Intraspecific divergence, assortative mating and hybridisation in the Amazonian frog, *Allobates femoralis*

Photo sourced from http://www.sareptiles.co.za/forum/viewtopic.php?f=75&t=20594

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This thesis is written in the form of a journal article from *Molecular Ecology.*

While the author guidelines for *Molecular Ecology* have been followed in respect to referencing, sections of this thesis vary from the typical format required for this journal to meet the thesis formatting requirements of Macquarie University. Consequently, narrower margins and 1.5 line spacing are used. To improve clarity, an extended introduction, methods and discussion are provided. Figures and tables have also been integrated into the text.

Declaration

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- My supervisor, Assoc Prof Adam Stow, for his advice with my methods, support with my lab work, and comments on drafts of this manuscript.

- Dr Siobhan Dennison, for teaching me how to run PCRs, optimise primers, and conduct analyses in MEGA and STRUCTURE.

All other research described in this report is my own original work.

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Abstract

The processes that have driven species diversity in the Amazon Basin remain unknown, or in contention for many taxa. For the brilliant-thighed frog, *Allobates femoralis*, I evaluated two competing vicariance-based hypotheses explaining the diversification of Amazonian biota*;* the riverine barrier hypothesis and the Pleistocene refugia hypothesis. I also investigated whether assortative mating maintains the barrier between distinct genetic lineages when physical contact is restored. Using fragments of 16S rRNA and cyt*b* mtDNA sourced from GenBank, I conducted a time-calibrated phylogenetic reconstruction using the Bayesian approach implemented in the *BEAST software. Node divergence dates and patterns of intraspecific divergence were found to be strongly associated with river locations, and therefore, compatible with the riverine barrier hypothesis. At several locations, however, contact zones occur between genetically distinct lineages of *A. femoralis*. In order to test whether assortative mating explains the lack of genetic recombination, I analysed mate choice in a population where individuals with different call types occur in sympatry. A data set of 8067 Single Nucleotide Polymorphisms and mtDNA (16S) sequences revealed significant genetic partitioning between colour morphs but not call type. These results provide a new insight into anuran mating systems, especially in respect to the traits which control mate choice.

Keywords: evolutionary diversification, Dendrobatoidea, mate choice, Neotropics, single nucleotide polymorphisms

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Contents

1. Introduction

The rapid depletion of worldwide biodiversity has been primarily attributed to human activities and population growth, with the human related impacts of habitat loss, introduced species, over-exploitation and pollution currently threatening many taxa (Frankham 2010). Consequently, a growing number of conservation programmes have been established. More recently, the importance of considering evolutionary processes when designing conservation strategies has been recognised, and with the advent of more efficient genetic technologies this information is more readily accessible (Frankham 2010; Frankham *et al.* 2010). Furthermore, the relevance of genetic variation to conservation is now better appreciated, both with respect to identifying genetically divergent parts of a species distribution, and evaluating the prospect of inbreeding, or outbreeding depression (Frankham *et al.* 2010). The acquisition of these data can be used to more effectively implement conservation strategies, with the aim of retaining as much genetic diversity as possible for threatened species (Frankham 2010; Frankham *et al.* 2010). To this end, knowledge of the processes that generate and maintain genetic diversity are helpful to the implementation of effective conservation strategies, and to predict the impacts of environmental change.

Investigation of the evolutionary processes that generate and maintain genetic diversity can be used to predict areas of high biodiversity and also the environmental features that, if impacted, might be detrimental to the production of future diversity (Moritz 2002; Eizaguirre & Baltazar-Soares 2014). These processes operate at a range of scales, extending from the broad influence of dispersal across landscapes, to the narrower role of behavioural adaptations and genetic compatibilities, which have evolved to prevent mismatched matings (Jennions & Petrie 1997). Although these processes are poorly understood for many regions globally, knowledge is especially limited for taxa in the Amazon Basin, despite a substantial amount of scientific attention (Simões *et al.* 2014). This is in part due to the high levels of biodiversity in this region (Duellman 1999; Da Silva *et al.* 2005).

The lowland forests of the Amazon Basin are among the most species rich on Earth (Gentry 1988; Wilson 1992). This diversity has fascinated biologists since the 19th century, and a number of hypotheses have been proposed to explain how, where and when Amazonian species originated. However, because no clear geographical features or palaeontological forces explain general patterns of diversification across a range of animal

groups, a convincing solution for the origins of Amazonian diversification has not yet been determined (Ribas *et al.* 2011). In addition, the large scale of the region, and poorly known patterns of intraspecific diversity and taxonomy, mean that the relationships between many parts of a species distribution are unknown (Simões *et al.* 2014). Research in this area is necessary for the effective implementation of conservation strategies.

1.1 Origins of Amazonian diversity

Although allopatric speciation is considered to be the primary mechanism underlying Amazonian diversity (Haffer 2008, Ribas *et al.* 2011), there is widespread debate about the barriers which led to divergence, and the time period over which this occurred (Wallace 1852; Haffer 1969; Räsänen *et al.* 1990; Ayres & Clutton-Brock 1992; Bush 1994; Rull 2008; Simões *et al.* 2008; Hoorn *et al.* 2010). Of all the hypotheses suggested, two are predominant in the literature. These are the riverine barrier hypothesis (Wallace 1852) and the Pleistocene refugia hypothesis (Haffer 1969).

1.1.1 Riverine barrier hypothesis

The riverine barrier hypothesis is the oldest evolutionary mechanism proposed to explain the diversity of the Amazon region (Colwell 2000). Although its foundations are based on early reports of Amazonian faunal distributions (Wallace 1852), this hypothesis has been adapted over time to reflect a contemporary understanding of geomorphological, ecological and genetic processes. Under this model, the development of the Amazonian river system divided continuous lowland forest, and effectively fragmented populations of what was a widespread species. With populations isolated on opposite sides of a large river, gene flow was inhibited, and each population underwent separate evolutionary pathways to become independently evolving lineages. As a result, according to this hypothesis, isolated populations are genetically distinct, and may eventually diverge to become separate species (Simões 2010).

Major Amazonian rivers have been documented to be effective barriers in a variety of organisms, and are recognised for controlling distributional boundaries in a substantial portion of Amazonian vertebrates (Ribas *et al.* 2011), including understorey birds (Capparella 1988; Ribas *et al.* 2011) primates (Ayres & Clutton-Brock 1992), and more recently, intraspecific divergence at both genetic markers and phenotypes for frog species located in different interfluves (Funk *et al.* 2007; Simões *et al.* 2008; Fouquet *et al.* 2012; Kaefer *et al.* 2013; Simões *et al.* 2014; Dias-Terceiro *et al.* 2015). Although the major

rivers of the Amazon have been shown to effectively separate many populations of forest biota, this hypothesis has received little critical attention (Gascon *et al.* 1998), and its role in promoting speciation has not yet gained wide acceptance (Ribas *et al.* 2011).

1.1.2 Pleistocene refugia hypothesis

The refuge hypothesis (Haffer 1969) has received a substantial amount of scientific attention since its inception, and for decades, was the most widely accepted model for Amazonian divergence (Rull 2008). Its foundations are based on modern biogeographic distributions of endemic avian species within Amazonia which were combined with historical rainfall data, to infer regions of climatic stability in which divergence may have occurred (Haffer 1969). Under this hypothesis, the climatic fluctuations associated with the glacial cycles of the Pleistocene caused large scale changes to vegetation, and subsequently shaped present day biota. The arid conditions experienced during glacial maxima were proposed to have driven forest fragmentation in the Neotropical lowlands. As forests survived only in areas of higher rainfall, their range shifted to these regions, while lowland areas were replaced with savannah. Vertebrate species are suggested to have followed suitable habitat to higher altitudes, and in doing so, mirrored this range shift. As a result, many plant and animal populations were isolated in forest remnants or 'refuges', where they adapted to meet local conditions. When humid conditions returned, forest regions expanded, as vegetation was able to again survive in the lower elevations. This expansion allowed populations to reconnect (Haffer 1969).

Haffer's (1969) finding that patterns of endemic avian taxa correspond to areas which experience greatest rainfall prompted a number of authors to model patterns of endemism for other animal groups. Consequently, refugia are suggested to have led to divergence in *Anolis* lizards (Vanzolini & Williams 1981), amphibians (Duellman 1982), Nymphalid butterflies (Brown 1982), and plants (Prace 1982). As patterns of overlap differ slightly between species, Haffer's original nine refugia were expanded, and more than forty are currently listed (Brown 1982).

1.2 Secondary contact of populations

The retraction of geographic and climatic barriers is believed to have led to the range expansion of organisms that had diverged in isolation (Haffer 2008). Strong mate choice is hypothesized to operate within these zones, with selective pressures predicted to act against the production of hybrid individuals (Losos & Leal 2013). This is believed to occur

for several reasons, the most important being outbreeding depression. As lineages have diverged under different environmental conditions, the production of hybrids may result in the loss of adaptations which are highly suited to a particular environment (Losos & Leal 2013). Additional explanations consider genetic compatibilities and 'good genes', which can minimise the production of sterile or unattractive offspring (Hobel & Gerhardt 2003; Jiang *et al.* 2013). Interestingly, individuals have been documented to be more choosy when selecting mates in zones where distinct lineages co-occur (Losos & Leal 2013). Within these zones, many animal species have been shown to select mates with traits that are phenotypically similar to themselves (Jiang *et al.* 2013). This non-random mating pattern – known as assortative mating – is important in a number of ways; it can drive speciation through the divergence of populations, and can maintain the genetic isolation of populations if they happen to come back into contact (Jiang *et al.* 2013).

For mate choice to operate effectively in secondary contact zones, lineages should differ in one or more detectable traits (Losos & Leal 2013). These must convey a minimum amount of information so that appropriate mating decisions can be made by the receiver. It has been suggested that mate choice operates as a two-step process, in which signals must encode sufficient information to enable the recognition of species identity and allow assessment of individual mate quality (Ptacek 2000; Thibert-Plante & Gavrilets 2013). Additionally, many species produce multiple cues to increase the likelihood that signals are received and interpreted correctly.

In non-random mating systems, sexual signals are under strong evolutionary pressures (Amézquita *et al.* 2011). Ultimately this causes the reproductive traits of different species to vary (Pfennig & Pfennig 2010). As a result, each species occupies its own signalling space in which the cues produced are distinct from those of heterospecifics (Pfennig & Pfennig 2010). As females adopt mating preferences which enhance the likelihood of mating with conspecifics, variation in ecological traits may alter female mate choices (Pfennig & Pfennig 2010). If this occurs, females are expected to mate assortatively to help ensure the production of viable offspring (Jiang *et al.* 2013). Over time, this can lead to reproductive isolation, and potentially to speciation (Pfennig & Pfennig 2010; Jiang *et al.* 2013). This method of speciation is not widely considered, with ecological speciation overlooked for the Amazon region (Beheregaray *et al.* 2014).

1.3 Anuran amphibians as a model

Anuran amphibians are excellent models to use when investigating the processes that generate and maintain genetic diversity. As they are generally philopatric and demonstrate low vagility (Duellman 1982; Blaustein *et al.* 1994; Beebee 1996), populations demonstrate pronounced genetic structuring and tend to retain high-resolution signals caused by historical events (Zeisset & Beebee 2008). By creating a time-calibrated phylogeographic analysis on Amazonian anuran species and comparing these to hypotheses of Amazonian diversification, we should be able to gain powerful insights regarding the evolutionary history of this region.

Anuran amphibians have been documented to use a number of sensory cues to communicate with conspecifics (Belanger & Corkum 2009). These cues can be acoustic, visual or chemical in nature, and are used to facilitate a number of interactions, including social behaviours, foraging and predator avoidance (Brizzi & Corti 2007; Belanger & Corkum 2009). These signals play a particularly important role in sexual communication, as they can be used to attract potential mates, locate signalling individuals, and assess sexual receptivity (Belanger & Corkum 2009). As anurans have played a central role in research on mating strategies and mate choice (Summers *et al.* 1999) and their calls can be used as reliable population markers (Amézquita *et al.* 2009), they can be used as an effective model to test the influence of secondary contact zones on mate choice.

1.4 Allobates femoralis

The brilliant-thighed frog, *Allobates femoralis* (Boulenger 1884) provides an excellent model system for investigating evolutionary processes. Because this species has a pan-Amazonian distribution, demonstrates a high level of philopatry and low vagility (Lougheed *et al.* 1999), and is thought to be incapable of crossing large geographical barriers (Fouquet *et al.* 2012), strong genetic patterns should be reflected within this species' evolutionary history. Because several evolutionary lineages have also been documented within this taxon (Grant *et al.* 2006; Santos *et al.* 2009), the processes that maintain phenotypic diversity between populations can be assessed. Assortative mate choice has been hypothesized to act strongly in contact zones where phenotypically distinct populations co-occur (Simões *et al.* 2012).

1.5 Study objectives

This study addresses two broad themes: an assessment of how genetic divergence is generated in *Allobates femoralis* and an investigation of whether assortative mating is a mechanism maintaining divergence when divergent lineages come into contact. Specifically I test two competing hypotheses of Amazonian diversification, the riverine barrier hypothesis and Pleistocene refugia hypothesis. I also evaluate two cues upon which assortative mating might be based, variation in call type and thigh-colouration.

This research adds important information to the history of Amazonian anuran diversification, provides a new insight into anuran mating systems, and highlights the extent of genetic structuring between distinct phenotypes. Knowledge of these processes will aid in the implementation of more effective conservation management within the Amazon region.

2. Methods

2.1 Intraspecific divergence

2.1.1 Data collection

A BLAST search was conducted to assess the availability of existing *A. femoralis* sequences. The mitochondrial genes, 16S and cytochrome *b* (cyt*b*), were selected for analysis as they are the two most commonly used markers for phylogenetic analysis in this species, and a large number of sequences exist for these two gene regions. Although sequences of the 12S rRNA and CO1 gene regions also exist, these markers were not employed in our analysis as the number of sequences available from this database and their respective sampling locations were insufficient to allow geographical hypothesis testing.

A total of 285 and 149 sequences for *A. femoralis* were downloaded from GenBank for 16S and cyt*b*, respectively. The geographic location for each sample was recorded, with any sequences lacking this information excluded from the analysis. In addition, one sequence of each gene was downloaded for both *A. zaparo* and *A. nidicola*. These individuals were selected as outgroups for divergence analysis as they are the closest species for which divergence time information exists (Santos *et al.* 2009), allowing the root and first internal node of the species tree to be calibrated.

2.1.2 Divergence time estimation

Divergence times and tree topology were estimated using *BEAST v.2.2.1 (Heled & Drummond 2010; Bouckaert *et al.* 2014). As this method requires the species/population of interest to be specified *a priori*, further filtering was conducted on the sequences described above, with any areas represented by a single gene excluded from the analysis. There were, however, two exceptions to this rule. Samples from Vai-Quem-Quer (16S) and Serra Azul (cyt*b*) were combined into a single group due to their close geographical proximity (<20 km). A second group was also created for two separate sampling sites at Altamira. It was assumed that these groupings would not affect the outcome of the analysis, as samples were taken from the same riverbank, and sequences between combined sites were highly similar, with Geneious output revealing sequences were 99.0% identical. This resulted in the testing of a total of 164 individuals from 27 populations (see Supplementary material, Table S1), along with the two outgroups which were identified as separate species.

