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Parthenogenesis doubles the rate of amino acid substitution in whiptail mitochondria

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Sexual reproduction is ubiquitous in the natural world, suggesting that sex must have extensive benefits to overcome the cost of males compared to asexual reproduction. One hypothesized advantage of sex with strong theoretical support is that sex plays a role in removing deleterious mutations from the genome. Theory predicts that transitions to asexuality should lead to the suppression of recombination and segregation and, in turn, weakened natural selection, allowing for the accumulation of slightly deleterious mutations. We tested this prediction by estimating the d*N*/d*S* ratios in asexual vertebrate lineages in the genus *Aspidoscelis* using whole mitochondrial genomes from seven asexual and five sexual species. We found higher d*N*/d*S* ratios in asexual *Aspidoscelis* species, indicating that asexual whiptails accumulate nonsynonymous substitutions due to weaker purifying selection. Additionally, we estimated nucleotide diversity and found that asexuals harbor significantly less diversity. Thus, despite their recent origins, slightly deleterious mutations accumulated rapidly enough in asexual lineages to be detected. We provide empirical evidence to corroborate the connection between asexuality and increased amino acid substitutions in asexual vertebrate lineages.

KEY WORDS: Molecular Evolution, Mutations, Genetic Variation, Sex, Selection -Natural.

The recognition of the paradox of sex has spearheaded efforts to identify the advantages of sex that make up for its high cost (Williams and Mitton 1973; Maynard-Smith 1978; Otto and Lenormand 2002). Theory predicts that the per-capita birth rate of asexual populations should rapidly cause the displacement of sexual populations. This is because asexual females only invest in daughters, unlike sexual individuals that must also invest in males; this is what John Maynard-Smith (1971, 1978) coined the "cost of males." Yet, asexual reproduction is rare in nature while sexual reproduction is pervasive, a pattern that has led to the long-standing and unresolved mystery that has been labeled as the queen of problems in evolutionary biology: "Why is there sex?" (Bell 1982; Barton and Charlesworth 1998).

The mutation-recombination model is one theory for the prevalence of sexual reproduction and is also supported by a substantial body of theoretical work (Otto and Gerstein 2006). Transitioning to asexuality abolishes the benefits of recombination and segregation and thrusts the whole genome into complete linkage (Hill and Robertson 1966). The tight association between all loci reduces the effective population size of linked genes, which weakens the efficacy of natural selection to act independently on individual loci and strengthens the influence of genetic drift on the fate of *de novo* mutations, resulting in an irreversible accumulation of mildly deleterious mutations (Muller's ratchet), a decline of individual fitness, and eventual population extinction (Muller 1964). In contrast to asexual reproduction, sex breaks the linkage between genes via recombination and segregation, thereby increasing the efficiency of selection to remove deleterious variants and increase the frequency of beneficial mutations.

Among vertebrates, only squamates (lizards and snakes) have multiple species that are obligate parthenogens, which exhibit clonal reproduction via meiosis (Vrijenhoek et al. 1989; Kearney et al. 2009). The hypothesized cellular mechanisms to produce genetically identical daughters via meiosis in obligately parthenogenetic whiptails requires a second doubling of DNA before entering meiosis (premeiotic endoreplication), allowing for the pairing of sister chromosomes to maintain heterozygosity, and finally the production of unreduced eggs during meiosis II (Cuellar 1971; Lutes et al. 2010; Newton et al. 2016). Obligately parthenogenetic lizards and snakes have arisen multiple times

independently across nine families but represent <1% of squamate diversity (Fujita et al. 2020). Whiptail lizards in the genus *Aspidoscelis* have 15 known all-female asexual species that have hybrid origins: hybridization events between two distinct extant sexual species resulted in initial diploid parthenogens, with backcrossing or even further hybridization with a third sexual species producing triploids (Neaves and Baumann 2011). Different diploid asexual whiptail species have independent origins from one another, though it is still unclear whether single or multiple hybridization events occurred within a species (Reeder et al. 2002).

