**Supplemental Materials**

**SUPPLEMENTAL METHODS:**

**Genome assembly:**

*Annotations using Maker:*

We annotated our genome using Maker version 3.01.02 [(Campbell et al. 2014)](https://paperpile.com/c/kXPKYZ/Big8Z). We used transcript evidence from *Ranitomeya imitator* to aid in assembly (“est2genome=1”). These data include: 1) a developmental series of tadpole skin across color morphs of captive bred *R. imitator* (this paper, see below), 2) liver, skin, and intestine samples from six different wild *R. imitator* populations (Stuckert et al. unpublished data), and 3) brain samples from captive bred *R. imitator* (Geralds et al. unpublished data). The developmental series data were used in order to accurately annotate genes that are expressed in the skin at different time points in order to target color genes. The addition of data from wild frogs and a variety of other tissue types were used to provide additional transcript evidence in an effort to recover more genes after annotation.

*Transcriptome assemblies*:

*Developmental series:*

We used data from the imitator developmental series we analyzed in this paper to make transcriptome across developmental time points in the skin. In order to generate an initial reference transcriptome we assembled 40 M randomly subsampled forward and reverse reads sampled across morphs and time points using seqtk (https://github.com/lh3/seqtk) and used the Oyster River Protocol version 1.1.1 (MacManes 2017) to assemble this dataset. Evidence indicates that there is a substantial diminishment of returns in terms of transcriptome assembly completeness from using over 20-30 million reads (MacManes 2017). Initial error correction was done using RCorrector 1.01, followed by adaptor removal and quality trimming using trimmomatic version 0.36 at a Phred score of ≤ 3 (Bolger et al. 2014) since overly aggressive quality trimming has been shown to reduce assembly completeness (MacManes 2014). The Oyster River Protocol (MacManes 2017) assembles a transcriptome by merging multiple assemblies constructed using a series of different transcriptome assemblers and kmer lengths. We constructed the Independent assemblies with Trinity version 2.4.0 (Grabherr et al. 2011), Shannon version 0.0.2 (Kannan et al. 2016), and SPAdes assembler version 3.11 using 35-mers (Bankevich et al. 2012). This deviates slightly from the Oyster River Protocol specified in MacManes (2017), which specifies kmer lengths of 55 and 75 for SPAdes assemblies, but that exceeds our 50 bp sequencing read length. We then merged these individual assemblies using OrthoFuser (MacManes 2017). Finally, we assessed transcriptome quality using BUSCO version 3.0.1 (Simão et al. 2015) and TransRate 1.0.3 (Smith-Unna et al. 2016).

*Transcriptomes from wild frogs of multiple populations:*

We *de novo* assembled transcriptomes from six populations along a transition zone from the orange banded morph of *R. imitator* (*R. summersi* mimic) through the yellow striped morph (lowland *R. variabilis* mimic). This includes two pure orange banded populations, two pure yellow striped populations, and two admixed populations with highly variable phenotypes. For each population we randomly chose one individual, concatenated Illumina HiSeq 4000 reads from the liver, intestines, and dorsal skin into a single readset per population and then used the Oyster River Protocol version 2.2.8 [(MacManes 2018)](https://paperpile.com/c/kXPKYZ/Cx1WH) to assemble population specific *de novo* transcriptomes. This is similar to the approach above in the section “*Developmental series*”, with some minor differences which we detail here. First, we assembled individual assemblies using Trinity version 2.8.5 [(Grabherr et al. 2011)](https://paperpile.com/c/kXPKYZ/22dBa), two iterations of SPAdes version 3.13.1 [(Bankevich et al. 2012)](https://paperpile.com/c/kXPKYZ/D5QpL) with kmer values of 55 and 75 respectively, and finally Trans-ABySS version 2.0.1 [(Robertson et al. 2010)](https://paperpile.com/c/kXPKYZ/LyAvQ). These individually built transcriptomes were then merged together using OrthoFuser (MacManes 2018). Unique contigs which were dropped in Orthofuser were recovered using a reciprocal blast search of the final assembly against the individual assemblies for unique contigs. We removed all contigs with expression lower than one Transcript Per Million (TPM) using the TPM=1 flag in the ORP. Contigs that were dropped due to low expression but which likely represent expressed genes were recovered by blasting these against the UniProt database. Finally, transcriptome quality was assessed using BUSCO version 3.0.1 (Simão et al. 2015) and TransRate 1.0.3 (Smith-Unna et al. 2016).

*Brain transcriptome assemblies:*

Geralds et al. (unpublished data) conducted an experiment looking at the effects of parental behaviors and tadpole begging on gene expression in the brains of adult *Ranitomeya imitator*. We randomly chose one individual from individuals that were interacting with begging tadpoles, and those that were not. We concatenated these data into a single forward and reverse read file, then used the Oyster River Protocol version 2.2.8 [(MacManes 2018)](https://paperpile.com/c/kXPKYZ/Cx1WH) to assemble the brain transcriptome. These details are the same as above in the “*Transcriptomes from wild frogs of multiple populations”* section.