Alignments for both 16S and cyt*b* were constructed using the ClustalW and translation alignment algorithms, respectively, in Geneious version 8.1.6 (http://www.geneious.com: (Kearse *et al.* 2012)) before being checked and trimmed manually. Final alignments were 405 bp long for 16S and 153 bp long for cyt*b.*

These alignments were input directly into the *BEAST template. Nucleotide substitution models and partitions for each gene were inferred using Partition Finder (Lanfear *et al.* 2012), which suggested that three unlinked substitution models should be run. 16S was analysed as a single subset with the HKY+G substitution model selected, while cyt*b* was partitioned into two groups which were run as linked loci; the first subset consisting of codons 1 and 2, run with the HKY+G model, and the second subset consisting of codon 3 with the TrN substitution model chosen. As different individuals were used for both genes, the tree remained unlinked. A relaxed clock model (uncorrelated lognormal) (Drummond *et al.* 2006) was employed as trial runs revealed that evolutionary rates were not homogenous among branches, and the Yule speciation process applied as a tree prior. Population functions were marked as linear with a constant root, and both genes were marked as being mitochondrial. Priors had a normal distribution, with the root and first internal node calibrated based on mean divergence times and 95% confidence intervals provided by Santos *et al.* (2009). Three analyses of 400 million generations each were run, with samples logged every 5,000 steps. Posterior distributions for all model parameters

were checked using the diagnostic software, Tracer v1.6 (Rambaut *et al.* 2014). Log files for each run were combined using LogCombiner, with a burn-in of 10% and resampling frequency of 400,000. A single consensus tree was produced with this output using TreeAnnotator and FigTree (http://tree.bio.ed.ac.uk/software/figtree/).

The final topology and node divergence times of the consensus tree were then compared to those expected under the riverine barrier and Pleistocene refugia hypothesis to determine whether the resulting patterns correspond to either hypothesis.

2.2 Assessing patterns of mate choice in both captive and 'wild' populations

Tissue samples collected by staff at the Instituto Nacional de Pesquisas da Amazônia (INPA) were sent to Macquarie University, where genetic analyses were undertaken. The samples received were from two linked experiments; the first, a set of muscle tissue and tadpole fin clips from a captive run of assortative mating trials, and the second, toe clips from 'wild' individuals whose parents had escaped from these trials and were permitted to mate in a 'wild' situation on campus.

2.2.1 Experimental design

Individuals involved in the mating trials were sourced from two areas within the Amazon Basin, in which populations with different call types exist in sympatry. Yellow-thighed individuals with one and four note call types were sourced from Seringal do Condor and Eirunepé, respectively, while red-thighed individuals with six note and four note calls, were sourced from Altamira and another population within the same interfluve. In order to determine whether females were mating assortatively in respect to call type, colour was controlled in the captive trials. Each female was given a choice of two males; one from her own population, and the other from the neighbouring region. A full experimental run was completed for the yellow-thighed individuals, with some of the offspring escaping from their terraria. A complete run, however, was not possible for all red-thighed individuals, as most of the adults involved in this experimental round escaped. Escapees were permitted to mate in a 'wild' situation on campus. This allowed the mate preference of individuals to be assessed in the absence geographical barriers. Tissue samples were taken in order to identify whether hybridisation could be occurring between frogs originating from different populations.

A total of 94 tissue samples were received. This included muscle tissue from 7 females, 13 candidate males, and 47 tadpoles (three individuals from each clutch produced) involved in the captive mating trials, as well as 27 toe clips from 'wild' individuals.

2.2.2 Laboratory Procedures

Total genomic DNA was extracted from preserved toe clips using a modified salting-out protocol (Sunnucks & Hales 1996). For each sample, the mitochondrial 16S rRNA gene was targeted, as previous work on amphibians has demonstrated that is performs well at the intraspecific level, and can be used to differentiate between closely related species (Vences *et al.* 2005; Lougheed *et al.* 2006; Simões 2010). The primers 16Sar and 16Sbr (Palumbi 1996) were used to amplify a fragment of this gene through polymerase chain reactions (PCR). PCRs had a final volume of 10 µl and contained 1.0 µl of DNA, 4.3 µl ddH_2O, 0.8 µl $MgCl_2$, 1.6 µl dNTPs, 2.0 µl GoTaq Flexi Buffer (Promega), 0.1 µl of each primer and 0.1 µl *Taq* Polymerase (Promega). Thermocycling began with an initial denaturation for 3 min at 94°C, followed by five touchdown cycles with 94°C denaturation of 30 s, annealing temperatures (55°C, 53°C, 51°C, 49°C, 47°C) for 30 s, and 72°C extension for 30s. An additional 35 cycles were conducted with a denaturation step of 94°C, an annealing temperature of 50°C, and followed by a final extension step of 72°C for 10 min. PCR products were visualised on 1% agarose gel. PCR-products were purified using a Qiagen PCR clean up kit, before being sequenced with an automatic high throughput ABI3730XL sequencer (Applied Biosystems). All PCR purification and sequencing was performed by Macrogen (Korea).

In addition, all preserved tissue samples were sent to Diversity Arrays Technology Pty. Ltd. (Canberra, Australia) for SNPs discovery and genotyping, under the standard DartSeq™ protocol described in Momigliano *et al.* (in review). This genotype-by-sequencing approach combines DArT markers (Jaccoud *et al.* 2001; Luikart *et al.* 2003) with next-generation sequencing on Illumina platforms (Sansaloni *et al.* 2011), to identify thousands of SNPs spaced homogeneously throughout the genome (Petroli *et al.* 2012).

Under this protocol, DNA was extracted from preserved tissue samples using a GenCatch™ Blood and Tissue Genomic Mini Prep Kit (Epoch Biolabs), before being incubated in a 1x solution of Multi-Core™ restriction enzyme buffer (Promega). All samples were visualised on 0.8% agarose gel pre-stained with GelRed™ (Biotium) to ensure they had both a high molecular weight and were free from nucleases which could potentially interfere with subsequent digestions. A combination of the restriction enzymes *PstI* and

SphI was then used to digest approximately 100 ng of DNA for each sample. *PstI* and *SphI* compatible adapters, with an Illumina flow cell attachment sequence, sequencing primer and a unique barcode sequence, were then ligated to each sample.

All samples were purified using a spin-column Qiagen PCR clean up kit, before being amplified by primers specific to the adapter and barcode sequences. PCRs began with an initial denaturation of 1 min at 94°C, followed by 30 cycles with 94°C denaturation of 20 s, annealing temperature 58°C for 30 s and 72°C extension for 45 s, and were concluded with final extension of 72°C for 7 min. Equimolar quantities of each sample were then pooled, diluted and denatured with NaOH to prepare for hybridisation within the flow cell. An Illumina HiSeq2500 platform (single read) sequenced the library using 77 cycles, resulting in fragments 69 bp long. To ensure SNPs calls were reproducible, technical replicates were created by carrying 40% of samples through a second run of the library preparation protocol and downstream analyses.

Raw sequence data were converted to .fastq files using the Illumina HiSeq2500 software, with samples de-mulitplexed based on the individual-specific ligated barcodes. All reads then underwent an individual quality assessment, in which they were checked for bacterial and viral contaminants using both GenBank and the in-house DArT database (Diversity Arrays Technology Pty. Ltd., Canberra), and any with PHRED (Ewing *et al.* 1998) quality scores <25 were removed. The remaining reads were identified and called by Diversity Arrays Technology Pty. Ltd. following the standard procedure in DArTSoft14™. Monomorphic sequence clusters were removed, and only SNPs which occurred in both homozygous and heterozygous forms were called. This pipeline also removed loci with very high read depths, so that only SNPS with a high balance or read counts in allelic pairs, with a reproducibility of >95% and read depth >5 were retained.

2.2.3 Data analyses

2.2.3.1 Inferring parentage in the captive mating trials

Single Nucleotide Polymorphisms (SNPs) were filtered so that only those with a call rate of 100% were used in determining parentage. This resulted in a data set of 1260 SNPs. The software programmes COLONY2 (Jones & Wang 2010) and CERVUS v. 3.0 (Kalinowski *et al.* 2007) were both employed to determine parentage. For each programme, adult samples were separated by sex, and simulations assumed that 75% of parents had been sampled.

The parameters in COLONY2 were set to analyse an outbreeding population in which both male and female mating systems were polygamous, and parental and sibship relationships are unknown. A single thread of medium length was run on full-likelihood. Parentage was inferred from the best maximum-likelihood maternal and paternal assignment plots, produced by the programme.

In CERVUS, a simulation of 10,000 offspring with an error rate of 0.01 was conducted. The simulation was initially run on the complete set of 1260 loci. The data set, however, had to be cut down substantially due to a "floating point error". The simulation was subsequently run on the maximum number of loci before this occurred, a final set of 380 loci. Parentage was inferred based on LOD values, and the number of mismatches between the parent-offspring trio.

In clutches where parentage did not match between programmes, full SNP data (1260 loci) was checked by eye, and the exclusion method was used to determine the most likely parent.

2.2.3.2 Population analyses of 'wild' individuals

16S sequences were initially aligned using the ClustalW algorithm in MEGA v.6.0 (Tamura *et al.* 2013), before being checked by eye and corrected manually where necessary. Gaps and substitutions were verified by comparisons of original chromatographs, before trimming to provide a region 410 bp in length. A Maximum Likelihood (ML) tree was constructed using the MEGA software, with branch support for the final topology computed with 5000 bootstrap replicates. Haplotype and nucleotide diversity was assessed with DnaSP v.5.10 (Librado & Rozas 2009).

The complete SNP dataset was filtered so only those from wild individuals with a call rate > 90% were used for analysis. This resulted in a set of 8067 SNPs. Discriminant analysis of principle components (DAPC) was used to define the number of genetically distinct groups based on this SNP dataset. This analysis was conducted using the R package adegenet (Jombart 2008), in R 3.2.0 (R development core team, www.r-project.org). As population information could not be specified *a priori*, *K* was selected using the find.clusters function and Bayesian Information Criterion (BIC).

STRUCTURE v.2.3.4 (Pritchard *et al.* 2000) was used to further assess population structuring and identify admixture. It was assumed that the number of possible genetic

clusters (*K*) formed by these samples was between 1 and 6 (twice the number of clusters suggested in our DAPC analysis). 10 iterations were undertaken for each run with 100,000 MCMC replicates, after 10,000 initial replicates were discarded as burn-in. The admixture model with independent allele frequencies was employed, and no prior populations were specified. The number of genetic clusters was determined with the Evanno method (Evanno *et al.* 2005) which was calculated by the online programme, Structure Harvester (Earl & Vonholdt 2012). Results allowed us to confirm the number of clusters suggested by the DAPC analysis described above, determine whether hybridisation occurred between populations, and identify admixed individuals.

3. Results

3.1 Intraspecific divergence

The Bayesian evolutionary analysis indicated that *A. femoralis* began to diverge approximately 3.75 million years ago, with all clades in place 0.4 million years ago (Figure 1). All clades were established prior to the last glacial maximum, with previous glaciations occurring throughout the Pleistocene seen to have little to no effect on the divergence of this species. In contrast, the genetic diversity of *A. femoralis* appears to be strongly related to river positions (Figure 2, Table 1), although in varying degrees. The Amazon River does not appear to have acted as a barrier to dispersal, with two clades represented by populations which occur on both the northern and southern sides of this river. The Madeira River, in comparison, appears to have acted as a barrier to dispersal at two different times. Populations inhabiting the northern and southern banks of the Madeira in the Pará region split 2.23 MYA, while populations inhabiting the upper regions split 0.4 MYA. The Madeira has not acted as a barrier to gene flow at Humaitá.

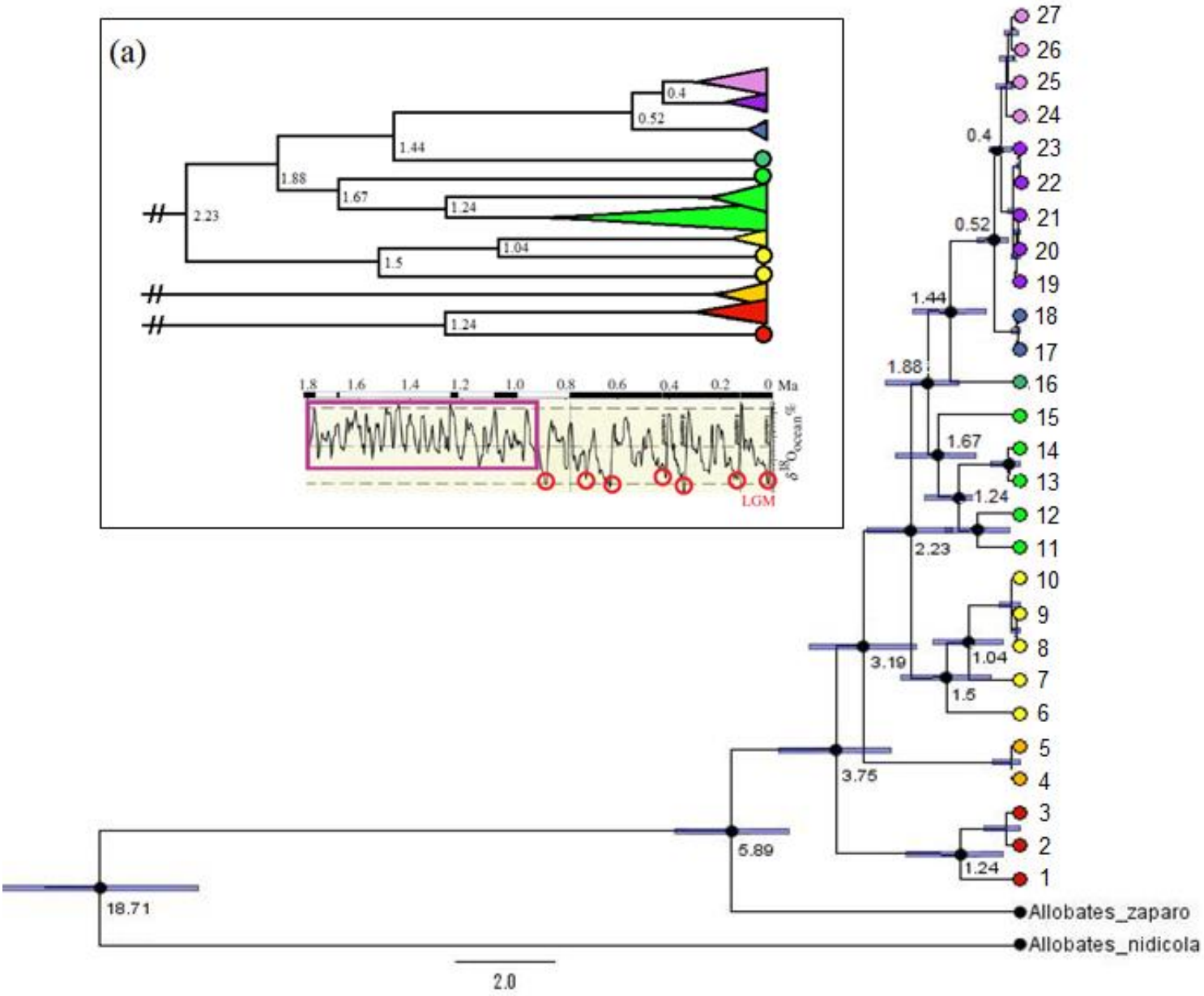

Figure 1. Time-calibrated phylogeny for 27 populations of Allobates femoralis, (a) plotted against the climate curve sourced from Ribas et al. (2011), to provide a phylogenetic test of the refuge hypothesis. Mean age estimates and 95% confidence intervals were based on fragments of the two mitochondrial genes, 16S rRNA and cytochrome b, and were obtained via *BEAST. Terminals are coloured in respect to clade. Populations are numbered according to Table 1.

