Supporting Information

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Table S1. Number of *Mc1r* alleles sampled from dark soil and white sands habitats for the three focal species

Species and habitat	Number of wild-type alleles	Number of derived alleles
Sceloporus undulatus		
Dark soil	58	0
White sands	30	26
FET P = 2 x 10^{-10}		
Aspidoscelis inornata		
Dark soil	41	3*
White sands	8	48
FET P = 7 x 10^{-15}		
Holbrookia maculata		
Dark soil	52	0
White sands	0	36
FET P = 3 x 10^{-16}		

Derived alleles are those with the $His^{208}Tyr$ substitution for *Sceloporus undulatus*, the $Thr^{170}Ile$ substitution for *Aspidoscelis inornata*, and the $Val^{168}Ile$ substitution for *Holbrookia maculata*. Fisher's Exact Test (FET) was highly significant for each species. *Note that derived alleles in dark soil *A. inornata* were always found in the heterozygous state.

Table S2. Functional characterization of Mc1r variants

			cAMP accumulation		Expression		
			Basal cAMP	E_{max}	EC_{50}	Total	Cell surface
Species	Phenotype	Amino acid difference wild-type vs. derived	(Fold over basal)		(pmol/L)	(% dark variant)	
Sceloporus undulatus	Dark	$His^{208}Tyr$ TMD5	4.7 ± 1.3 (7)	29.9 ± 9.7 (7)	296 ± 107 (7)	100 (3)	100 (5)
	Blanched		**1.5 ± 0.1** (7)	22.8 ± 4.5 (7)	706 ± 300 (7)	101 ± 6 (3)	**80 ± 2** (5)
Aspidoscelis inornata	Dark	$Thr^{170}Ile$ TMD4	21.3 ± 4.8 (6)	59.9 ± 15.8 (6)	752 ± 187 (6)	100 (3)	100 (3)
	Blanched		**8.1 ± 1.9** (6)	**22.6 ± 4.2** (6)	674 ± 360 (6)	100 ± 5 (3)	107 ± 3 (3)
Holbrookia maculata	Dark	$Val^{168}Ile$ TMD4	8.9 ± 2.1 (6)	39.4 ± 10.1 (6)	1272 ± 955 (6)	100 (3)	100 (6)
	Blanched		8.3 ± 1.9 (6)	38.8 ± 9.5 (6)	534 ± 208 (6)	100 ± 5 (3)	103 ± 2 (6)

For cAMP accumulation assay, COS-7 cells were transfected with expression vectors coding for all variants listed. We measured basal cAMP, E_{max}, and EC_{50} values from concentration–response curves (1 fM–10 μM) of the natural agonist α-MSH. Basal cAMP and E_{max} values are given as x-fold over basal cAMP accumulation of control-transfected cells (basal cAMP in assays from different species: *Sceloporus undulatus*, 48 ± 44 amol/cell; *Aspidoscelis inornata*, 4 ± 1 amol/cell; *Holbrookia maculata*, 56 ± 51 amol/cell). Data are presented as means ± SEM of independent experiments (number indicated in parentheses), each carried out at least in duplicate. Numbers in bold indicate a significant mean value difference between wild-type and derived variant of the respective species ($P < 0.05$; two-tailed, paired Student *t* test). For expression analyses in ELISA studies, COS-7 cells were transfected, total cellular expression levels (intracellular plus plasma membrane expression) were measured by a sandwich ELISA, and cell-surface expression levels (plasma membrane expression) were measured by an indirect cellular ELISA. Specific optical density (OD) readings (OD value of HA-tagged construct minus OD value of control-transfected cells) are given as a percentage of the wild-type allele from the respective species. For the total expression ELISA, the nonspecific $OD_{492\ nm}$ values of control (GFP)-transfected COS-7 cells were 0.127 ± 0.007 (set 0%), and the specific $OD_{492\ nm}$ values of the wild-type *Mc1r* alleles were *Sceloporus undulatus*, 0.941 ± 0.014; *Aspidoscelis inornata*, 0.954 ± 0.058; *Holbrookia maculata*, 0.954 ± 0.015 (set 100% for the respective species). For cell-surface expression ELISA, the nonspecific $OD_{492\ nm}$ values of control-transfected COS-7 cells were *Sceloporus undulatus*, 0.037 ± 0.007; *Aspidoscelis inornata*, 0.038 ± 0.012; *Holbrookia maculata*, 0.036 ± 0.006 (set 0% for the respective species), and the specific $OD_{492\ nm}$ values of the wild-type *Mc1r* alleles were *Sceloporus undulatus*, 0.996 ± 0.080; *Aspidoscelis inornata*, 0.703 ± 0.017; *Holbrookia maculata*, 1.105 ± 0.0875 (set 100% for the respective species). Data are presented as means ± SEM of independent experiments (number indicated in parentheses), each carried out in triplicate. Numbers in bold indicate a significant mean value difference between wild-type and derived variant of the respective species ($P < 0.05$; two-tailed, paired Student *t* test).