Sex chromosomes in mammals and birds provide insight into the long-term (>10 million years) evolutionary trajectory of the genome in the absence of recombination (Graves 2006). However, obligately parthenogenetic lizards provide an opportunity to examine mutation accumulation at much more recent timescales (<1 million years). Furthermore, parthenogenetic whiptail lizards are exceptional systems to address questions surrounding the evolution of sex because they serve as a contrasting model to how sexual reproduction influences genome evolution (Kearney et al. 2009; Fujita et al. 2020). Due to their high abundance and wide distribution throughout the southwestern US and northern Mexico, asexual whiptail lizards (*Aspidoscelis*) are excellent model systems to investigate the fundamental cellular mechanisms of parthenogenesis and the genomic consequence of asexuality (Cuellar 1971; Lutes et al. 2010; Newton et al. 2016). A few studies have begun leveraging parthenogenetic genomes to test whether weakened selection and mutation accumulation are characteristic of asexual vertebrate populations (Moritz 1991; Fujita et al. 2007; Boussau et al. 2011).

Most studies investigating the genetic consequences of asexuality using DNA sequence data have primarily focused on asexual invertebrates and plants. These studies have yielded mixed results when examining whether or not mutation accumulation occurs in asexual lineages. Two studies using asexual stick insects in the genus *Timema* found a higher rate of nonsynonymous to synonymous mutations in both mitochondrial and nuclear coding genes of asexual populations (Henry et al. 2012; Bast et al. 2018). The New Zealand snail *Potamopyrgus antipodarum* has mixed populations of sexual and asexual individuals. The results demonstrated an increased point-mutation accumulation in mitochondrial coding genes, with asexual snails having a d*N*/d*S* ratio two times higher than sexuals (Neiman et al. 2010). Transcriptome- and genome-wide studies respectively found that two distinct asexual plant species exhibited a higher load of deleterious mutations than sexual plant species (Hollister et al. 2015; Lovell et al. 2017). On the other hand, recent studies using whole genomic- and transcriptomic-wide data have found evidence that is contrary to the expectation of mutation accumulation in asexual populations. Transcriptome-wide data from oribatid mites revealed that purifying selection was more effective in the extremely old asexual lineages (~10 million years old) than in sexual lineages (Brandt et al. 2017, 2021). Nonrecombining portions of the genome are expected to accumulate transposable elements (TE) and other repetitive content that leads to genomic degradation such as in the Y-chromosomes (Junakovic et al. 1998; Bachtrog 2013). Yet, genome-wide data from several distinct asexual crustacean species had no evidence of increased TE content (Bast et al. 2016). Similarly, asexual aphid lineages show no evidence of increased mutation accumulation in mitochondrial genes, and only one lineage had increased mutation accumulation in nuclear genes (Normark and Moran 2000). The mixed results between studies show that the expected effect of suppressing recombination across the whole genome does not always lead to the detrimental genetic consequences we expect based on our predicted theory (Jaron et al. 2021). The differences among asexual species, such as the cellular mechanism of asexuality, the age of the asexual lineage, and the method of transitioning to asexuality, could affect the genetic diversity across distinct asexual species.

Our study uses whole mitochondrial genome data from asexual and sexual whiptail lizards to investigate our prediction that parthenogenetic lineages accumulate mutations faster than sexual lineages. We calculated d*N*/d*S* ratios between asexual and sexual lineages, carried out selection tests on asexual lineages to see if these lineages have undergone positive selection, estimated nucleotide diversity levels between asexual and sexual groups, and estimated the age of asexual lineages.