***Gene expression:***

*Ranitomeya imitator:*

Frogs from our study populations were collected by Understory and captive bred in Peru prior to shipping captive-bred frogs to a breeding facility in Canada. We purchased individuals that were captive bred in Canada. The frogs used in this study have a similar phenotype to those of individuals found in captivity from their source populations. As with any captive stock sourced from the wild, there is likely an initial bottleneck and corollary reduction in overall heterozygosity, however morphs were not selectively bred to produce desired phenotypes.

Breeding *R. imitator* pairs were placed in 5-gallon terraria containing small (approximately 13 cm) PVC pipes capped on one end and filled halfway with water. We removed tadpoles from the tanks after the male transported them into the pools of water and hand reared them. Although in the wild female *R. imitator* feed their tadpoles unfertilized eggs, tadpoles can survive and thrive on other food items (Brown et al. 2008). We raised experimental tadpoles on a diet of Omega One Marine Flakes fish food mixed with Freeze Dried Argent Cyclop-Eeze, which they received three times a week, with full water changes twice a week until sacrificed for analyses at 2, 4, 7, and 8 weeks of age. At two weeks, tadpoles are limbless, patternless, and a light gray color with two dark black eyeballs. At 4 weeks tadpoles are a slightly darker gray and have back limb buds. Tadpoles had developed their pattern and some coloration as well as reached the onset of metamorphosis at around week 7, and had metamorphosed, were resorbing the tail, and had their froglet patterns at 8 weeks old. Pattern development continues as juveniles and subadults frogs as they grow into the ultimate pattern they possess as adults. Our four sampling periods correspond to roughly Gosner stages 25, 27, 42, and 44 (Gosner 1960). Tadpoles were raised in a homogenous environment, and thus tadpoles were generally at the same developmental stage at the point of sacrifice. Due to inherent variation, some individuals may have been one Gosner stage off the norm. We sequenced a minimum of three individuals at each time point from the Sauce, Tarapoto, and Varadero populations (except for Tarapoto at 8 weeks), and two individuals per time point from the Huallaga population. Individuals within the same time points were sampled from different family groups (Table 1).Individually barcoded samples were pooled and sequenced using 50 bp paired end reads on three lanes of the Illumina HiSeq 2500 at the New York Genome Center. This yielded on average 24.45M reads per library ± 8.6M sd (range: 10.1-64.M).

*Ranitomeya fantastica* and *Ranitomeya variabilis*:

We set up a captive colony consisting of between 6 and 10 wild collected individuals per locality, which was maintained at the Tarapoto Research Center (INIBICO. jr. Ventanilla s/n Sector Ventanilla. Banda de Shilcayo. San Martin, Peru). Male-female pairs were placed into individual terrariums which were misted with rainwater and fed with fruit flies daily. Artificial egg deposition sites consisting of short sections of PVC pipe (~10 cm in length) were positioned within each terrarium and we checked terraria for eggs biweekly. When eggs were found, they were transferred into petri dishes to monitor their development. Upon hatching, tadpoles were placed individually into 25 ml plastic cups filled with rain water and fed daily with a pinch of a 50/50 mix of spirulina and nettle leaf powder. Three tadpoles per stage (7, 14, 35, 49 and 56 days after hatching) were fixed in an RNAlater (Ambion) solution. To do so, tadpoles were first euthanized in a 250 mg/L benzocaine hydrochloride bath, then rinsed with distilled water before the whole tadpole was placed in RNAlater in a 2.5ml Eppendorf tube. The Eppendorf tube with sample in RNAlater was then stored at 4°C for 6h before being frozen at -20°C for long-term storage. This protocol was approved by the Peruvian Servicio Forestal y de Fauna Silvestre through the authorization number 232-2016- SERFOR/DGGSPFFS.

|  |  |  |  |
| --- | --- | --- | --- |
| Species | Morph | Age (weeks) | n |
| *Ranitomeya imitator* | Redheaded | 2 | 2 |
| 4 | 2 |
| 7 | 2 |
| 8 | 2 |
| White-Banded | 2 | 3 |
| 4 | 3 |
| 7 | 3 |
| 8 | 3 |
| Spotted | 2 | 4 |
| 4 | 4 |
| 7 | 4 |
| 8 | 1 |
| Striped | 2 | 3 |
| 4 | 3 |
| 7 | 3 |
| 8 | 3 |
| *Ranitomeya fantastica* | Redheaded | 1 | 3 |
| 2 | 3 |
| 4 | 3 |
| 5 | 3 |
| 7 | 3 |
| 8 | 3 |
| White-Banded | 4 | 3 |
| 5 | 3 |
| 7 | 3 |
| 8 | 3 |
| *Ranitomeya variabilis* | Spotted | 1 | 3 |
| 2 | 3 |
| 4 | 3 |
| 5 | 3 |
| 7 | 3 |
| 8 | 3 |
| 9 | 3 |
| Striped | 1 | 3 |
| 2 | 3 |
| 4 | 3 |
| 5 | 3 |
| 7 | 3 |
| 8 | 3 |

Supplemental Table 1. Sample sizes for gene expression of model species *R. fantastica* and *R. variabilis.*

**SUPPLEMENTAL RESULTS:**

*Genome sequence data:*

We produced a large set of sequence data. This includes approximately 228,115,533,900 10X bases, 22,076,177,973 Oxford Nanopore bases, and 297,076,730,441 PacBio bases.