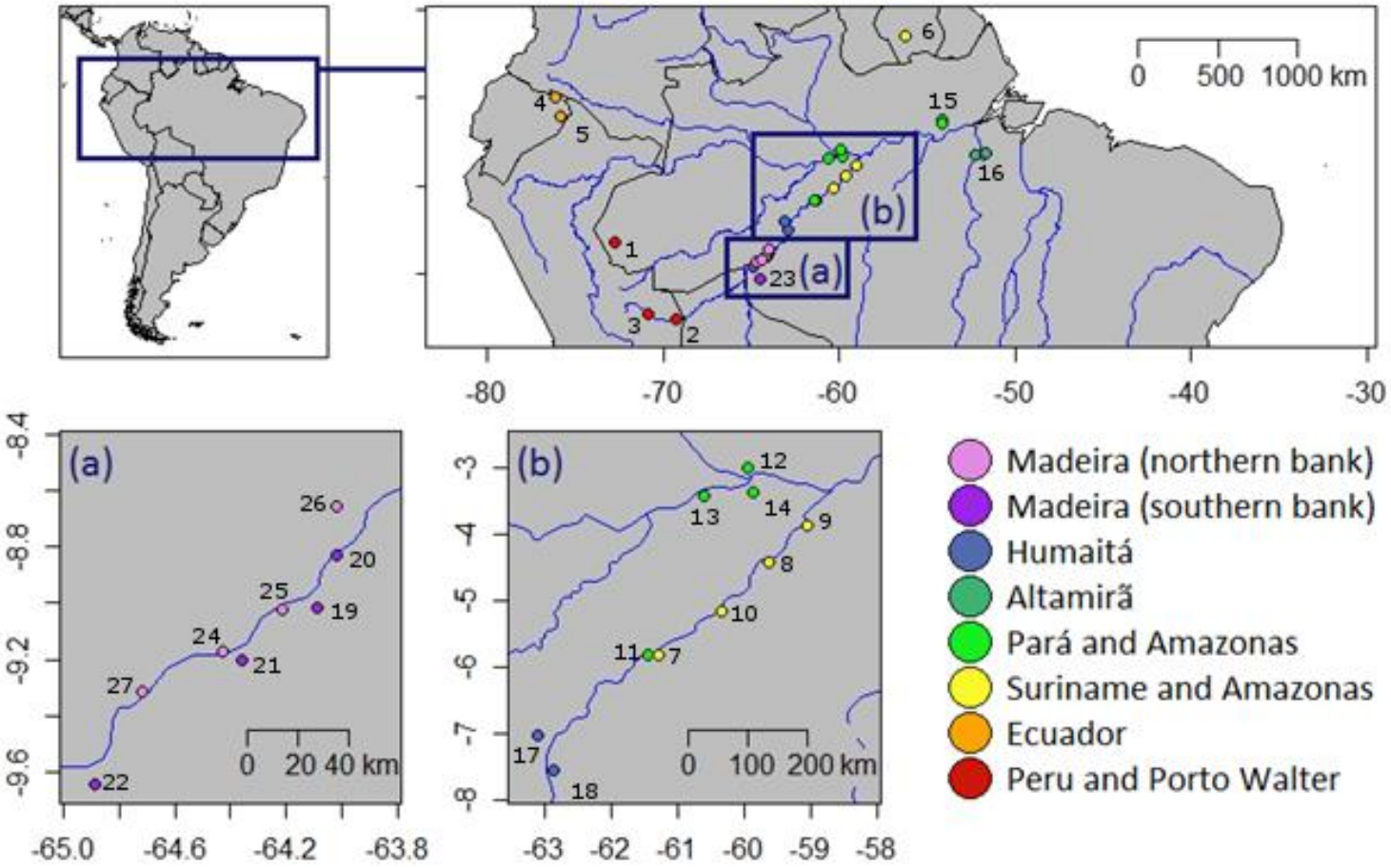

Figure 2. Geographic location of the 27 *Allobates femoralis* populations used in our intraspecific divergence analyses. Populations have been colour coded in respect to their resulting clade (see Figure 1) to illustrate the connection between major rivers and patterns of genetic diversity. Larger scale maps provide a closer look at the spread of genetic diversity at the (a) upper and (b) lower reaches of the Madeira River. Sample locations are numbered according to Table 1.

Table 1. Populations of *Allobates femoralis* used for intraspecific divergence analyses. The respective coordinates of each population, and sample sizes for each mitochondrial gene are provided.

Locality	**Coordinates**	**16S**	**cyt*b***
1. Porto Walter	8.25° S, 72.76666° W	5	3
2. Madre de Dios	12.5914° S, 69.3258° W	4	4
3. Boca Manu	12.25° S, 70.9° W	1	1
4. Northern Ecuador	0.0° S, 76.16666° W	3	3
5. Southern Ecuador	1.1007° S, 75.8069° W	1	1
6. Suriname	3.5° N, 56.2799° W	1	1
7. Manicoré	5.8231° S, 61.2986° W	16	5
8. Borba	4.4342° S, 59.6236° W	5	5
9. Nova Olinda de Norte	3.8744° S, 59.0461° W	5	1
10. Novo Aripuanã	5.1503° S, 60.3467° W	8	6
11. Democracia	5.8058° S, 61.4453° W	7	7
12. Manaus	3.0079° S, 59.9393° W	1	1
13. Manaquiri	3.4272° S, 60.6150° W	6	6
14. Careiro	3.3708° S, 59.8683° W	7	7
15. Vai-Quem-Quer/Serra Azul	1.2822° S, 54.1297° W / 1.4395° S, 54.1531° W	8	3
16. Altamirã	3.2432° S, 52.2440° W / 3.1275° S, 51.7097° W	5	5
17. Humaitá (left bank)	7.0228° S, 63.1028° W	6	6
18. Humaitá (right bank)	7.5488° S, 62.8772° W	4	4
19. Morrinho (right bank)	9.0199° S, 64.2172° W	5	5
20. Santo Antônio (right bank)	8.6550° S, 64.0195° W	8	8
21. Jaci-Paraná (right bank)	9.2045° S, 64.3620° W	8	8
22. Mutum-Paraná (right bank)	9.6414° S, 64.8859° W	7	4
23. Guajará-Mirim	10.31666° S, 64.55° W	3	2
24. Jaci-Paraná (left bank)	9.1694° S, 64.4289° W	11	7
25. Morrinho (left bank)	9.0199° S, 64.2172° W	7	7
26. Santo Antônio (left bank)	8.6550° S, 64.0195° W	4	4
27. Lower Jirau	9.3114° S, 64.7172° W	9	8
	Total	155	122

3.2 Captive mating trials

Genotypes were successfully reported for 92 individuals, with SNPs recorded for all but two tadpoles. Parentage analyses revealed that offspring were produced in all trials, with each female producing up to four clutches. Mating strategies were found to vary between the two colour groups. Yellow-thighed females were found to mate with males from both the one and four-note populations. A total of 11 clutches were produced within this experimental group. Five clutches (45%) were produced assortatively in respect to call type, with the remaining six (55%) produced under a disassortative mating pattern (Table 2, 3). In contrast, red-thighed females were found to mate purely disassortatively, with all clutches fathered by males from the four-note population. Although some movement was detected between terraria (see Supplementary material, Table S2), this did not alter the final results of the trials.

The two approaches used to infer parentage (COLONY and CERVUS) identified the same mother and father in all but two clutches, in which different mothers were recognised by the software programmes (Table S2). Consequently, the data from these two trials was disregarded.

Table 2. Results of the *Allobates femoralis* captive mating trials. Cells indicate the cage reference ID, number of clutches produced, call type for the mother and father of each clutch, and state whether clutches were produced assortatively with respect to call repertoire.

Experiment	Clutch	Call type of mother	Call type of father	Assortative mating
1	1	1	4	No
	2	1	1	Yes
	3	1	1	Yes
2	1	1	1	Yes
3	1	1	1	Yes
	2	1	4	No
4	1	1	1	Yes
	2	1	4	No
5	1	1	4	No
	2	1	4	No
	3	1	4	No
6	1	6	4	No
	2	6	4	No
9	1	6	4	No
	2	6	4	No

Table 3. The number and percentage of clutches produced both assortatively and disassortatively in the *Allobates femoralis* mating trials. Results are provided for the two experimental groups tested.

Tested call types	Total clutches produced	Assortative mating	Disassortative mating	Percent Assortative	Percent Disassortative
1 and 4 note	11	5	6	45.5	54.5
4 and 6 note	6	0	6	0.0	100.0

3.3 'Wild' population structure

3.3.1 Mitochondrial sequence data

The 16S rRNA gene region was successfully amplified for 25 individuals. Sequences were found to contain 9 variable sites, all of which were parsimony informative. Two unique haplotypes were detected (Table 4). These largely mirrored the original combinations examined in the captive trials. One haplotype was found to consist purely of yellow-thighed individuals with one and four note calls, while the other haplotype comprised of red-thighed individuals with four or six note calls, along with two individuals, one with a previously untested combination: yellow-thighs with a six note call (Figure 3).

3.3.2 SNP loci

DAPC yielded three clusters (Figure 4a), which were shown to be genetically distinct. Each cluster was grouped based on thigh colouration, with red and yellow-thighed individuals comprising two exclusive groups at either end of the range (Table 5). The intermediate cluster was found to comprise of four individuals, two of each colour.
STRUCTURE analysis revealed a best-fit of $K = 2$ (Figure 4b). Clusters were again partitioned strongly by colouration, with box plots revealing that the middle cluster defined in the DAPC consisted of four admixed individuals (Figure 4c). Each individual had a 50% probability of assignment for each of the two clusters.

Table 4. The two unique haplotypes detected in 'wild' samples of *A. femoralis.* All nine variable sites are listed. Letters are used to indicate nucleic acid, while the numbers provided indicate the site (in base pairs) at which each variation occurs.

Site:	150	152	192	203	204	222	238	275	335
Haplotype 1	G	C	A	T	T	T	T	A	C
Haplotype 2	A	T	G	A	C	C	C	G	T

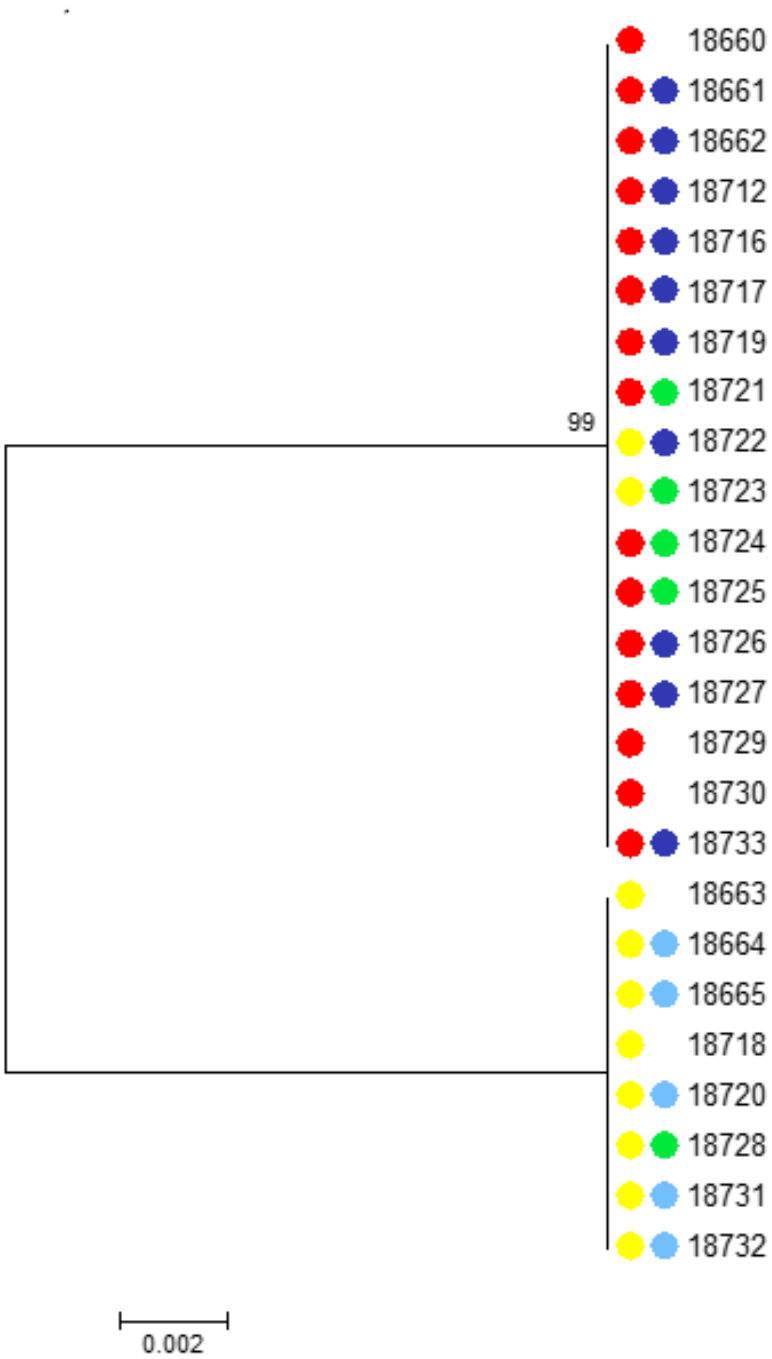

Figure 3. Maximum Likelihood (ML) tree constructed for 'wild' *Allobates femoralis* captured on campus at INPA, based on fragments of the 16S rRNA gene region. Support values correspond to ML bootstrap probability. Red and yellow coloured terminals correspond to the thigh-colour of each individual. The light blue, dark blue and green circles represent 1, 4 and 6 note call types respectively.