Methods

We sampled multiple populations of both asexual and sexual whiptail species throughout the southwestern United States and received additional tissue samples from the Burke Museum and American Museum of Natural History collections. We collected individuals by hand, lasso, or using rubber bands in accordance with our IACUC protocol (A13.010). We humanely euthanized each individual and collected liver and skeletal tissue, and stored them in RNA later (Thermo Fisher Scientific, Waltham, MA). Specimens were preserved using 10% formalin, stored in ethanol, and deposited at the Amphibian & Reptile Diversity Research Center at the University of Texas at Arlington. We sequenced 86 individuals: 18 *A. tesselata*, 14 *A. neotesselata*, 14 *A. marmorata*, 10 *A. inornata*, 8 *A. gularis*, 7 *A. exsanguis*, 5 *A. sexlineata*, 4 *A. neomexicana*, 2 *A. dixoni*, and *A. laredoensis*, 1 *A. velox*, and *A. scalaris*. Supporting information Table S1 has the locality data associated with each sequenced sample.

We extracted DNA from liver tissue using a standard phenol-chloroform protocol and measured the concentrations using a

Qubit 2.0 (Invitrogen, Carlsbad, CA). To sequence the whole mitochondrial genome, we followed the protocol from Roelke et al. (2018). Briefly, we used exonucleases that specifically remove double-stranded linear DNA. The remaining intact circular mitochondrial genomes are then isolated using SeraPure beads (Rohland and Reich 2012) and resuspended in fresh buffer (10 mM Tris, pH 8.0). We amplified the mitochondrial genome using Φ29 DNA polymerase before following standard Illumina library preparation to generate genomic libraries. Each individual was dual-labeled with inline barcodes and standard Illumina indices before sequencing on the Illumina MiSeq (Illumina) to generate 300 bp paired-end reads.

Our sequenced reads were filtered using the FASTX-Toolkit to remove low-quality reads and reads shorter than 50 bp long. We assembled the genomes using the CLC Genomics WB 9 (Qiagen, Germantown, MD) short-read assembler. We used the Mitos web server (Bernt et al. 2013) to annotate the mitochondrial genome for protein-coding, RNA, and tRNA genes. Because the origins of parthenogenesis in vertebrates are essentially founder events, we expect to see limited genetic diversity in parthenogenetic whiptails compared to sexual lineages. To examine this, we calculated nucleotide diversity (π) for each whiptail species using DNAsp version 6 (Librado and Rozas 2009).

We used the Geneious R6 aligner to align each individual protein-coding gene and both rRNA genes. We then used a concatenated alignment to identify the best partitioning scheme and evolutionary models with PartitionFinder 2 (Lanfear et al. 2017) using the following parameters: linked branch lengths, the greedy algorithm, BIC for our selection model, and the following models of evolution that can be implemented in BEAST. To estimate divergence times and to obtain a rooted tree, we used a Bayesian statistical framework with BEAST version 2.6.6 (Bouckaert et al. 2014) with *Ameiva undulata*, *Teius teyou*, *Gymnophthalmus speciosus*, and *Podarcis muralis* used as outgroups. We used two calibration points with uniform priors: (1) fossil of an ancestor for living cnemidophorines (GHUNLPam21745, 9.0-10.0 Ma) with lower and upper bounds of 9 and 86 Ma, respectively (Albino et al. 2013; Tucker et al. 2017); and (2) the node of Gymnophthalmoidea (Teiidae + Gymnophthalmidae) with lower and upper bounds of 70 and 86 Ma, based on previous squamate studies (Vidal and Hedges 2009; Pyron 2010; Mulcahy et al. 2012; Tucker et al. 2017). We performed the analysis on our concatenated mitochondrial dataset since the mitochondrial genes are linked together, using a GTR+I nucleotide substitution model (as predicted by PartitionFinder), a log-normal relaxed molecular clock, and a birth-death model. We ran the analysis of three independent runs with 20,000,000 generations, sampling every 1000 generations, producing a total of 20,000 trees. The runs were assessed using Tracer version 1.6 (Rambaut 2009) to examine convergence and runs were combined in LogCombiner version 2.6.6. A burn-in of 10% was discarded, and a maximum clade credibility tree with median heights was created from the remaining trees. To produce an unrooted phylogenetic tree needed for carrying out the d*N*/d*S* analysis, we used MrBayes and PartitionFinder 2 with the same parameters described above (Fig. 1). We first removed three samples that failed to assemble into the complete ~16 kb genome as we could not allow missing data for our d*N*/d*S* analysis. Additionally, we removed the outgroups from the concatenated dataset because it can only be classified as sexual (Neiman et al. 2010).

