)
Bits
P. maniculatus (LA) VHLTDAEKALVTGLWGKVKPEEIGGEALGRLLAVYPWTQRFFDSFGDLSSASAIMGNAKVKAHGKKVIDSFSEGIKHLDNIKGTFASLSELHCDKLHVDPENFKLLGNMIVIVMAHHLGKDFTPAAQSAYQKVVSGVATALAHKYH
P. maniculatus (HA)
San Luis Obispo mice
CR12T1A
CR12T1B
CR13T1A
CR13T1B
CR15T1A
CR15T1B
CR16T1A
CR16T1B
CR17T1A
CR17T1B
CR18T1A
CR18T1B
B) HBB-T2
P. maniculatus
(n = 458 alleles)
Bits
P. maniculatus (LA) VHLTDAEKALVTGLWGKVKPEEIGGEALGRLLAVYPWTQRFFDSFGDLSSASAIMGNAKVKAHGKKVIDSFSEGIKHLDNIKGTFASLSELHCDKLHVDPENFKLLGNMIVIVMAHHLGKDFTPAAQSAYQKVVSGVATALAHKYH
P. maniculatus (HA)
San Luis Obispo mice
CR12T2A
CR12T2B
CR13T2A
CR13T2B
CR15T2A
CR15T2B
CR16T2A
CR16T2B
CR17T2A
CR17T2B
CR18T2A
CR18T2B



P. maniculatus A (n=4)
P. maniculatus B (n=6)
P. maniculatus C (n=4)
P. maniculatus D (n=20)
P. maniculatus E (n=15)
P. keeni (n=4)
P. polionotus (n=2)
P. leucopus (n=2)
P. aztecus (n=2)
P. melanophrys (n=2)
DPG-str
KCl-str
(DPG+KCl)-str
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
Anion sensitivity (Δlog-P50)

Table S1. Specimens of *Peromyscus* used in the survey of polymorphism in the *HBA-T1*, *HBA-T2*, *HBB-T1*, and *HBB-T2* globin genes. Population samples of *P. maniculatus* are numbered in accordance with the map of collection localities shown in fig. 1*A*.

Species	County/borough, state (number of individual mice)	Locality	Coordinates	Elevation (m)
P. maniculatus	1. Humboldt Co., CA (*n*=8)	vicinity of Arcata	40.87, -124.08	38
	2. Marin Co., CA (*n*=5)	Point Reyes National Seashore	38.07, -122.81	12
	3. Monterey Co., CA (*n*=5)	Hastings Natural History Reservation	36.60, -121.90	8
	4. San Luis Obispo Co., CA (*n*=6)	Camp Roberts	35.78, -120.79	208
	5. Merced Co., CA (*n*=6)	vicinity of Snelling	37.51, -120.40	99
	6. Tuolemne Co., CA (*n*=6)	Upper Lyell Canyon, Yosemite National Park	37.78, -119.26	3006
	7. Mariposa Co., CA (*n*=6)	Lake Vogelsang, Yosemite National Park	37.79, -119.35	3134
	8. Mono Co., CA (*n*=8)	White Mountain Peak, White Mountains	37.63, -118.26	3800
	9. White Pine Co., NV (*n*=5)	Mt. Washington, South Snake Range	38.90, -114.30	3315
	10. Coconino Co., AZ (*n*=6)	Humphreys Peak, San Francisco Peaks	35.35, -111.68	3500
	11. Wayne Co., UT (*n*=5)	Boulder Mountain, Aquarius Plateau	38.17, -111.54	3260
	12. Clear Creek Co., CO (*n*=38)	Mt. Evans, Colorado Front Range	39.66, -105.60	4347
	13. Yuma Co., CO (*n*=26)	Bonny Lake State Park	39.62, -102.17	1158
	14. Pawnee Co., KS (*n*=6)	Larned National Monument	38.19, -99.22	620
	15. Lancaster Co., NE (*n*=12)	Nine-Mile Prairie	40.87, -96.81	406
P. keeni	Wrangell Borough, AK (*n*=7)	Wrangell Island	56.39, -132.09	21
P. leucopus	Saunders Co., NE (*n*=11)	Lee G. Simmons Conservation Park	41.04, -96.37	332

Table S2. Interparalog divergence between the tandemly linked *HBA-T1* and *HBA-T2* genes of *P. maniculatus* and *P. keeni*. Divergence data are from population samples in which both alleles of the *HBA-T1* and *HBA-T2* genes were separately cloned and sequenced. Data from *P. leucopus* are not included here because this species possesses a single adult-expressed α-globin gene, *HBA-T1*. *k*, average number of nucleotide differences between the two paralogs; D_{xy} = average number of nucleotide substitutions per site between paralogs; D_a = number of net nucleotide substitutions per site between paralogs; K_S = nucleotide divergence at silent sites (Jukes-Cantor corrected).