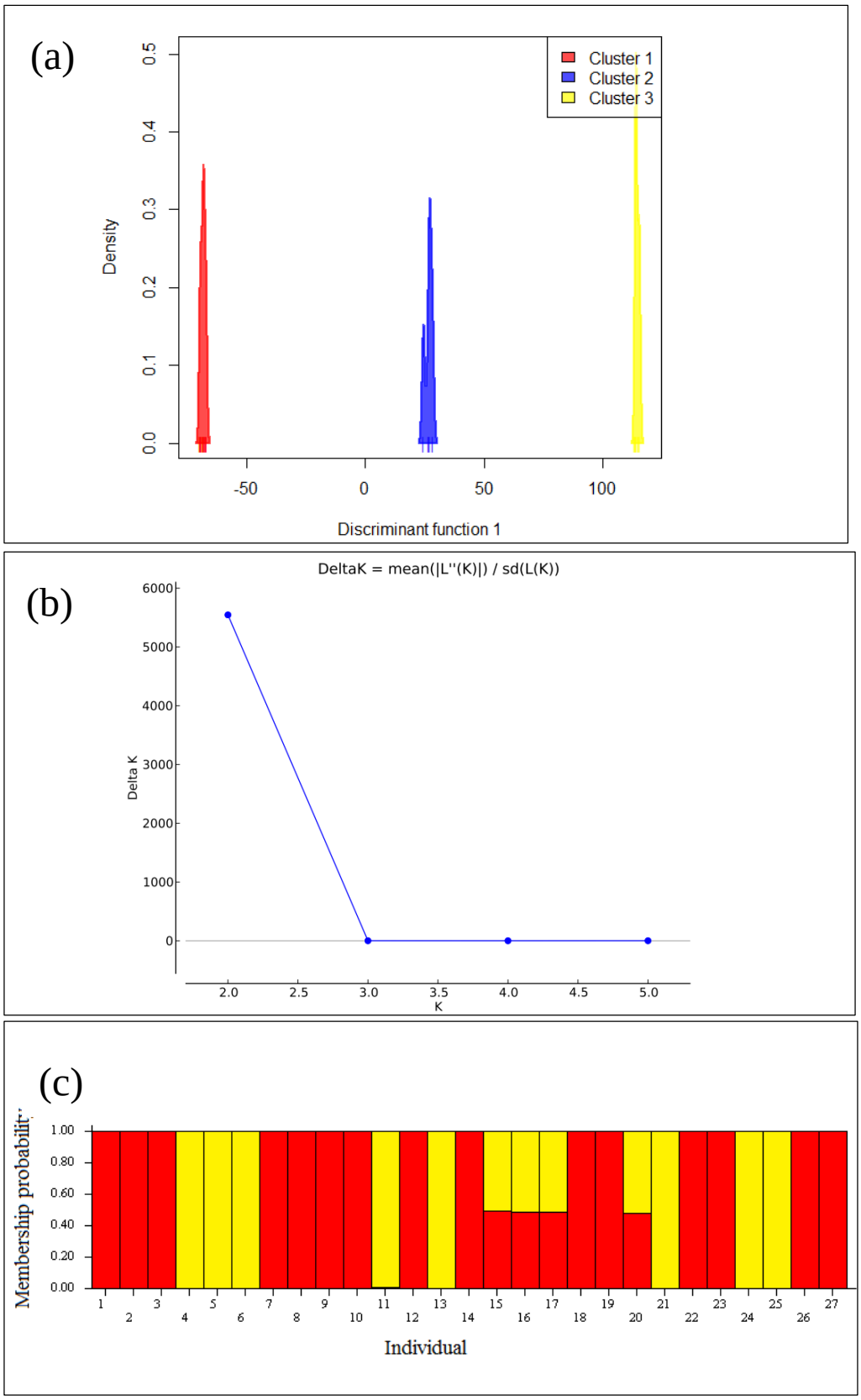

Figure 4. DAPC and STRUCTURE analyses of 'wild' *Allobates femoralis* captured on campus at INPA. The colours used for each cluster and population assignment, corresponds to thigh-colouration. (a) DAPC histogram showing the potential number of genetic clusters (K), as K = 3. (b) ΔK values for the number of clusters examined, showing a best-fit value of K = 2. (c) STRUCTURE bar plot showing population assignments of each individual. Individual ID and phenotypes are provided in Table 5.

Table 5. Summary of the clusters identified through DAPC. The automatic number assigned by adegenet is matched with the ID and phenotype of each individual. These results were mirrored in STRUCTURE, with Cluster 2 comprising of admixed individuals.

Cluster	Assigned number	Individual ID	Colour	Call
1	1	18660	Red	*
	2	18661	Red	4
	3	18662	Red	4
	7	18712	Red	4
	8	18713	Red	*
	9	18716	Red	4
	10	18717	Red	4
	12	18719	Red	4
	14	18721	Red	6
	18	18725	Red	6
	19	18726	Red	4
	22	18729	Red	*
	23	18730	Red	*
	26	18733	Red	4
	27	18734	Red	4
2	15	18722	Yellow	4
	16	18723	Yellow	6
	17	18724	Red	6
	20	18727	Red	4
3	4	18663	Yellow	*
	5	18664	Yellow	1
	6	18665	Yellow	1
	11	18718	Yellow	*
	13	18720	Yellow	1
	21	18728	Yellow	6
	24	18731	Yellow	1
	25	18732	Yellow	1

4. Discussion

4.1 Processes that generate genetic diversity in Allobates femoralis

Our time-calibrated phylogeny suggests that diversification of *A. femoralis* occurred between 3.75 and 0.4 million years ago (Figure 1), and shows that patterns of intraspecific divergence are strongly associated with river locations (Figure 2). As a result, we believe that riverine barriers have played a significant role in promoting genetic diversity within this species.

The divergence times suggested for lineages of *A. femoralis* are consistent with those proposed for the Plio-Pleistocene establishment of the Amazonian drainage system (Rossetti *et al.* 2005; Latrubesse *et al.* 2010). As in Ribas *et al.* (2011), our data indicate a separation between clades inhabiting the northern and southern banks of the Amazon River between 2.7 and 2.0 million years ago, and the Madeira approximately 2.0 million years ago. The formation of the Tapajós, approximately 1.3 million years ago, can also be used to explain divergence between populations inhabiting Altamira and Humaitá.

While these divergence dates roughly correspond with those expected for the formation of the Amazonian river system, not all of our findings are consistent with those expected under the riverine barrier hypothesis. In particular, the assumptions that major rivers act as strong vicariant barriers, and that genetic differentiation is more pronounced between populations inhabiting opposite riverbanks than the same one (Gascon *et al.* 1998), are violated. Our results show several instances in which populations belonging to the same clade are found on either side of a major river. This shows that these river channels have not permanently prevented the dispersal of individuals between riverbanks. This pattern is particularly pronounced in populations inhabiting Amazonas and Humaitá (Figures 1, 2). In addition, the most recent divergence date deviates from that expected under the Plio-Pleistocene model of river formation. Although the genetic partitioning between clades inhabiting the left and right banks of the mid-Madeira is highly pronounced, the date estimated for the separation of these lineages is approximately 0.5 million years less than what is expected under this hypothesis. While this date violates the assumption that riverine barriers have acted as vicariant barriers since their establishment, we believe that these patterns are likely to be caused by dispersal events which are linked to river formation and dynamics.

Variation in water flow and sediment deposition have been proposed to lead to channel reorientation, which may have facilitated divergence across river channels at several locations (Latrubesse 2003; Wilkinson *et al.* 2010). Regular shifts in course have been suggested to occur throughout the upper to middle reaches of the Madeira River (Simões 2010) as well as throughout the mid-section of the Amazon (Costa *et al.* 2001). These channel reorientation events can result in the passive 'transport' of individuals across what seems to be major rivers (Noonan & Wray 2006; Haffer 2008). This could explain the patterns seen in the mid-Amazon, particularly at Manaus and Democracia, as well as the later dispersal event from the right to left bank of the mid-Madeira. Interestingly, similar patterns in which the lower reaches of the Madeira were a more effective barrier to gene flow than the mid-Amazon, have been documented in several other *Allobates* species, including *A. parleovarzensis*, *A. nidicola* and *A. masniger* (Kaefer *et al.* 2013).

The glacial cycles of the Pleistocene had a substantial effect on Amazonian climate and vegetation, and are proposed to have driven the formation of refugia through this region (Mayle *et al.* 2004; Bonaccorso *et al.* 2006). In particular, the profound changes which occurred during the extremity of the last glacial maximum are proposed to have been responsible for driving the divergence seen in modern communities (Bush 1994). Despite the pronounced environmental effects associated with these glacial cycles, our data suggest that Pleistocene glaciation and refugia did not promote intraspecific divergence in *A. femoralis*.

All clades of *A. femoralis* were established prior to the last glacial maximum, with no lineages undergoing further divergence after this event (Figure 1a). Further analysis revealed that the majority of *A. femoralis* clades were established before the onset of all Pleistocene glacial cycles, with subsequent diversification events found to be unrelated to the timing of subsequent glacial maxima. The three clades which diversified most recently (Humaitá, Madeira left bank and Madeira right bank), existed through four of the seven major glacial maxima. However, as divergence time estimates for these nodes do not correspond with those of the glacial maxima, it is assumed that Pleistocene refugia did not influence intraspecific divergence in these groups. Therefore, we can assume that if the glacial cycles of Pleistocene influenced forest distribution throughout Amazonia, the disturbance experienced was insufficient to have disrupted gene flow between clades of *A. femoralis*. Consequently, no distinct genetic patterns linked to these glacial events and refugia have emerged. Although major climatic fluctuations have occurred prior to the Pleistocene, it has been suggested that these have had little effect on the creation and

maintenance of refugia due to reduced climatic responses and a warmer global climate (Ravelo *et al.* 2004). Consequently, climatic changes which predate the Pleistocene have not been considered.

Our results show that the timing of intraspecific diversification within *A. femoralis* is inconsistent with the refuge hypothesis. With the majority of divergence dates largely predating the glacial cycles of the Pleistocene, it is apparent that climatic cycles had little to no effect in generating genetic divergence within this species. As major features of these rivers are believed to have been well established before the climatic fluctuations of the Pleistocene occurred (Haffer 1969; Ribas *et al.* 2011), it is more likely that riverine barriers have triggered diversification.

Although our data do not fit all the stringent predictions of the riverine barrier hypothesis (Gascon *et al.* 1998), it is clear that patterns of intraspecific divergence are strongly associated with river locations (Figure 2). As the majority of divergence times correspond to those expected under the Plio-Pleistocene model of river formation, and discrepancies explained by divergence events due to channel reorientation, we conclude that this hypothesis is best suited to explain intraspecific divergence in *A. femoralis*. This conclusion adds to the growing body of literature which supports the Madeira and Amazon Rivers as effective barriers which generate both phenotypic and genetic diversity in *A. femoralis* (Simões *et al.* 2008; Simões *et al.* 2014) as well as a number of other species (Capparella 1988; Funk *et al.* 2007; Ribas *et al.* 2011; Fouquet *et al.* 2012; Kaefer *et al.* 2013).

Our appraisal, however, should be interpreted carefully. Amazonian diversification is a complex process and it is important to recognise that while a single factor may explain a lot of this divergence, it is likely that many others have also influenced this (Bush 1994). Further analyses should consider coupling mtDNA with nuclear markers in order for chronological events in the history of a population to be assessed (Zhang & Hewitt 2003). This will provide further insight into the origins of Amazonian biota and the extent of genetic diversity within this region.

4.2 Processes that maintain genetic diversity

In this study, we investigate mate choice in both captive and wild situations. This is considered to be beneficial because females are generally found to be choosier under laboratory conditions, whereas in wild situations, they have been found to mate with the

closest signalling male (Richards-Zawacki *et al.* 2012), likely due to higher energy costs and decreased chance of locating the appropriate mate (Jennions & Petrie 1997; Thibert-Plante & Gavrilets 2013). It provides us with a broader understanding of patterns of mate choice, and in doing so, allows investigation of the processes which maintain genetic diversity. We found little evidence to suggest that assortative mating in *A. femoralis* occurs on the basis of call type, with genetic data from both the captive mating trials and wild caught individuals suggesting that mating occurs randomly with respect to this trait (Table 2, 3).

The design of the captive mating trials allowed mate preferences to be explicitly assessed in relation to call type. These revealed contrasting patterns of mate choice between the two experimental groups. Patterns of paternity revealed that yellow-thighed females were not particularly selective of their mates in respect to call type, with 55% of clutches produced found to have been fathered by males with a call type different to their own. All clutches produced by red-thighed females, in comparison, were found to only have been fathered by males with the opposite call type, revealing a disassortative mating pattern. However, because the majority of adult individuals involved in this experimental group escaped, our summary is based on the reproductive output of two males and two females. Due to the resulting small sample size, definitive conclusions cannot be drawn from the breeding trials of the red-thighed individuals.

Although data were not supplemented by visual records of pairings between individuals, we believe the exclusive use of genetic techniques provides an adequate representation of parentage within these trials, and the processes that maintain genetic diversity within populations (Hauser *et al.* 2011). As no obvious genetic structuring exists between individuals with different call types (Figure 3), we can predict that disassortative mating in respect to call type is unlikely to lead to outbreeding depression. Therefore, all matings are expected to have resulted in the successful production of offspring.

While mate preferences were assessed directly in the captive mating trials, they had to be inferred on the basis of phenotypic and genetic data for wild individuals. If assortative mating occurs in respect to call type in this system, we can expect the call types of the females tested to be predominant among their offspring (Wade *et al.* 1994; Shackleton *et al.* 2005). We found call types were distributed approximately evenly between frogs of different colour, with wild individuals mirroring phenotypes of the original captive trials.

These findings further suggest that no directional mating patterns occur with respect to call type.

Our finding that assortative mating does not occur in respect to call type is surprising, considering the assumption that it is the primary trait on which mate choice is based in anurans (Waldman & Bishop 2004; Wells & Schwartz 2006). There is a wealth of literature highlighting that females are highly discriminative of mates based on advertisement calls, particularly in areas characterised with marked character displacement (Littlejohn 1965; Littlejohn & Loftus-Hills 1968; Fouquette 1975; Gerhardt & Doherty 1988). However, the differentiation in the acoustic traits of *A. femoralis* is proposed to have been driven by genetic drift rather than strong selective pressures (Amézquita *et al.* 2009). This study and our research indicate that call type may be of less significance in mate selection in *A. femoralis* than previously believed.

Call types may not act as effective barriers to reproduction in *A. femoralis* for several reasons. It is possible that mate selection is based on a number of cues, rather than call type alone. To increase the likelihood that signals are received and interpreted correctly, many species produce multiple cues which can influence mating decisions. Typically, the number of cues exhibited increases in environments in which signals may be masked by biotic or abiotic factors (Brizzi and Corti, 2007). As a result, the explicit analysis of advertisement calls may provide us with an incomplete understanding of this study system.