We carried out our d*N*/d*S* analysis on our concatenated alignment of 13 protein-coding genes using the branch model implemented in the codeml program from the PAML version 4.9 package (Yang 2007). Codeml works by calculating the fitness of our specified model of evolution to a phylogenetic data set and uses likelihood ratio tests to assess which model of evolution provides the best fit to the data. We used codeml to compare the goodness of fit of our specified evolutionary models to our unrooted phylogenetic tree and protein-coding sequence data. These ranged from the simplest models where the entire whiptail phylogeny experienced the same rate of evolution to more complex models that allow sexual and asexual whiptail lineages to evolve at different rates. We first ran codeml's one-ratio maximum likelihood model (M0: one omega ratio for all branches) to estimate a single d*N*/d*S* value for our unrooted whiptail phylogeny. The second model we ran was the two-ratio model, where sexual and asexual lineages were allowed to have different d*N*/d*S* values (M2: two or more d*N*/d*S* ratios for branches). We then carried out a likelihood ratio test to assess whether the two-ratio or the one-ratio model provided a better fit to the data. An elevated d*N*/d*S* value for the asexual lineages and a significantly better fit of the two-ratio model to the data would imply that asexual whiptails accumulate deleterious mutations faster than sexual whiptails (Neiman et al. 2010). Additionally, we estimated the d*N*/d*S* ratio per gene using the M0 and M2 model to test for an elevated nonsynonymous substitutions in asexuals.

The young age of asexual whiptails lineages (Cullum 1997; Birky 2010) can confound the differences between sexual and asexual d*N*/d*S* due to differences in branch length between sexual and asexual whiptail lineages. The time lag between the origin of new mutations and their removal by purifying selection results in a higher estimate of mutation accumulation in terminal branches (Neiman et al. 2010). To address this issue, we used codeml to evaluate the fitness of two additional models to our data that allowed the terminal and internal branches to have different evolutionary rates (terminal vs. internal branches). The additional model we ran was similar to Neiman et al. 2010, where we categorized branches in our unrooted phylogenetic tree as terminal sexual, terminal asexual, internal sexual, and internal asexual (four-ratio model). We used likelihood ratio tests to compare