Table S3. Samples of *Sceloporus undulatus* and *Aspidoscelis inornata* used for the Fig. 3 comparison between *Mc1r* genotype (determined by sequencing) and color phenotype (determined by spectrophotometer)

Specimen catalog no.	Habitat type	AUC
Sceloporus undulatus		
MVZ 252867	Dark soil	4,181
MVZ 252868	Dark soil	3,582
MVZ 252869	Dark soil	4,214
MVZ 252870	Dark soil	4,147
MVZ 252871	Dark soil	3,855
MVZ 252872	Dark soil	5,217
MVZ 252873	Dark soil	4,290
MVZ 252874	Dark soil	3,918
MVZ 252875	Dark soil	5,313
MVZ 252876	Dark soil	4,917
MVZ 252877	Dark soil	3,175
MVZ 252937	Dark soil	3,453
MVZ 252941	Dark soil	4,359
MVZ 253020	Dark soil	3,705
MVZ 253021	Dark soil	4,414
MVZ 253023	Dark soil	2,296
MVZ 253025	Dark soil	4,073
MVZ 253026	Dark soil	3,604
MVZ 253028	Dark soil	2,136
MVZ 253029	Dark soil	2,776
MVZ 253030	Dark soil	2,814
MVZ 252957	Ecotone	7,791
MVZ 252961	Ecotone	6,740
MVZ 252962	Ecotone	8,694
MVZ 252968	Ecotone	5,514
MVZ 252973	Ecotone	6,482
MVZ 252975	Ecotone	5,215
MVZ 252976	Ecotone	7,078
MVZ 252978	Ecotone	7,270
MVZ 252979	Ecotone	8,238
MVZ 252984	Ecotone	9,845
MVZ 252986	Ecotone	6,293
MVZ 252994	Ecotone	6,152
MVZ 253007	Ecotone	6,923
MVZ 253016	Ecotone	6,048
MVZ 252939	White sands	6,867
MVZ 252940	White sands	9,200
MVZ 252946	White sands	11,023
MVZ 252949	White sands	10,037
MVZ 252951	White sands	7,962
MVZ 252952	White sands	8,965
MVZ 252966	White sands	6,571
MVZ 252989	White sands	10,173
MVZ 252991	White sands	7,977
MVZ 252998	White sands	5,770
MVZ 252999	White sands	6,854
MVZ 253000	White sands	9,314
MVZ 253001	White sands	6,083
MVZ 253004	White sands	6,496
MVZ 253008	White sands	4,110
MVZ 253014	White sands	5,928
EBR 492	White sands	6,629
Aspidoscelis inornata		
MVZ 252632	Dark soil	4,705
MVZ 252633	Dark soil	4,141
MVZ 252634	Dark soil	4,911
MVZ 252635	Dark soil	2,655
MVZ 252636	Dark soil	4,755
MVZ 252637	Dark soil	3,347

Table S3. (Cont.)

Specimen catalog no.	Habitat type	AUC
MVZ 252638	Dark soil	4,138
MVZ 252639	Dark soil	4,022
MVZ 252640	Dark soil	3,220
MVZ 252642	Dark soil	3,902
MVZ 252698	Dark soil	4,552
MVZ 252699	Dark soil	4,331
MVZ 252667	Ecotone	4,112
MVZ 252671	Ecotone	5,896
MVZ 252673	Ecotone	4,836
MVZ 252674	Ecotone	6,408
MVZ 252675	Ecotone	6,375
MVZ 252676	Ecotone	5,938
MVZ 252677	Ecotone	7,028
MVZ 252679	Ecotone	4,969
MVZ 252680	Ecotone	4,978
MVZ 252688	Ecotone	8,027
MVZ 252690	Ecotone	5,503
MVZ 252691	Ecotone	5,339
MVZ 252694	Ecotone	4,854
MVZ 252695	Ecotone	3,242
MVZ 252696	Ecotone	3,433
MVZ 252670	White sands	9,803
MVZ 252672	White sands	7,615
MVZ 252682	White sands	7,235
MVZ 252683	White sands	8,862
MVZ 252684	White sands	7,200
MVZ 252685	White sands	9,170
MVZ 252686	White sands	9,359
MVZ 252687	White sands	8,462
MVZ 252689	White sands	8,435
MVZ 252692	White sands	7,956
MVZ 252693	White sands	5,114
EBR 339	White sands	8,267

MVZ specimen ID numbers refer to accession numbers at the Museum of Vertebrate Zoology, University of California, Berkeley. EBR specimen ID numbers reference samples housed at the University of Idaho. AUC, area under the spectral curve for the visible spectrum (400–700 nm).