Neotropical poison frogs have been documented to demonstrate diverse and often multisensory signals (Summers et al., 1999, Wells, 2007), and *A. femoralis* is no exception. As well as the use of both advertisement and courtship calls (Montanarin *et al.* 2011), visual displays, including leg-stretching, throat displays and limb lifting, have been documented to occur in some populations of this species. As this species is diurnal, it is possible that species recognition through visual means may play a more important role in mate selection than call type.

A number of factors are suggested to be involved in mate selection (Brizzi & Corti 2007; Coleman 2009). While we have attempted to control as many factors as possible so that only differences in call type were assessed, there may have been factors out of our control. This may have influenced the resulting mating patterns. These factors could potentially include visual displays (Wells 2007; Montanarin *et al.* 2011), chemosensory cues (Belanger & Corkum 2009), male-male agonistic behaviour (Narins *et al.* 2003;

Narins *et al.* 2005; Erdtmann *et al*. 2011) and male vigour. Further studies are required to evaluate whether mate choice occurs with respect to call type within this species.

The role visual communication plays in mate choice has received increasing attention in recent years. As the largest range of visual signals are believed to occur in the dendrobatoids (De Luna *et al.* 2010), these organisms have been thoroughly investigated (Summers *et al.* 1999; Reynolds & Fitzpatrick 2007; De Luna *et al.* 2010). Studies have revealed that colouration influences mating decisions in the strawberry poison frog, *Oophaga pumilio* (Summers *et al.* 1999; Reynolds & Fitzpatrick 2007; Richards-Zawacki & Cummings 2011; Richards-Zawacki *et al.* 2012), with females preferring to mate with morphs of the same colour (Summers *et al.* 1999) and of similar melanistic spotting patterns (Reynolds & Fitzpatrick 2007). This mating preference is demonstrated to be stronger in regions where polymorphic populations occur (Richards-Zawacki & Cummings 2011). However, no-choice experiments reveal that hybrids can be produced between lineages, and that no obvious detrimental effects were found to reduce fitness in admixed individuals (Dugas & Richards-Zawacki 2015)).

As colour was controlled between treatments in captive trials, we cannot assess the effect of thigh colouration on mate choice in these individuals. However, in the wild caught individuals, we could evaluate whether this trait had an effect on mate choice. Both our mitochondrial and SNP data revealed the extent of genetic partitioning between lineages based on colouration, showing that it was strong and significant between colour morphs (Figure 3, 4). Genetic groups were found to be entirely distinct, with no overlap occurring between these groups.

The pattern demonstrated, particularly with the population assignments of SNP data, suggests that assortative mating occurs on the basis of colour. This finding is, again, surprising as the colouration pattern of *A. femoralis* has been suggested to play no particular role in intraspecific recognition (Amézquita *et al.* 2009). Instead the bright colour patches on the thighs of this species have been proposed to play a more important role in heterospecific communication, by advertising toxicity to potential predators (Darst *et al.* 2006). However, as *A. femoralis* has been documented to engage in leg-stretching displays during mating interactions (Montanarin *et al.* 2011), behavioural observations suggest that thigh-colouration may play a more important role in mate recognition and mate choice than previously believed.

High variation in thigh colour has been previously documented in *A. femoralis* (Simões *et al.* 2008; Simões *et al.* 2010; Simões *et al.* 2014). However as differences in this trait are uncorrelated with both the geographic and genetic distance of populations, local selective forces were proposed to have driven this divergence (Amézquita *et al.* 2009).

Given the extent of genetic partitioning between colour morphs, the mating pattern displayed here is understandable. We should expect females to be mating with males with similar characteristics so that the negative effects associated with outbreeding are avoided (Frankham *et al.* 2010). Although this genetic partitioning appears to be strongly based on colour, this mating pattern could be a result of genetic distance between populations, as frogs captured for the captive mating trials were sourced from two provinces located approximately 2,000 km apart. Further studies should address whether female *A. femoralis* will select mates of different colour from populations that are geographically closer, which are therefore of similar genetic composition. This will allow us to determine whether it is colour or geographic distance which influences this mating pattern.

Although assortative mating occurs on the basis of colouration, the presence of admixed individuals reveals that this mating pattern is not strict. Of the 27 wild individuals sampled, 4 were found to be hybrids. The position of the cluster in the DAPC, as well as the 50% probability of assignment to either the red or yellow-thighed populations (Figure 4c), indicates that these individuals are the first generation of admixed individuals produced as part of these mate choice experiments.

Theory suggests that admixed individuals should not be produced if lineages are genetically distinct. The negative effects associated with outbreeding depression should reduce fitness in these circumstances, which may result in admixed individuals being either sterile, ecologically unfit or unattractive (Griffith & Immler 2009). Previous hybridisation events, however, have been documented in *A. femoralis* at the core of contact zone with a closely related sister species, *A. hodli* (Simões *et al.* 2012). As successful matings have been documented to occur between these species, it has been suggested that pre-zygotic barriers are ‘leaky’ and that post-zygotic barriers may be more likely to influence the production of hybrid offspring (Simões *et al.* 2012). This raises a number of questions regarding hybrid survival and viability between lineages of *A. femoralis*, particularly whether postzygotic barriers may be responsible for maintaining genetic diversity between lineages.

It is important to stress that all wild individuals collected were adults and that this sampling pattern may have led to a biased results. Consequently, we cannot guarantee that a full complement of hybrids were represented within this study. Perhaps low survival rates of admixed individuals may have influenced the number of hybrids collected. Further studies are required to assess the mating success of admixed individuals. This will provide a broader understanding of whether matings between lineages are less productive than those within lineages, and whether hybrid offspring are sterile, less productive or suffer developmental problems.

Our assortative mating experiments provide a new and interesting insight to the mating system of *A. femoralis*. The finding that mate choice occurs on the basis of thigh-colouration rather than call type completely remodels our expectations of the traits on which mate choice in anurans occurs. In addition, the discovery of hybrid individuals between genetically distinct lineages highlights that we do not completely understand the processes at work within this system. Further analyses of the *A. femoralis* mating system are required to gain a broader insight of anuran mating systems. These future studies will provide a better understanding the processes underlying mate choice, and its role in maintaining the genetic diversity of populations.

5. References

Amézquita A, Flechas SV, Lima AP, Gasser H, Hödl W (2011) Acoustic interference and recognition space within a complex assemblage of dendrobatid frogs. *Proceedings of the National Academy of Sciences*, **108**, 17058-17063.

Amézquita A, Lima AP, Jehle R, Castellanos L, Ramos Ó, Crawford AJ, Gasser H, Hödl (2009) Calls, colours, shape, and genes: a multi-trait approach to the study of geographic variation in the Amazonian frog *Allobates femoralis*. *Biological Journal of the Linnean Society*, **98**, 826-838.

Ayres JM, Clutton-Brock TH (1992) River Boundaries and Species Range Size in Amazonian Primates. *The American Naturalist*, **140**, 531-537.

Beebee TJC (1996) *Ecology and conservation of amphibians*. Chapman & Hall, London.

Beheregaray LB, Cooke GM, Chao NL, Landguth EL (2014) Ecological speciation in the tropics: insights from comparative genetic studies in Amazonia. *Frontiers in Genetics*, **5**, 477.

Belanger RM, Corkum LD (2009) Review of Aquatic Sex Pheromones and Chemical Communication in Anurans. *Journal of Herpetology*, **43**, 184-191.

Blaustein AR, Wake DB, Sousa WP (1994) Amphibian Declines: Judging Stability, Persistence, and Susceptibility of Populations to Local and Global Extinctions. *Conservation Biology*, **8**, 60-71.

Bonaccorso E, Koch I, Peterson AT (2006) Pleistocene fragmentation of Amazon species' ranges. *Diversity and Distributions*, **12**, 157-164.

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A, Drummond AJ (2014) BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. *PLoS Comput Biol*, **10**, e1003537.

Boulenger GA (1884) On a collection of frogs from Yurimaguas, Huallaga River, Northern Peru. *Proceedings of the Zoological Society of London*, **1883**.

Brizzi R, Corti C (2007) Cutaneous antipredatory secretions and pheromones in anurans and urodeles. *Marine and freshwater behaviour and physiology*, **40**, 225-231.

Brown KSJ (1982) Paleoecological and regional patterns of evolution in neotropical forest butterflies. In: *Biological Diversification in the Tropics* (ed Prace GT), pp. 25 - 308. Columbia University Press, New York.

Bush MB (1994) Amazonian Speciation: A Necessarily Complex Model. *Journal of Biogeography*, **21**, 5-17.

Capparella A (1988) Genetic variation in Neotropical birds: implications for the speciation process. In: *Congress International Ornithology*, pp. 1658 - 1664.

Clough M, Summer K (2000) Phylogenetic systematic and the biogeography of poison frogs: Evidence from mitochondrial DNA sequences. *Biological Journal of the Linnean Society*, **19**, 11-15.

Coleman SW (2009) Taxonomic and sensory biases in the mate-choice literature: there are far too few studies of chemical and multimodal communication. *Acta Ethologica*, **12**, 45-48.

Colwell RK (2000) A barrier runs through it … or maybe just a river. *Proceedings of the National Academy of Sciences*, **97**, 13470-13472.

Costa JBS, Léa Bemerguy R, Hasui Y, da Silva Borges Mc (2001) Tectonics and paleogeography along the Amazon river. *Journal of South American Earth Sciences*, **14**, 335-347.

Da Silva JMC, Rylands AB, Da Fonseca GAB (2005) The Fate of the Amazonian Areas of Endemism. *Conservation Biology*, **19**, 689-694.

Darst CR, Cummings ME, Cannatella DC (2006) A mechanism for diversity in warning signals: conspicuousness versus toxicity in poison frogs. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 5852-5857.

de Luna AG, Hödl W, Amézquita A (2010) Colour, size and movement as visual subcomponents in multimodal communication by the frog Allobates femoralis. *Animal Behaviour*, **79**, 739-745.

Dias-Terceiro RG, Kaefer IL, de Fraga R, de Araújo MC, Simões PI, Lima AP (2015) A Matter of Scale: Historical and Environmental Factors Structure Anuran Assemblages from the Upper Madeira River, Amazonia. *Biotropica*, 1–8.

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A (2006) Relaxed Phylogenetics and Dating with Confidence. *PLoS Biol*, **4**, e88.

Duellman WE (1999) *Patterns od distribution of Amphibians: a global perspective*. John Hopkins University Press, Baltimore.

Duellman WE (1982) Quaternary climatic-ecological fluctuations in the lowland tropics: frogs and forest. In: *Biological diversification in the tropics* (ed Prance GT), pp. 641 - 657. Columbia University Press, New York.

Dugas MB, Richards-Zawacki CL (2015) A captive breeding experiment reveals no evidence of reproductive isolation among lineages of a polytypic poison frog. *Biological Journal of the Linnean Society*.

Earl D, vonHoldt B (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359-361.

Eizaguirre C, Baltazar-Soares M (2014) Evolutionary conservation—evaluating the adaptive potential of species. *Evolutionary Applications*, **7**, 963-967.

Erdtmann LK, Simões PI, Mello AC, Lima AP (2011) Do natural differences in acoustic signals really interfere in conspecific recognition in the pan-Amazonian frog *Allobates femoralis*? *Behaviour*, **148**, 485-500.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology*, **14**, 2611-2620.

Ewing B, Green P (1998) Base-calling of automated sequencer traces using phred. II. Error probabilities. *Genome Research*, **8**, 186-194.

Fouquet A, Noonan BP, Rodrigues MT, Pech N, Gilles A, Gemmell NJ (2012) Multiple Quaternary Refugia in the Eastern Guiana Shield Revealed by Comparative Phylogeography of 12 Frog Species. *Systematic Biology*, **61**, 461-489.

Fouquette MJ, Jr. (1975) Speciation in Chorus Frogs. I. Reproductive Character Displacement in the Pseudacris nigrita Complex. *Systematic Zoology*, **24**, 16-23.

Frankham R (2010) Challenges and opportunities of genetic approaches to biological conservation. *Biological Conservation*, **143**, 1919-1927.

Frankham R, Ballou JD, Briscoe DA (2010) *Introduction to conservation genetics* Cambridge University Press, New York.

Frost DR, Grant T, Faivovich J, Bain RH, Haas A, Haddad CFB, de Sá RO, Channing A, Wilkinson M, Donnellan SC, Raxworthy CJ, Campbell JE, Blotto BL, Moller P, Drewes RC, Nussbaun RA, Lynch JD, Green DM, Wheeler WC (2006) The amphibian tree of life. *Bulletin of the American Museum of Natural History*, **279**, 1-370.

Funk WC, Caldwell JP, Peden CE, Padial JM, De la Riva I, Cannatella DC (2007) Tests of biogeographic hypotheses for diversification in the Amazonian forest frog, *Physalaemus petersi*. *Molecular Phylogenetics and Evolution*, **44**, 825-837.

Gascon C, Lougheed SC, Bogart JP (1998) Patterns of Genetic Population Differentiation in Four Species of Amazonian Frogs: A Test of the Riverine Barrier Hypothesis. *Biotropica*, **30**, 104-119.

Gentry AH (1988) Tree species richness of upper Amazonian forests. *Proceedings of the National Academy of Sciences*, **85**, 156-159.

Gerhardt HC, Doherty JA (1988) Acoustic communication in the gray treefrog, Hyla versicolor: evolutionary and neurobiological implications. *Journal of Comparitive Physiology A*, **162**, 261–278.

Grant T, Caldwell JP, Frost DR, Gagliardo R, Haddad CFB, Kok PJR, Means DB, Noonan BP, Schargel WE, Wheeler W (2006) *Phylogenetic systematics of dart-poison frogs and their relatives (Amphibia, Athesphatanura, Dendrobatidae). Bulletin of the AMNH ; no. 299*. American Museum of Natural History, New York.

Griffith SC, Immler S (2009) Female infidelity and genetic compatibility in birds: the role of the genetically loaded raffle in understanding the function of extrapair paternity. *Journal of avian biology*, **40**, 97-101.

Haffer J (2008) Hypotheses to explain the origin of species in Amazonia. *Brazilian Journal of Biology*, **68**, 917-947.

Haffer J (1969) Speciation in Amazonian Forest Birds. *Science*, **165**, 131-137.

Hauser L, Baird M, Hilborn R, Seeb LW, Seeb JE (2011) An empirical comparison of SNPs and microsatellites for parentage and kinship assignment in a wild sockeye salmon (Oncorhynchus nerka) population. *Molecular Ecology Resources*, **11**, 150-161.

Heled J, Drummond AJ (2010) Bayesian Inference of Species Trees from Multilocus Data. *Molecular Biology and Evolution*, **27**, 570-580.

Hobel G, Gerhardt HC (2003) Reproductive character displacement in the acoustic communication system of green tree frogs (*Hyla cinerea*). *Evolution*, **57**, 894-904.

Hoorn C, Wesselingh FP, ter Steege H, Bermudez MA, Mora A, Sevink J, Sanmartín I, Sanchez-Meseguer A, Anderson CL, Figueiredo JP, Jarmillo C, Riff D, Negri FR, Hooghiemstra H, Lundberg J, Stadler T, Särkinen T, Antonelli A (2010) Amazonia Through Time: Andean Uplift, Climate Change, Landscape Evolution, and Biodiversity. *Science*, **330**, 927-931.