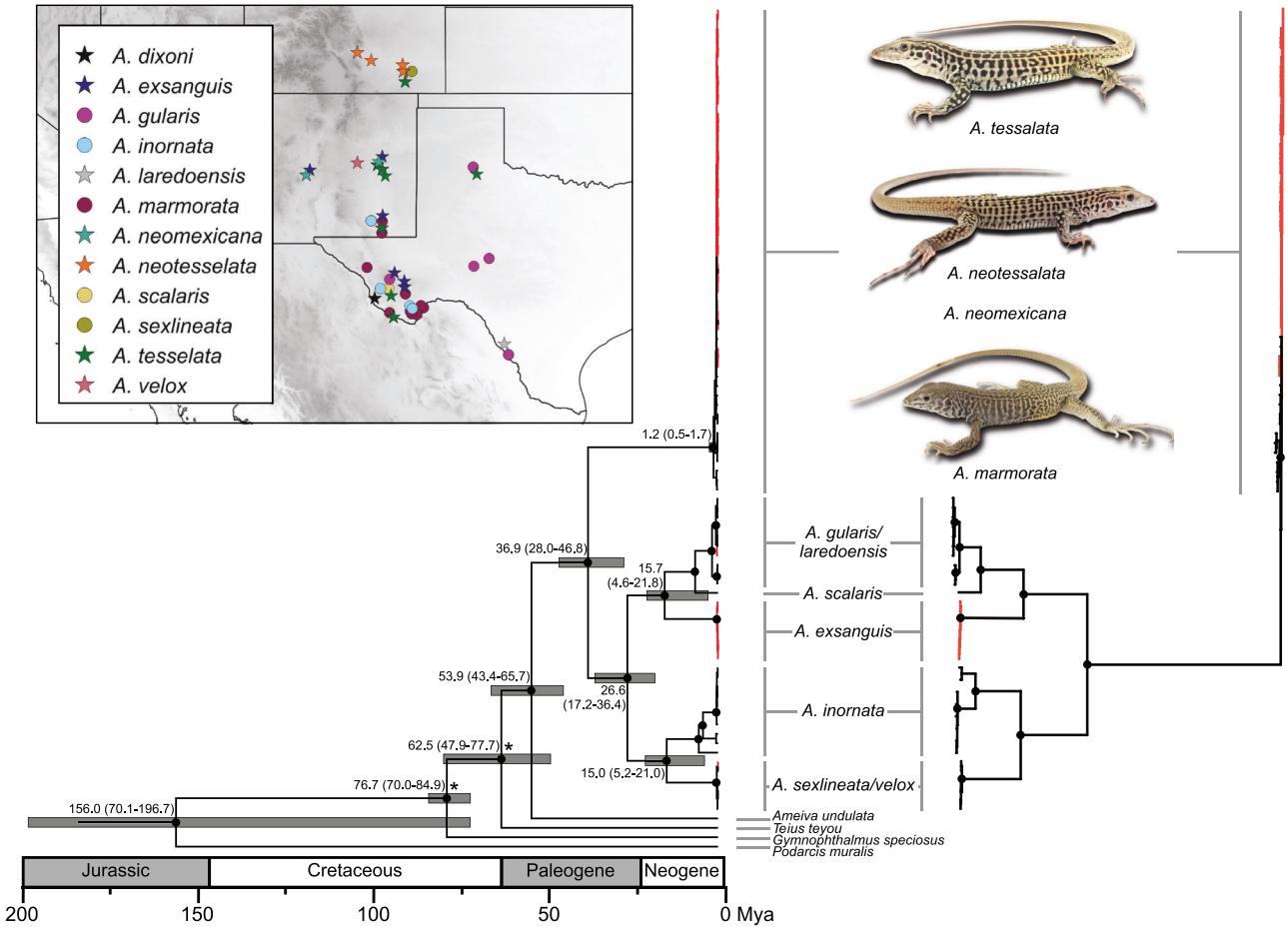

Figure 1. **Sampling map of all individuals used in genetic analyses (asexuals denoted with stars; sexuals denoted with circles). Samples of *A. neotesselata* (ADL 4935–4938) from Washington are not shown in the map, but were used for all analyses. Bayesian chronogram (left) and unrooted Bayesian phylogram (right) were made from our concatenated dataset. Red branches on both trees indicate asexual individuals, whereas black branches represent sexual individuals. Node/fossil calibrations are indicated by *. Median ages, 95% highest probability densities (HPD), and bars representing the 95% HPDs are provided. Nodes on both trees with high support (posterior probability ≥ 0.95) are indicated by black dots. The unrooted Bayesian phylogram had posterior probabilities >0.50 and the tree topology was used for all d*N*/d*S* ratio analyses.**

the fit of the model of the terminal versus internal two ratio-model to our one-ratio model and compare the four-ratio model to both two-ratio models (asexual vs. sexual and terminal vs. internal) and the one-ratio model. As a simple test to make sure neutral evolution did not produce our empirical results, we generated a simulated nucleotide data set using the Evolver codon setting from the PAML V4.9 package (Yang 2007), and we reran the one-ratio vs. two-ratio model and compared the results from our simulation to our empirical dataset. Evolver generated our simulated DNA sequences by using our unrooted tree, and using the Monte Carlo simulation model, and we kept all other parameters at default.