Population	Length (bp)[a]	*N*	No. fixed differences	No. shared polymorphisms	*k*	D_{xy}	D_a	K_S
P. maniculatus								
Humboldt Co., CA	1495	32	7	24	52.69	0.035	0.020	0.042
Mono Co., CA	1402	32	0	114	65.71	0.047	0.014	0.043
Clear Creek Co., CO	1362	68	0	119	40.96	0.030	0.005	0.031
Yuma Co., CO	1354	68	0	193	65.70	0.049	0.003	0.056
P. keeni								
Wrangell Island, AK	1453	24	0	0	8.667	0.006	0.001	0.015

[a]Excluding alignment gaps.

Table S3. Interparalog divergence between the tandemly linked *HBB-T1* and *HBB-T2* genes of *P. maniculatus*, *P. keeni*, and *P. leucopus*. Divergence data are from population samples in which both alleles of the *HBB-T1* and *HBB-T2* genes were separately cloned and sequenced. *k*, average number of nucleotide differences between the two paralogs; D_{xy} = average number of nucleotide substitutions per site between paralogs; D_a = number of net nucleotide substitutions per site between paralogs; K_S = nucleotide divergence at silent sites (Jukes-Cantor corrected).

Population	Length (bp)[a]	*N*	No. fixed differences	No. shared polymorphisms	*k*	D_{xy}	D_a	K_S
P. maniculatus								
Humboldt Co., CA	1241	32	15	13	36.66	0.0295	0.0188	0.0401
Marin Co., CA	1277	20	0	47	36.13	0.0283	0.0060	0.0378
Monterey Co., CA	1265	20	0	77	110.54	0.0874	0.0102	0.0973
San Luis Obispo Co., CA	1291	24	28	0	28.08	0.0218	0.0217	0.0284
Merced Co., CA	1154	24	0	512	134.79	0.1168	0.0028	0.1172
Tuolemne Co, CA	1251	24	12	35	95.43	0.0763	0.0172	0.0884
Mariposa Co., CA	1266	24	0	39	43.15	0.0341	0.0139	0.0451
Mono Co., CA	1211	32	0	52	39.47	0.0326	0.0113	0.0441
White Pine Co., NV	1259	20	10	18	46.27	0.0368	0.0172	0.0497
Coconino Co., AZ	1268	20	0	27	35.34	0.0279	0.0075	0.0377
Wayne Co., UT	1253	18	18	20	42.43	0.0339	0.0185	0.0462
Clear Creek Co., CO	1115	56	0	26	29.01	0.0260	0.0078	0.0363
Yuma Co., CO	1113	60	1	39	28.07	0.0252	0.0106	0.0342
Pawnee Co., KS	1281	20	47	3	56.33	0.0440	0.0395	0.0590
P. keeni								
Wrangell Island, AK	1195	24	13	0	42.33	0.0354	0.0199	0.0423
P. leucopus								
Saunders Co., NE	1243	20	15	15	33.53	0.0270	0.0176	0.0366

[a]Excluding alignment gaps.

Table S4. Estimates of trait-specific genetic variances and heritabilities (h^2) for the pooled sample of Hb variants in *P. maniculatus*.

	Posterior mean of genetic variance	Posterior mean of h^2 (95% CI)
$P_{50(stripped)}$	0.21	0.27 (0.03-0.66)
$P_{50(+KCl)}$	0.49	0.23 (0.02-0.62)
$P_{50(+DPG)}$	0.36	0.32 (0.04-0.76)
$P_{50(KCl+DPG)}$	0.41	0.22 (0.02-0.61)
Δlog-$P_{50(KCl-str)}$	0.34	0.28 (0.03-0.76)
Δlog-$P_{50(DPG-str)}$	1.05	0.72 (0.25-0.94)
Δlog-$P_{50([KCl+DPG]-str)}$	0.39	0.33 (0.04-0.76)

Table S5. Trait-specific genetic variances for 21 amino acid polymorphisms in the *HBA* and *HBB* genes of *P. maniculatus*. The association analysis did not include three *HBB* sites that were polymorphic in the species as a whole (β21, β33, and β56), as these three sites were monomorphic in the set of phenotyped specimens. Biochemical phenotypes include P_{50} values under four experimental treatments, and measures of sensitivity to allosteric effectors (see *Materials and Methods* for details).