Jaccoud D, Peng K, Feinstein D, Kilian A (2001) Diversity Arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Research*, **29**, e25.

Jennions MD, Petrie M (1997) Variation in mate chioce and mating preferences: a review of causes and consequences. *Biological Reviews*, **72**, 283-327.

Jiang Y, Bolnick DI, Kirkpatrick M (2013) Assortative Mating in Animals. *The American Naturalist*, **181**, E125-E138.

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, **24**, 1403-1405.

Jones OR, Wang J (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources*, **10**, 551-555.

Kaefer I, Tsuji-Nishikido B, Mota E, Farias I, Lima A (2013) The Early Stages of Speciation in Amazonian Forest Frogs: Phenotypic Conservatism Despite Strong Genetic Structure. *Evolutionary Biology*, **40**, 228-245.

Kalinowski ST, Taper ML, Marshall TC 2007) Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, **16**, 1099-1106.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**, 1647-1649.

Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses. *Molecular Biology and Evolution*, **29**, 1695-1701.

Latrubesse EM (2003) *The Late-Quaternary Palaeohydrology of large South American fluvial systems. Palaeohydrology: Understanding Global Change*. John Wiley & Sons Ltd, Hoboken, USA.

Latrubesse EM, Cozzuol M, da Silva-Caminha SAF, Rigsby CA, Absy ML, Jaramillo C (2010) The Late Miocene paleogeography of the Amazon Basin and the evolution of the Amazon River system. *Earth-Science Reviews*, **99**, 99-124.

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451-1452.

Littlejohn MJ (1965) Premating isolation in the Hyla ewingi complex (Anura: Hylidae). *Evolution*, **19**, 234-243.

Littlejohn MJ, Loftus-Hills JJ (1968) An experimental evaluation of premating isolation in the Hyla ewingi complex (Anura: Hylidae). *Evolution*, **22**, 659–663.

Losos JB, Leal M (2013) The evolution of species recognition signals. *Molecular Ecology*, **22**, 3879-3881.

Lougheed S, Austin J, Bogart J, Boag P, Chek A (2006) Multi-character perspectives on the evolution of intraspecific differentiation in a neotropical hylid frog. *BMC Evolutionary Biology*, **6**, 23.

Lougheed SC, Gascon C, Jones DA, Bogart JP, Boag PT (1999) Ridges and rivers: a test of competing hypotheses of Amazonian diversification using a dart-poison frog (*Epipedobates femoralis).Proceedings of the Royal Society of London*, **266**, 1829-1835.

Luikart G, England PR, Tallmon D, Jordan S, Taberlet P (2003) The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics*, **4**, 981-994.

Mayle FE, Beerling DJ, Gosling WD, Bush MB (2004) Responses of Amazonian ecosystems to climatic and atmospheric carbon dioxide changes since the last glacial maximum. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **359**, 499-514.

Momigliano P, Harcourt R, Robbins W, Jaiteh V, Mahardika GN, Sembiring A, Stow A (in reviw) The first analysis of adaptive genetic variation for a shark reveals islands of selection within a continuous population.

Montanarin A, Kaefer IL, Lima AP (2011) Courtship and mating behaviour of the brilliant-thighed frog Allobates femoralis from Central Amazonia: implications for the study of a species complex. *Ethology Ecology & Evolution*, **23**, 141-150.

Moritz C (2002) Strategies to Protect Biological Diversity and the Evolutionary Processes That Sustain It. *Systematic Biology*, **51**, 238-254.

Narins PM, Hödl W, Grabul DS (2003) Bimodal signal requisite for agonisitc behaviour in a dart-poison frog, *Epipedobates femoralis*. *Proceedings of the National Academy of Sciences*, **100**, 557-580.

Narins PM, Grabul DS, Soma KK, Gaucher P, Hödl W (2005) Cross-modal integration on a dart-poison frog. *Proceedings of the National Academy of Sciences*, **102**, 2425-2429.

Noonan BP, Wray KP (2006) Neotropical diversification: the effects of a complex history on diversity within the poison frog genus Dendrobates. *Journal of Biogeography*, **33**, 1007-1020.

Palumbi SR (1996) Nucleic acids II: the polymerase chain reaction. In: *Molecular systematics* pp. 205-247. Sinauer, Sunderland.

Petroli CD, Sansaloni CP, Carling J, Steane DA, Vaillancourt RE, Myburg AA, da Silva OB, Jr., Pappas GJ, Jr., Kilian A, Grattapaglia D (2012) Genomic Characterization of DArT

Markers Based on High-Density Linkage Analysis and Physical Mapping to the *Eucalyptus* Genome. *PLoS ONE*, **7**, e44684.

Pfennig DW, Pfennig KS (2010) Character Displacement and the Origins of Diversity. *The American Naturalist*, **176**, S26-S44.

Prace GT (1982) Forest refuges evidence from woody angiosperms. In: *Biological Diversification in the Tropics* (ed Prance GT), pp. 137 - 156. Columbia University Press, New York.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of Population Structure Using Multilocus Genotype Data. *Genetics*, **155**, 945-959.

Ptacek MB (2000) The role of mating preferences in shaping interspecific divergence in mating signals in vertebrates. *Behavioural Processes*, **51**, 111-134.

Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) Tracer v1.6. Available from http://beast.bio.ed.ac.uk/Tracer.

Räsänen ME, Salo JS, Jungnert H, Pittman LR (1990) Evolution of the Western Amazon Lowland Relief: impact of Andean foreland dynamics. *Terra Nova*, **2**, 320-332.

Ravelo AC, Andreasen DH, Lyle M, Olivarez Lyle A, Wara MW (2004) Regional climate shifts caused by gradual global cooling in the Pliocene epoch. *Nature*, **429**, 263-267.

Reynolds RG, Fitzpatrick BM (2007) Assortative mating in poison-dart frogs based on an ecologically important trait. *Evolution*, **61**, 2253-2259.

Ribas CC, Aleixo A, Nogueira ACR, Miyaki CY, Cracraft J (2011) A palaeobiogeographic model for biotic diversification within Amazonia over the past three million years. *Proceeding of the Royal Society B*.

Richards-Zawacki CL, Cummings ME (2011) Intraspecific reproductive character displacement in a polymorphic poison dart frog, *Dendrobrate pumilio*. *Evolution*, **65**, 259-267.

Richards-Zawacki CL, Wang IJ, Summers K (2012) Mate choice and the genetic basis for colour variation in a polymorphic dart frog: inferences from a wild pedigree. *Molecular Ecology*, **21**, 3879-3892.

Roberts JL, Brown JL, von May R, Arizabal W, Schulte R, Summer K (2006) Genetic divergence and speciation in lowland and montane Peruvian poison frogs. *Molecular Phylogenetics and Evolution*, **41**, 149-164.

Rossetti DdF, Mann de Toledo P, Góes AM (2005) New geological framework for Western Amazonia (Brazil) and implications for biogeography and evolution. *Quaternary Research*, **63**, 78-89.

Rull V (2008) Speciation timing and neotropical biodiversity: the Tertiary–Quaternary debate in the light of molecular phylogenetic evidence. *Molecular Ecology*, **17**, 2722-2729.

Sansaloni C, Petroli C, Jaccoud D, Carling J, Detering F, Grattapaglia D, Kilian A. *BMC Proceedings.* 2011.

Santos JC, Cannatella DC (2011) Phenotypic integration emerges from aposematism and scale in poison frogs. *Proceedings of the National Academy of Sciences USA*, **108**, 6175-6180.

Santos JC, Coloma LA, Summers K, Caldwell JP, Ree R, Cannatella DC (2009) Amazonian Amphibian Diversity Is Primarily Derived from Late Miocene Andean Lineages. *PLoS Biol*, **7**, e1000056.

Shackleton M, Jennions M, Hunt J (2005) Fighting success and attractiveness as predictors of male mating success in the black field cricket, Teleogryllus commodus: the effectiveness of no-choice tests. *Behavioral Ecology and Sociobiology*, **58**, 1-8.

Simões P, Lima A, Farias I (2012) Restricted natural hybridization between two species of litter frogs on a threatened landscape in southwestern Brazilian Amazonia. *Conservation Genetics*, **13**, 1145-1159.

Simões PI (2010) Diversificação do complexo Allobates femoralis (Anura, Dendrobatidae) em florestas da Amazônia brasileira: desvendando padrões atuais e históricos. Instituto Nacional de Pesquisas da Amazônia.

Simões PI, Lima AP, Farias IP (2010) The description of a cryptic species related to the pan-Amazonian frog *Allobates femoralis* (Boulenger 1883) (Anura: Aromobatidae). *Zootaxa*, **2406**, 1-28.

Simões PI, Lima AP, Magnusson WE, Hödl W, Amézquita A (2008) Acoustic and Morphological Differentiation in the Frog Allobates femoralis: Relationships with the Upper Madeira River and Other Potential Geological Barriers. *Biotropica*, **40**, 607-614.

Simões PI, Stow A, Hödl W, Amézquita A, Farias IP, Lima AP (2014) The value of including intraspecific measures of biodiversity in environmental impact surveys is highlighted by the Amazonian brilliant-thighed frog (*Allobates femoralis*). *Tropical Conservation Science*, **7**, 811-828.

Summers K, Symula R, Clough M, Cronin T (1999) Visual mate choice in poison frogs. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **266**, 2141-2145.

Sunnucks P, Hales DF (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus Sitobion (Hemiptera: Aphididae). *Molecular Biology and Evolution*, **13**, 510-524.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, **30**, 2725-2729.

Thibert-Plante X, Gavrilets S (2013) Evolution of mate choice and the so-called magic traits in ecological speciation. *Ecology Letters*, **16**, 1004-1013.

Vanzolini PE, Williams EE (1981) The vanishing refuge: a mechanism for ecogeographic speciation. *Pap,is Avulsos de Zoologia*, **34**, 251-255.

Vences M, Thomas M, van der Meijden A, Chiari Y, Vieietes DR (2005) Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. *Frontiers in Zoology*, **2**.

Wade MJ, Patterson H, Chang NW, Johnson NA (1994) Postcopulatory, prezygotic isolation in flour beetles. *Heredity (Edinb)*, **72**, 163-167.

Waldman B, Bishop PJ (2004) Chemical communication in an archaic anuran amphibian. *Behavioral Ecology*, **15**, 88-93.

Wallace RA (1852) On the monkeys of the Amazon. *Proceedings of the Zoological Society*, **17**, 107-110.

Wells KD (2007) *The ecology and behavior of amphibians*. The University of Chicago Press, Chicago.

Wells KD, Schwartz JJ (2006) The Behavioral Ecology of Anuran Communication. In: *Hearing and Sound Communication in Amphibians* (eds Narins P, Feng A, Fay R, Popper A), pp. 44-86. Springer New York.

Wilkinson MJ, Marshall LG, Lundberg JG, Kreslavsky MH (2010) *Megafan environments in northern South America and their impact on Amazon Neogene aquatic ecosystems. Amazonia, landscape and species evolution*. Blackwell Publishing, Oxford.

Wilson EO (1992) *The diversity of life*. Norton, New York.

Zeisset I, Beebee TJC (2008) Amphibian phylogeography: a model for understanding historical aspects of species distributions. *Heredity*, **101**, 109-119.

Zhang D-X, Hewitt GM (2003) Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. *Molecular Ecology*, **12**, 563-584.

6. Supplementary material

Author Guidelines
General Information

Molecular Ecology publishes papers that use molecular genetic techniques to address consequential questions in ecology, evolution, behaviour and conservation. Studies may employ neutral markers for inference about ecological and evolutionary processes, or examine ecologically important genes and their products.

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To facilitate viewing on screen, please format your manuscript with 2.5 cm side margins, 3 cm top and bottom margins, and clear paragraph delimitations. All manuscripts must have page numbers and continuous line numbers. The main body of text must be double-spaced, with Tables and Figure captions, References, Appendices and Supporting Information single-spaced.

References

Please see a recent issue of the journal for reference formats.

From the current issue:
In text references appear in chronological order and are cited as follows
(Rousset 1997, 2000; Pritchard *et al.* 2000; Waples & Gaggiotti 2006).
All authors cited in text are provide in the final reference list. This is arranged in alphabetical order.

Journal article - Rousset F (2000) Genetic differentiation between individuals. *Journal of Evolutionary Biology*, **13**, 58-62.

Book - Rousset F (2004) *Genetic Structure and Selection in Subdivided Populations*. Princeton University Press, Princeton, New Jersey.

Book chapter - Rousset F (2007) Inferences from spatial population genetics. In: *Handbook of Statistical Genetics* (eds Balding DJ, Bishop M, Cannings C), pp. 945, UK.

Table S1. The source, population location, respective coordinates, GenBank ID and population tag for all samples used in the intraspecific divergence analyses of *Allobates femoralis*.