If asexual whiptails lineages exhibit a higher d*N*/d*S* ratio than their sexual counterpart, we had to test that the inflated ratio was not caused by positive selection but by an increase of nonsynonymous substitutions (d*N*). To do so, we ran the RELAX and aBSREL selection test on our dataset found in the Hypothesis Testing using Phylogenies version 2.3 software package (Pond et al. 2005). The RELAX selection test asks whether the strength of natural selection has been intensified or relaxed along specified set test branches. In our RELAX selection test, we labeled asexual branches as our test branches and sexual branches as references branches. RELAX uses the parameter k, which is the selection intensity parameter, where $k > 1$ indicates selection has been intensified and $k < 1$ selection has been relaxed along our specified test branches (Wertheim et al. 2015). The aBSREL model tested if asexual branches in our phylogeny have evolved under positive selection. To do so, aBSREL tests whether a proportion of sites have evolved under positive selection in our specified branches of interest. aBSREL then runs Likelihood Ratio Tests to compare the adaptive model ($\omega > 1$) to the null model (Smith et al. 2015).

Table 1. Summary of PAML analyses and comparisons of d*N*/d*S* ratios in coding mitochondrial DNA genes in sexual versus asexual whiptails.

Coding genes	Number of free parameters	ln(L)	*p*-values	d*N*/d*S*
One Ratio	142	−36392.0908	NA	0.0429
Two Ratio (sex vs. asex)	143	−36380.4102	vs. one ratio: 1.343e-06	Sex: 0.03925
				Asex: 0.07251
Two Ratio (T vs. I)	143	−36359.4881	vs. one ratio: 6.7494e-16	I: 0.03750
				T: 0.1018
Four Ratio (SexT, AsexT, SexI, AsexI)	146	−36344.8913	vs. one ratio: 1.530e-19	SexT: 0.0912
			vs. two ratio (A vs. S): 2.559e-15	AsexT: 0.1903
			vs. two (T vs. I): 2.039e-06	SexI: 0.0380
				AsexI: 0.0613

"T" indicates terminal branches and "I" indicates internal branches.

Results

DE NOVO ASSEMBLY, GENE ANNOTATIONS, AND PHYLOGENETIC ANALYSIS

We sequenced 86 whiptail mitochondrial genomes. Three samples were missing several genes and failed to assemble into the complete ∼16 kb mitochondrial genome. Mitos successfully annotated protein-coding genes and identified the start and stop codons, both rRNA genes, and all tRNA genes. To conduct the d*N*/d*S* analysis, we removed the three individuals with incomplete mitochondrial genome assemblies to generate an unrooted tree (Fig. 1). The divergence dating analysis of our concatenated dataset strongly supports an Eocene divergence (36.9 Mya [95% highest probability densities [HPD]: 28.0-46.8 Mya]) between the *Aspidoscelis marmorata* group (including *marmorata* and the parthenogenetic *tesselata*, *neotesselata*, and *neomexicana*) from the remaining *Aspidoscelis* in our study. The *marmorata* clade dates to within the Pleistocene (1.26 Mya [95% HPD: 0.54–1.77 Mya]), as does the origin of *A. tesselata* (0.36 Mya [95% HPD: 0.1–0.4 Mya]. Other parthenogenetic lineages (*exsanguis* and *laredoensis*) have Pleistocene origins. The ESS values for our combined runs were all ≥200.