Site	$P_{50(stripped)}$	$P_{50(+KCl)}$	$P_{50(+DPG)}$	$P_{50(KCl+DPG)}$	Δlog-$P_{50(KCl-str)}$	Δlog-$P_{50(DPG-str)}$	Δlog-$P_{50([KCl+DPG]-str)}$
α5	1.44×10^{-4}	1.57×10^{-4}	8.39×10^{-4}	2.65×10^{-4}	5.94×10^{-4}	2.22×10^{-3}	9.63×10^{-4}
α10	3.53×10^{-4}	9.17×10^{-8}	5.40×10^{-4}	5.12×10^{-4}	2.30×10^{-4}	3.37×10^{-3}	2.68×10^{-3}
α12	1.67×10^{-4}	3.17×10^{-5}	8.72×10^{-4}	2.84×10^{-4}	2.73×10^{-4}	1.12×10^{-2}	1.45×10^{-3}
α15	2.39×10^{-4}	6.97×10^{-5}	1.54×10^{-3}	5.44×10^{-4}	5.04×10^{-4}	2.32×10^{-2}	2.60×10^{-3}
α23	1.62×10^{-4}	4.00×10^{-5}	8.90×10^{-4}	3.09×10^{-4}	2.86×10^{-4}	1.20×10^{-2}	1.47×10^{-3}
α34	2.82×10^{-4}	6.49×10^{-5}	1.75×10^{-3}	5.28×10^{-4}	4.95×10^{-4}	2.42×10^{-2}	2.71×10^{-3}
α50	1.60×10^{-5}	4.28×10^{-4}	2.86×10^{-3}	2.02×10^{-4}	7.82×10^{-4}	4.96×10^{-3}	4.08×10^{-4}
α57	1.07×10^{-4}	3.35×10^{-4}	6.99×10^{-5}	2.73×10^{-5}	1.13×10^{-4}	2.54×10^{-7}	6.04×10^{-6}
α60	1.17×10^{-4}	3.57×10^{-4}	6.25×10^{-5}	2.92×10^{-5}	1.20×10^{-4}	3.29×10^{-7}	5.91×10^{-6}
α64	2.34×10^{-4}	3.84×10^{-7}	5.31×10^{-4}	3.44×10^{-4}	1.80×10^{-4}	4.68×10^{-3}	1.60×10^{-3}
α71	1.27×10^{-3}	1.03×10^{-3}	3.87×10^{-5}	1.79×10^{-4}	6.69×10^{-6}	5.10×10^{-4}	2.00×10^{-4}
α78	7.27×10^{-4}	1.91×10^{-4}	2.53×10^{-6}	7.33×10^{-7}	3.34×10^{-5}	5.48×10^{-4}	4.15×10^{-4}
α113	1.55×10^{-3}	8.73×10^{-4}	1.16×10^{-4}	3.88×10^{-6}	1.57×10^{-6}	8.71×10^{-4}	1.05×10^{-3}
α115	1.45×10^{-3}	7.81×10^{-4}	9.31×10^{-5}	9.44×10^{-8}	6.04×10^{-6}	1.00×10^{-3}	1.17×10^{-3}
α116	4.04×10^{-4}	3.95×10^{-7}	3.38×10^{-7}	6.05×10^{-5}	4.30×10^{-4}	6.45×10^{-6}	6.83×10^{-5}
β6	1.27×10^{-6}	1.75×10^{-3}	1.55×10^{-4}	9.82×10^{-5}	5.03×10^{-3}	3.73×10^{-4}	9.13×10^{-5}
β25	1.48×10^{-5}	1.32×10^{-5}	1.43×10^{-5}	1.82×10^{-5}	4.89×10^{-6}	1.89×10^{-5}	8.83×10^{-6}
β62	5.39×10^{-5}	1.31×10^{-3}	7.20×10^{-4}	2.10×10^{-4}	8.53×10^{-4}	3.92×10^{-4}	6.66×10^{-5}
β72	6.57×10^{-5}	1.60×10^{-3}	9.03×10^{-4}	2.39×10^{-4}	1.02×10^{-3}	4.88×10^{-4}	9.84×10^{-5}
β128	6.04×10^{-5}	9.74×10^{-4}	8.99×10^{-4}	8.96×10^{-5}	5.97×10^{-4}	1.09×10^{-3}	1.23×10^{-5}
β135	2.71×10^{-5}	7.50×10^{-4}	1.09×10^{-3}	1.83×10^{-5}	4.85×10^{-4}	2.24×10^{-3}	1.38×10^{-6}
median	1.62×10^{-4}	3.35×10^{-4}	5.40×10^{-4}	1.79×10^{-4}	2.86×10^{-4}	1.00×10^{-3}	4.08×10^{-4}