Source	Location	Region/Country	Coordinates	Cyt*b*	16S	Population Tag
Lougheed *et al.* 1999	Altamirã	Pará, Brazil	*	AF163928	*	Altamira
Lougheed *et al.* 1999	Altamirã	Pará, Brazil	*	AF163929	*	Altamira
Lougheed *et al.* 1999	Altamirã	Pará, Brazil	*	AF163930	*	Altamira
Lougheed *et al.* 1999	Altamirã	Pará, Brazil	*	AF163931	*	Altamira
Lougheed *et al.* 1999	Altamirã	Pará, Brazil	*	AF163932	*	Altamira
Simões *et al.* 2014	Altamirã	Pará, Brazil	3.2432° S, 52.2440° W	*	KF310936	Altamira
Simões *et al.* 2014	Altamirã	Pará, Brazil	3.2432° S, 52.2440° W	*	KF310937	Altamira
Simões *et al.* 2014	Altamirã	Pará, Brazil	3.2432° S, 52.2440° W	*	KF310938	Altamira
Simões *et al.* 2014	Altamirã	Pará, Brazil	3.1275° S, 51.7097° W	*	KF310952	Altamira
Simões *et al.* 2014	Altamirã	Pará, Brazil	3.1275° S, 51.7097° W	*	KF310953	Altamira
Roberts *et al.* 2006	Boca Manu	Cuzco, Peru	12.25° S, 70.9° W	DQ523139	DQ523069	Boca Manu
Simões 2010	Borba	Amazonas, Brazil	4.4342° S, 59.6236° W	JF690045	JF689971	Borba
Simões 2010	Borba	Amazonas, Brazil	4.4342° S, 59.6236° W	JF690046	JF689972	Borba
Simões 2010	Borba	Amazonas, Brazil	4.4342° S, 59.6236° W	JF690047	JF689973	Borba
Simões 2010	Borba	Amazonas, Brazil	4.4342° S, 59.6236° W	JF690048	JF689974	Borba
Simões 2010	Borba	Amazonas, Brazil	4.4342° S, 59.6236° W	JF690049	JF689975	Borba
Simões 2010	Careiro	Amazonas, Brazil	3.3708° S, 59.8683° W	JF690033	JF689984	Careiro
Simões 2010	Careiro	Amazonas, Brazil	3.3708° S, 59.8683° W	JF690031	JF689985	Careiro
Simões 2010	Careiro	Amazonas, Brazil	3.3708° S, 59.8683° W	JF690032	JF689986	Careiro
Simões 2010	Careiro	Amazonas, Brazil	3.3708° S, 59.8683° W	JF690035	JF689987	Careiro
Simões 2010	Careiro	Amazonas, Brazil	3.3708° S, 59.8683° W	JF690036	JF689988	Careiro
Simões 2010	Careiro	Amazonas, Brazil	3.3708° S, 59.8683° W	JF690037	JF689989	Careiro
Simões 2010	Careiro	Amazonas, Brazil	3.3708° S, 59.8683° W	JF690034	JF689990	Careiro
Clough & Summers 2000	Cuyabeno	Sucumbíos, Ecuador	*	*	AF128572	Northern_Ecuador
Grant *et al.* 2006	Cuyabeno	Sucumbíos, Ecuador	0.0° S, 76.16666° W	DQ502525	*	Northern_Ecuador
Grant *et al.* 2006	Cuyabeno	Sucumbíos, Ecuador	0.0° S, 76.16666° W	DQ502526	DQ502094	Northern_Ecuador
Grant *et al.* 2006	Cuyabeno	Sucumbíos, Ecuador	0.0° S, 76.16666° W	DQ502661	DQ502228	Northern_Ecuador

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Source	Location	Region/Country	Coordinates	Cyt*b*	16S	Population Tag
Simões 2010	Democracia	Amazonas, Brazil	5.8058° S, 61.4453° W	JF690024	JF689977	Democracia
Simões 2010	Democracia	Amazonas, Brazil	5.8058° S, 61.4453° W	JF690026	JF689979	Democracia
Simões 2010	Democracia	Amazonas, Brazil	5.8058° S, 61.4453° W	JF690027	JF689980	Democracia
Simões 2010	Democracia	Amazonas, Brazil	5.8058° S, 61.4453° W	JF690028	JF689981	Democracia
Simões 2010	Democracia	Amazonas, Brazil	5.8058° S, 61.4453° W	JF690030	JF689982	Democracia
Simões 2010	Democracia	Amazonas, Brazil	5.8058° S, 61.4453° W	JF690029	JF689983	Democracia
Frost *et al.* 2006	Guajará-Mirim	Rondônia, Brazil	10.31666° S, 64.55° W	*	DQ283045	Guajara-Mirim
Grant *et al.* 2006	Guajará-Mirim	Rondônia, Brazil	10.31666° S, 64.55° W	DQ502520	*	Guajara-Mirim
Grant *et al.* 2006	Guajará-Mirim	Rondônia, Brazil	10.31666° S, 64.55° W	DQ502519	DQ502088	Guajara-Mirim
Santos *et al.* 2009	Guajará-Mirim	Rondônia, Brazil	10.31666° S, 64.55° W	*	EU342537	Guajara-Mirim
Simões 2010	Humaitá (left bank)	Amazonas, Brazil	7.0228° S, 63.1028° W	JF690018	JF689997	Humaita_left_bank
Simões 2010	Humaitá (left bank)	Amazonas, Brazil	7.0228° S, 63.1028° W	JF690019	JF689998	Humaita_left_bank
Simões 2010	Humaitá (left bank)	Amazonas, Brazil	7.0228° S, 63.1028° W	JF690020	JF689999	Humaita_left_bank
Simões 2010	Humaitá (left bank)	Amazonas, Brazil	7.0228° S, 63.1028° W	JF690022	JF690000	Humaita_left_bank
Simões 2010	Humaitá (left bank)	Amazonas, Brazil	7.0228° S, 63.1028° W	JF690021	JF690001	Humaita_left_bank
Simões 2010	Humaitá (left bank)	Amazonas, Brazil	7.0228° S, 63.1028° W	JF690023	JF690002	Humaita_left_bank
Simões 2010	Humaitá (right bank)	Amazonas, Brazil	7.5488° S, 62.8772° W	JF690109	JF690003	Humaita_right_bank
Simões 2010	Humaitá (right bank)	Amazonas, Brazil	7.5488° S, 62.8772° W	JF690110	JF690004	Humaita_right_bank
Simões 2010	Humaitá (right bank)	Amazonas, Brazil	7.5488° S, 62.8772° W	JF690111	JF690005	Humaita_right_bank
Simões 2010	Humaitá (right bank)	Amazonas, Brazil	7.5488° S, 62.8772° W	JF690112	JF690006	Humaita_right_bank
Simões *et al.* 2010	Jaci-Paraná	Rondônia, Brazil	9.1694° S, 64.4289° W	*	GU017450	Jaci-Parana_left_bank
Simões *et al.* 2010	Jaci-Paraná	Rondônia, Brazil	9.1694° S, 64.4289° W	*	GU017453	Jaci-Parana_left_bank
Simões *et al.* 2010	Jaci-Paraná	Rondônia, Brazil	9.1694° S, 64.4289° W	*	GU017456	Jaci-Parana_left_bank
Simões *et al.* 2010	Jaci-Paraná	Rondônia, Brazil	9.1694° S, 64.4289° W	*	GU017457	Jaci-Parana_left_bank
Simões 2010	Jaci-Paraná (left bank)	Rondônia, Brazil	9.1694° S, 64.4289° W	JF690062	GU017451	Jaci-Parana_left_bank
Simões 2010	Jaci-Paraná (left bank)	Rondônia, Brazil	9.1694° S, 64.4289° W	JF690064	GU017452	Jaci-Parana_left_bank
Simões 2010	Jaci-Paraná (left bank)	Rondônia, Brazil	9.1694° S, 64.4289° W	JF690063	GU017454	Jaci-Parana_left_bank
Simões 2010	Jaci-Paraná (left bank)	Rondônia, Brazil	9.1694° S, 64.4289° W	JF690066	GU017455	Jaci-Parana_left_bank
Simões 2010	Jaci-Paraná (left bank)	Rondônia, Brazil	9.1694° S, 64.4289° W	JF690065	JF689938	Jaci-Parana_left_bank
Simões 2010	Jaci-Paraná (left bank)	Rondônia, Brazil	9.1694° S, 64.4289° W	JF690068	JF689939	Jaci-Parana_left_bank
Simões 2010	Jaci-Paraná (left bank)	Rondônia, Brazil	9.1694° S, 64.4289° W	JF690067	JF689940	Jaci-Parana_left_bank

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Source	Location	Region/Country	Coordinates	Cyt*b*	16S	Population Tag
Simões 2010	Jaci-Paraná (right bank)	Rondônia, Brazil	9.2045° S, 64.3620° W	JF690102	JF689963	Jaci-Parana_right_bank
Simões 2010	Jaci-Paraná (right bank)	Rondônia, Brazil	9.2045° S, 64.3620° W	JF690103	JF689964	Jaci-Parana_right_bank
Simões 2010	Jaci-Paraná (right bank)	Rondônia, Brazil	9.2045° S, 64.3620° W	JF690101	JF689966	Jaci-Parana_right_bank
Simões 2010	Jaci-Paraná (right bank)	Rondônia, Brazil	9.2045° S, 64.3620° W	JF690097	JF689967	Jaci-Parana_right_bank
Simões 2010	Jaci-Paraná (right bank)	Rondônia, Brazil	9.2045° S, 64.3620° W	JF690098	JF689968	Jaci-Parana_right_bank
Simões 2010	Jaci-Paraná (right bank)	Rondônia, Brazil	9.2045° S, 64.3620° W	JF690099	JF689969	Jaci-Parana_right_bank
Simões 2010	Jaci-Paraná (right bank)	Rondônia, Brazil	9.2045° S, 64.3620° W	JF690100	JF689970	Jaci-Parana_right_bank
Grant *et al.* 2006	Kayser airstrip	Sipaliwini, Suriname	3.5° N, 56.2799° W	DQ502678	DQ502246	Suriname
Simões *et al.* 2010	Lower Jirau	Rondônia, Brazil	9.3114° S, 64.7172° W	*	GU017446	Lower_Jirau
Simões 2010	Lower Jirau	Rondônia, Brazil	9.3114° S, 64.7172° W	JF690074	GU017447	Lower_Jirau
Simões 2010	Lower Jirau	Rondônia, Brazil	9.3114° S, 64.7172° W	JF690075	GU017448	Lower_Jirau
Simões 2010	Lower Jirau	Rondônia, Brazil	9.3114° S, 64.7172° W	JF690076	GU017449	Lower_Jirau
Simões 2010	Lower Jirau	Rondônia, Brazil	9.3114° S, 64.7172° W	JF690073	JF689933	Lower_Jirau
Simões 2010	Lower Jirau	Rondônia, Brazil	9.3114° S, 64.7172° W	JF690077	JF689934	Lower_Jirau
Simões 2010	Lower Jirau	Rondônia, Brazil	9.3114° S, 64.7172° W	JF690078	JF689935	Lower_Jirau
Simões 2010	Lower Jirau	Rondônia, Brazil	9.3114° S, 64.7172° W	JF690079	JF689936	Lower_Jirau
Simões 2010	Lower Jirau	Rondônia, Brazil	9.3114° S, 64.7172° W	JF690080	JF689937	Lower_Jirau
Simões 2010	Manaquiri	Amazonas, Brazil	3.4272° S, 60.6150° W	JF690043	JF689991	Manaquiri
Simões 2010	Manaquiri	Amazonas, Brazil	3.4272° S, 60.6150° W	JF690038	JF689992	Manaquiri
Simões 2010	Manaquiri	Amazonas, Brazil	3.4272° S, 60.6150° W	JF690039	JF689993	Manaquiri
Simões 2010	Manaquiri	Amazonas, Brazil	3.4272° S, 60.6150° W	JF690040	JF689994	Manaquiri
Simões 2010	Manaquiri	Amazonas, Brazil	3.4272° S, 60.6150° W	JF690041	JF689995	Manaquiri
Simões 2010	Manaquiri	Amazonas, Brazil	3.4272° S, 60.6150° W	JF690042	JF689996	Manaquiri
Simões 2010	Manicoré	Amazonas, Brazil	5.8231° S, 61.2986° W	JF690060	JF690007	Manicore
Simões 2010	Manicoré	Amazonas, Brazil	5.8231° S, 61.2986° W	JF690059	JF690008	Manicore
Simões 2010	Manicoré	Amazonas, Brazil	5.8231° S, 61.2986° W	JF690056	JF690009	Manicore
Simões 2010	Manicoré	Amazonas, Brazil	5.8231° S, 61.2986° W	JF690057	JF690010	Manicore
Simões 2010	Manicoré	Amazonas, Brazil	5.8231° S, 61.2986° W	JF690058	JF690011	Manicore
Simões *et al.* 2014	Manicoré	Amazonas, Brazil	5.8231° S, 61.2986° W	*	KF310985	Manicore
Simões *et al.* 2014	Manicoré	Amazonas, Brazil	5.8231° S, 61.2986° W	*	KF310986	Manicore
Simões *et al.* 2014	Manicoré	Amazonas, Brazil	5.8231° S, 61.2986° W	*	KF310987	Manicore

continued next page

Source	Location	Region/Country	Coordinates	Cyt*b*	16S	Population Tag
Simões *et al.* 2014	Manicoré	Amazonas, Brazil	5.8231° S, 61.2986° W	*	KF310988	Manicore
Simões *et al.* 2014	Manicoré	Amazonas, Brazil	5.8231° S, 61.2986° W	*	KF310989	Manicore
Simões *et al.* 2014	Manicoré	Amazonas, Brazil	5.8231° S, 61.2986° W	*	KF310990	Manicore
Simões *et al.* 2014	Manicoré	Amazonas, Brazil	5.8231° S, 61.2986° W	*	KF310992	Manicore
Simões *et al.* 2014	Manicoré	Amazonas, Brazil	5.8231° S, 61.2986° W	*	KF310993	Manicore
Simões *et al.* 2014	Manicoré	Amazonas, Brazil	5.8231° S, 61.2986° W	*	KF310994	Manicore
Simões *et al.* 2014	Manicoré	Amazonas, Brazil	5.8231° S, 61.2986° W	*	KF310995	Manicore
Roberts *et al.* 2006	Mazuko	Madre de Dios, Peru	12.591° S, 69.326° W	DQ523125	DQ523055	Madre_de_Dios
Simões 2010	Morrinho (left bank)	Rondônia, Brazil	9.0199° S, 64.2172° W	JF690081	JF689951	Morrinho_left_bank
Simões 2010	Morrinho (left bank)	Rondônia, Brazil	9.0199° S, 64.2172° W	JF690082	JF689952	Morrinho_left_bank
Simões 2010	Morrinho (left bank)	Rondônia, Brazil	9.0199° S, 64.2172° W	JF690087	JF689953	Morrinho_left_bank
Simões 2010	Morrinho (left bank)	Rondônia, Brazil	9.0199° S, 64.2172° W	JF690083	JF689954	Morrinho_left_bank
Simões 2010	Morrinho (left bank)	Rondônia, Brazil	9.0199° S, 64.2172° W	JF690084	JF689955	Morrinho_left_bank
Simões 2010	Morrinho (left bank)	Rondônia, Brazil	9.0199° S, 64.2172° W	JF690085	JF689956	Morrinho_left_bank
Simões 2010	Morrinho (left bank)	Rondônia, Brazil	9.0199° S, 64.2172° W	JF690088	JF689957	Morrinho_right_bank
Simões 2010	Morrinho (left bank)	Rondônia, Brazil	9.0199° S, 64.2172° W	JF690086	JF689958	Morrinho_left_bank
Simões 2010	Morrinho (right bank)	Rondônia, Brazil	9.0158° S, 64.0914° W	JF690093	JF689947	Morrinho_right_bank
Simões 2010	Morrinho (right bank)	Rondônia, Brazil	9.0158° S, 64.0914° W	JF690094	JF689948	Morrinho_right_bank
Simões 2010	Morrinho (right bank)	Rondônia, Brazil	9.0158° S, 64.0914° W	JF690095	JF689949	Morrinho_right_bank
Simões 2010	Morrinho (right bank)	Rondônia, Brazil	9.0158° S, 64.0914° W	JF690096	JF689950	Morrinho_right_bank
Simões *et al.* 2010	Mutum-Paraná (right bank)	Rondônia, Brazil	9.6414° S, 64.8859° W	*	GU017458	Mutum-Parana_right_bank
Simões *et al.* 2010	Mutum-Paraná (right bank)	Rondônia, Brazil	9.6414° S, 64.8859° W	*	GU017459	Mutum-Parana_right_bank
Simões *et al.* 2010	Mutum-Paraná (right bank)	Rondônia, Brazil	9.6414° S, 64.8859° W	*	GU017462	Mutum-Parana_right_bank
Simões 2010	Mutum-Paraná (right bank)	Rondônia, Brazil	9.6414° S, 64.8859° W	JF690107	GU017460	Mutum-Parana_right_bank
Simões 2010	Mutum-Paraná (right bank)	Rondônia, Brazil	9.6414° S, 64.8859° W	JF690106	GU017461	Mutum-Parana_right_bank
Simões 2010	Mutum-Paraná (right bank)	Rondônia, Brazil	9.6414° S, 64.8859° W	JF690108	JF689941	Mutum-Parana_right_bank
Simões 2010	Mutum-Paraná (right bank)	Rondônia, Brazil	9.6414° S, 64.8859° W	JF690105	JF689942	Mutum-Parana_right_bank
Simões *et al.* 2014	Nova Olinda de Norte	Amazonas, Brazil	3.8744° S, 59.0461° W	*	KF310981	Nova_Olinda_de_Norte
Simões *et al.* 2014	Nova Olinda de Norte	Amazonas, Brazil	3.8744° S, 59.0461° W	*	KF310982	Nova_Olinda_de_Norte
Simões *et al.* 2014	Nova Olinda de Norte	Amazonas, Brazil	3.8744° S, 59.0461° W	*	KF310983	Nova_Olinda_de_Norte
Simões *et al.* 2014	Nova Olinda de Norte	Amazonas, Brazil	3.8744° S, 59.0461° W	*	KF310984	Nova_Olinda_de_Norte