dN/*dS* ANALYSIS AND NUCLEOTIDE DIVERSITY

We found that the two-ratio model (M2) fit our data better when comparing the two-ratio to a one-ratio model (M0) that only allows for one d*N*/d*S* for the entire tree. PAML showed that asexuals have ∼2× higher d*N*/d*S* than sexual whiptail lineages (d*N*/d*S* = 0.0725 for asexuals vs. 0.0395 for sexuals; Table 1). The four-ratio model allows four d*N*/d*S* estimates between internal and terminal branches for both asexuals and sexual whiptails. The four-ratio model had a significantly better fit to the data than the two-ratio model (asexual vs. sexual) and one-ratio model (Table 1). Our d*N*/d*S* estimates per gene found that six out of the 13 mitochondrial coding genes in asexual lineages had a significantly higher d*N*/d*S* than their sexual lineages (Table 2). We found that the other seven genes had no differences in d*N*/d*S* between both groups. While asexual whiptail lineages had an overall elevated d*N*/d*S*, we generally found higher levels of polymorphisms in sexual whiptail species. *Aspidoscelis sexlineata* is the only sexual whiptail species with a slightly lower nucleotide diversity of $\pi = 0.0012$ than the asexual *A. tesselata* species with $\pi = 0.0017$. We found the sexual species with the highest nucleotide diversity was *A. gularis* ($\pi = 0.0160$), while the asexual species with the lowest nucleotide diversity was the triploid *A. neotesselata* ($\pi = 0.0006$) (Table 3). We ran both M0 and M2 models on our simulated nucleotide dataset generated by Evolver and found no significant differences in d*N*/d*S* estimate between asexual and sexual whiptail lizards, indicating that our results deviate from the null expectation of our simulations.

Table 2. Six out of the thirteen protein-coding genes had a higher d*N*/d*S* in asexual lineages.

Gene	One ratio d*N*/d*S*	Two ratio d*N*/d*S*	*p*-values
COB	0.0173	Sex: 0.0140 Asex: 0.0517	0.0079
COX III	0.0271	Sex: 0.0227 Asex: 0.0719	0.0413
NAD I	0.0200	Sex: 0.0160 Asex: 0.0532	0.0132
NAD II	0.0485	Sex: 0.0428 Asex: 0.0909	0.0296
NAD IV	0.0536	Sex: 0.0461 Asex: 0.1035	0.0084
NAD V	0.0508	Sex: 0.0461 Asex: 0.0960	0.0223

SELECTION TEST

To ensure that the elevated d*N*/d*S* we found in asexual lineages was due to weakened purifying selection and not positive

Table 3. Nucleotide diversity (π and θW) was estimated from using 13 protein-coding genes for asexual and sexual whiptails.

Species	No. of individuals	Nucleotide diversity π	θW
A. neotesselata	14	0.0006	6E-04
A. exsanguis	7	0.001	0.001
A. neomexicana	4	0.0018	0.002
A. sexlineata	5	0.0012	0.001
A. tesselata	17	0.0017	0.002
A. marmorata	13	0.0047	0.006
A. inornata	8	0.015	0.014
A. gularis	8	0.016	0.019

selection, we carried out selection tests to determine if our asexual branches underwent positive selection. Both RELAX and aBSREL selection tests found no evidence of positive selection in asexuals. Our test of relaxation or intensification among asexual branches using the RELAX model found significant (p-value $<$ 0.05) relaxation in asexual lineages with a $k = 0.39$. aBSREL tested our branches of interest for diversifying selection and found no significant evidence of episodic diversifying selection in our asexual branches. To determine significance aBSREL assessed the alternative to the null model using the Likelihood Ratio Test at a threshold of $p \leq 0.05$, after correcting for multiple testing.

Discussion

Our study used whole mitochondrial genomes to study the genomic consequence of asexuality in parthenogenetic lizards. We found that the transition to asexuality led to relaxed natural selection in parthenogenetic lizards and the build-up of nonsynonymous mutations. Our findings support theoretical predictions that the loss of sex should lead to an irreversible build-up of deleterious mutations due to a reduction in the efficiency of purifying selection, and sex facilitates the removal of harmful mutations.