continued next page

Source	Location	Region/Country	Coordinates	Cyt*b*	16S	Population Tag
Simões 2010	Nova Olinda do Norte	Amazonas, Brazil	3.8744° S, 59.0461° W	JF690044	JF689976	Nova_Olinda_de_Norte
Simões *et al.* 2014	Novo Aripuanã	Amazonas, Brazil	5.1503° S, 60.3467° W	*	KF310967	Novo_Aripuana
Simões *et al.* 2014	Novo Aripuanã	Amazonas, Brazil	5.1503° S, 60.3467° W	*	KF310968	Novo_Aripuana
Simões 2010	Novo Aripuanã	Amazonas, Brazil	5.1503° S, 60.3467° W	JF690050	JF690012	Novo_Aripuana
Simões 2010	Novo Aripuanã	Amazonas, Brazil	5.1503° S, 60.3467° W	JF690051	JF690014	Novo_Aripuana
Simões 2010	Novo Aripuanã	Amazonas, Brazil	5.1503° S, 60.3467° W	JF690052	JF690015	Novo_Aripuana
Simões 2010	Novo Aripuanã	Amazonas, Brazil	5.1503° S, 60.3467° W	JF690053	JF690016	Novo_Aripuana
Simões 2010	Novo Aripuanã	Amazonas, Brazil	5.1503° S, 60.3467° W	JF690054	JF690017	Novo_Aripuana
Santos & Cannatella 2011	Parque Nac. Yasuni	Ecuador	1.1007° S, 75.8069° W	HQ290531	*	Southern_Ecuador
Santos *et al.* 2009	Parque Nac. Yasuni	Ecuador	1.1007° S, 75.8069° W	*	EU342535	Southern_Ecuador
Grant *et al.* 2006	Porto Walter	Acre, Brazil	8.25° S, 72.76666° W	DQ502523	DQ502091	Porto_Walter
Grant *et al.* 2006	Porto Walter	Acre, Brazil	8.25° S, 72.76666° W	DQ502524	DQ502092	Porto_Walter
Grant *et al.* 2006	Porto Walter	Acre, Brazil	8.25° S, 72.76666° W	DQ502664	DQ502231	Porto_Walter
Santos *et al.* 2009	Porto Walter	Acre, Brazil	8.25° S, 72.76666° W	*	EU342532	Porto_Walter
Santos *et al.* 2009	Porto Walter	Acre, Brazil	8.25° S, 72.76666° W	*	EU342533	Porto_Walter
Grant *et al.* 2006	Puerto Maldonado	Madre de Dios, Peru	*	DQ502415	DQ501990	Madre_de_Dios
Grant *et al.* 2006	Puerto Maldonado	Madre de Dios, Peru	*	DQ502439	DQ502014	Madre_de_Dios
Grant *et al.* 2006	Puerto Maldonado	Madre de Dios, Peru	*	DQ502440	DQ502015	Madre_de_Dios
Grant *et al.* 2006	Adolfo Ducke	Amazonas, Brazil	3.0079° S, 59.9393° W	DQ502545	DQ502113	Manaus
Simões 2010	Santo Antônio (left bank)	Rondônia, Brazil	8.6550° S, 64.0195° W	JF690069	JF689959	Santo_Antonio_left_bank
Simões 2010	Santo Antônio (left bank)	Rondônia, Brazil	8.6550° S, 64.0195° W	JF690072	JF689960	Santo_Antonio_left_bank
Simões 2010	Santo Antônio (left bank)	Rondônia, Brazil	8.6550° S, 64.0195° W	JF690071	JF689961	Santo_Antonio_left_bank
Simões 2010	Santo Antônio (left bank)	Rondônia, Brazil	8.6550° S, 64.0195° W	JF690070	JF689962	Santo_Antonio_left_bank
Simões 2010	Santo Antônio (right bank)	Amazonas, Brazil	8.8309° S, 64.0206° W	JF690089	JF689943	Santo_Antonio_right_bank
Simões 2010	Santo Antônio (right bank)	Amazonas, Brazil	8.8309° S, 64.0206° W	JF690090	JF689944	Santo_Antonio_right_bank
Simões 2010	Santo Antônio (right bank)	Amazonas, Brazil	8.8309° S, 64.0206° W	JF690091	JF689945	Santo_Antonio_right_bank
Simões 2010	Santo Antônio (right bank)	Amazonas, Brazil	8.8309° S, 64.0206° W	JF690092	JF689946	Santo_Antonio_right_bank
Simões *et al.* 2014	Serra Azul	Pará, Brazil	1.2822° S, 54.1297° W	*	KF310996	Vai-Quem-Quer/Serra_Azul
Simões *et al.* 2014	Serra Azul	Pará, Brazil	1.2822° S, 54.1297° W	*	KF310997	Vai-Quem-Quer/Serra_Azul
Simões *et al.* 2014	Serra Azul	Pará, Brazil	1.2822° S, 54.1297° W	*	KF310998	Vai-Quem-Quer/Serra_Azul
Simões *et al.* 2014	Serra Azul	Pará, Brazil	1.2822° S, 54.1297° W	*	KF310999	Vai-Quem-Quer/Serra_Azul

continued next page

Source	Location	Region/Country	Coordinates	Cyt*b*	16S	Population Tag
Simões *et al.* 2014	Serra Azul	Pará, Brazil	1.2822° S, 54.1297° W	*	KF311000	Vai-Quem-Quer/Serra_Azul
Simões *et al.* 2014	Serra Azul	Pará, Brazil	1.2822° S, 54.1297° W	*	KF311001	Vai-Quem-Quer/Serra_Azul
Simões *et al.* 2014	Serra Azul	Pará, Brazil	1.2822° S, 54.1297° W	*	KF311002	Vai-Quem-Quer/Serra_Azul
Simões *et al.* 2014	Serra Azul	Pará, Brazil	1.2822° S, 54.1297° W	*	KF311003	Vai-Quem-Quer/Serra_Azul
Lougheed *et al.* 1999	Vai-Quem-Quer	Pará, Brazil	1.4395° S, 54.1531° W	AF163914	*	Vai-Quem-Quer/Serra_Azul
Lougheed *et al.* 1999	Vai-Quem-Quer	Pará, Brazil	1.4395° S, 54.1531° W	AF163916	*	Vai-Quem-Quer/Serra_Azul
Grant *et al.* 2006	*	Brazil	*	DQ502533	DQ502101	Allobates_nidicola
Grant *et al.* 2006	*	Ecuador	*	DQ502450	DQ502022	Allobates_zaparo

Table S2. Results of the captive mating trials for each individual according to both COLONY2 and CERVUS. Pink cells represent females, blue cells indicate the candidate fathers, and orange cell highlight parents which were not included in the original experimental combination.

Experiment Info				COLONY results - SNPs		CERVUS results - SNPs		Parents		Program	Mate choice		
Colour	Call	Cage	Individual	Mother	Father	Mother	Father	Mother	Father	Mismatches	Call type of mother	Call type of father	Assortative?
Yellow	1	1	14432	*	*	*	*	*	*	*	*	*	*
Yellow	1	1	14430	*	*	*	*	*	*	*	*	*	*
Yellow	4	1	14441	*	*	*	*	*	*	*	*	*	*
Yellow	*	1	01C101	14432	14441	14432	14441	14432	14441	No	1	4	No
Yellow	*	1	01C102	14432	14441	14432	14441	14432	14441	No	1	4	No
Yellow	*	1	01C103	14432	14441	14432	14441	14432	14441	No	1	4	No
Yellow	*	1	01C201	14432	14430	14432	14430	14432	14430	No	1	1	Yes
Yellow	*	1	01C202	14432	14430	14432	14430	14432	14430	No	1	1	Yes
Yellow	*	1	01C203	14432	14430	14432	14430	14432	14430	No	1	1	Yes
Yellow	*	1	01C301	14429	14430	14429	14430	14429	14430	No	1	1	Yes
Yellow	*	1	01C302	14429	14430	14429	14430	14429	14430	No	1	1	Yes
Yellow	*	1	01C303	14429	14430	14429	14430	14429	14430	No	1	1	Yes
Yellow	1	2	14436	*	*	*	*	*	*	*	*	*	*
Yellow	1	2	14437	*	*	*	*	*	*	*	*	*	*
Yellow	4	2	14435	*	*	*	*	*	*	*	*	*	*
Yellow	*	2	02C101	14436	14437	14436	14437	14436	14437	No	1	1	Yes
Yellow	*	2	02C102	14436	14437	14436	14437	14436	14437	No	1	1	Yes
Yellow	*	2	02C103	14436	14437	14436	14437	14436	14437	No	1	1	Yes
Yellow	1	3	14425	*	*	*	*	*	*	*	*	*	*
Yellow	1	3	14426	*	*	*	*	*	*	*	*	*	*
Yellow	4	3	14427	*	*	*	*	*	*	*	*	*	*
Yellow	*	3	03C101	*	*	*	*	14425	14426	No	1	1	Yes
Yellow	*	3	03C102	14425	14426	14425	14426	14425	14426	No	1	1	Yes
Yellow	*	3	03C103	*	*	*	*	14425	14426	No	1	1	Yes

continued next page

Experiment Info				COLONY results - SNPs		CERVUS results - SNPs		Parents		Program	Mate choice		
Colour	Call	Cage	Individual	Mother	Father	Mother	Father	Mother	Father	Mismatches	Call type of mother	Call type of father	Assortative?
Yellow	*	3	03C201	14425	14434	14425	14434	14425	14434	No	1	4	No
Yellow	*	3	03C202	14425	14434	14425	14434	14425	14434	No	1	4	No
Yellow	*	3	03C203	14425	14434	14425	14434	14425	14434	No	1	4	No
Yellow	1	4	14429	*	*	*	*	*	*	*	*	*	*
Yellow	4	4	14434	*	*	*	*	*	*	*	*	*	*
Yellow	1	4	14433	*	*	*	*	*	*	*	*	*	*
Yellow	*	4	04C101	14429	14433	14429	14433	14429	14433	No	1	1	Yes
Yellow	*	4	04C102	14429	14433	14429	14433	14429	14433	No	1	1	Yes
Yellow	*	4	04C103	14429	14433	14429	14433	14429	14433	No	1	1	Yes
Yellow	*	4	04C201	14432	14434	14432	14434	14432	14434	No	1	4	No
Yellow	*	4	04C202	14432	14434	14432	14434	14432	14434	No	1	4	No
Yellow	*	4	04C203	14432	14434	14432	14434	14432	14434	No	1	4	No
Yellow	1	5	14420	*	*	*	*	*	*	*	*	*	*
Yellow	4	5	14419	*	*	*	*	*	*	*	*	*	*
Yellow	1	5	14418	*	*	*	*	*	*	*	*	*	*
Yellow	*	5	05C101	14420	14419	14420	14419	14420	14419	No	1	4	No
Yellow	*	5	05C102	14420	14419	14420	14419	14420	14419	No	1	4	No
Yellow	*	5	05C103	14420	14419	14420	14419	14420	14419	No	1	4	No
Yellow	*	5	05C201	14420	14419	14420	14419	14420	14419	No	1	4	No
Yellow	*	5	05C202	14420	14419	14420	14419	14420	14419	No	1	4	No
Yellow	*	5	05C203	14420	14419	14420	14419	14420	14419	No	1	4	No
Yellow	*	5	05C301	14420	14419	14420	14419	14420	14419	No	1	4	No
Yellow	*	5	05C302	14420	14419	14420	14419	14420	14419	No	1	4	No
Red	6	6	14421	*	*	*	*	*	*	*	*	*	*
Red	4	6	14439	*	*	*	*	*	*	*	*	*	*
Red	*	6	06C101	14440	14438	14440	14438	14440	14438	No	6	4	No
Red	*	6	06C102	14440	14438	14440	14438	14440	14438	No	6	4	No
Red	*	6	06C103	14440	14438	14440	14438	14440	14438	No	6	4	No

continued next page

Experiment Info				COLONY results - SNPs		CERVUS results - SNPs		Parents		Program	Mate choice		
Colour	Call	Cage	Individual	Mother	Father	Mother	Father	Mother	Father	Mismatches	Call type of mother	Call type of father	Assortative?
Red	6	6	14421	*	*	*	*	*	*	*	*	*	*
Red	4	6	14439	*	*	*	*	*	*	*	*	*	*
Red	*	6	06C201	14440	14438	14440	14438	14440	14438	No	6	4	No
Red	*	6	06C202	14440	14438	14440	14438	14440	14438	No	6	4	No
Red	*	6	06C203	14440	14438	14440	14438	14440	14438	No	6	4	No
Red	6	8	14440	*	*	*	*	*	*	*	*	*	*
Red	6	8	14442	*	*	*	*	*	*	*	*	*	*
Red	4	8	14438	*	*	*	*	*	*	*	*	*	*
Red	*	8	08C101	14440	14438	14421	14438	unknown	14438	Yes - females	6	4	No
Red	*	8	08C102	14440	14438	14421	14438	unknown	14438	Yes - females	6	4	No
Red	*	8	08C103	14440	14438	14421	14438	unknown	14438	Yes - females	6	4	No
Red	*	8	08C201	14440	14438	14421	14438	unknown	14438	Yes - females	6	4	No
Red	*	8	08C202	14440	14438	14421	14438	unknown	14438	Yes - females	6	4	No
Red	*	8	08C203	*	*	*	*	*	*	Yes - females	6	4	No
Red	*	9	09C101	1440	14439	1440	14439	1440	14439	No	6	4	No
Red	*	9	09C102	1440	14439	1440	14439	1440	14439	No	6	4	No
Red	*	9	09C103	1440	14439	1440	14439	1440	14439	No	6	4	No
Red	*	9	09C201	1440	14439	1440	14439	1440	14439	No	6	4	No