Our empirical data and results are similar to those of other younger and ancient asexual lineages (Neiman et al. 2010; Bast et al. 2018) and other nonrecombining genomes such as sex chromosomes. Nonrecombining sex chromosomes have evolved multiple times and accumulated both point and structural mutations, and many have degenerated to house just a few protein-coding genes (Vicoso 2019). The degradation of the mammalian Y and avian W sex chromosomes from the lack of recombination provided some of the best evidence for the long-term consequences of clonal inheritance (Fujita et al. 2020). Even the recently formed neo-Y sex chromosome that occurred one million years ago (Bachtrog and Charlesworth 2002) in *Drosophila miranda* has rapidly accumulated mutations and repetitive DNA (Bachtrog et al. 2008). In whiptails, studies have identified structural mutations in the mitochondrial genome in several asexual whiptail species that exhibit tandem duplications that are adjacent to tRNA, rRNA, or protein-coding genes (Moritz and Brown 1987; Stanton et al. 1994). Our results of increased rates of nonsynonymous mutations (mutation accumulation) in parthenogenetic asexual whiptail mitochondrial genomes are consistent with the findings of these other studies.

Our findings reveal that asexual whiptails exhibit lower levels of polymorphism compared to sexual species. Factors that can generate a disparity of genetic diversity between asexual and sexual populations include: the age of the asexual lineage, the number of hybridization events that occurred during the formation of the parthenogenetic lineages (the severity of the bottleneck during the origin of parthenogenesis), and the heterozygosity generated by those hybridization events.

Our phylogenetic analysis can address the first of these three factors. It is important to note that, while we used similar methods to Tucker et al. 2017 to estimate divergence dates, their analysis places the divergence between the cnemidophorines and *Teius* (a node which they denote as Teiinae) at ~35 Mya, whereas our analysis places this split at ~62.5 Mya, which is closer to their estimated node of Teiidae. This may be due to our using only two nodes for calibration within this group and/or less taxonomic coverage throughout this group. Nevertheless, we were still able to show that the origins of asexual populations are much younger than those of the sexual populations. For example, parthenogenetic *A. tesselata* have a recent origin well within the Pleistocene (0.36 [0.1-0.4] Mya), which is the general pattern with regard to the age of asexual lineages in vertebrates (Fujita et al. 2020). Further evidence for the recency of *A. tesselata* is that the maternal lineage, *A. marmorata*, is still extant.

Based on our sampling across its distribution, we inferred a single origin of *A. tesselata* (or at most, a few origins). With so few origins, we expect such a bottleneck to result in low nucleotide diversity in *A. tesselata*. Compared to *A. marmorata*, this is exactly what we see; *A. marmorata* has nearly three times as much nucleotide diversity (θ_W) than *A. tesselata*. Furthermore, *A. neotesselata* has a nuclear diversity nearly ten times less than *A. marmorata*, which is consistent with an even more recent origin than *A. tesselata*. These patterns of reduced diversity and recent hybrid origins that we see in *A. tesselata* contrasts with some other parthenogenetic systems such as the darwinulid ostracods and several lineages of asexual oribatid mites. In these latter systems, divergence has accumulated via asexual speciation (William Birky and Barraclough 2009), with the existence of ancient (>10 million years) parthenogenetic lineages implying that they have avoided the long-term consequences of Muller's Ratchet (Heethoff et al. 2009; Schön et al. 2009). Given the contrasting origins of ancient asexuals and parthenogenetic lizards, it

is difficult to directly compare the evolutionary mechanisms that generate genetic diversity between the two groups.

High heterozygosity is often a signature of genomes from asexual organisms compared to their sexual relatives (Fujita et al. 2020). There are two main mechanisms that can produce this high heterozygosity in asexual lineages compared to sexual lineages. First, in the absence of recombination, haplotypes can diverge in what is known as the Meselson effect in ancient asexuals (Birky 1996; Welch et al. 2000). Recent genomic data have demonstrated the Meselson effect and the long-term evolution in oribatid mites (Brandt et al. 2021). Second, hybridization between divergent sexual progenitors instantaneously produces asexual lineages with high heterozygosity; this is the mechanism seen in nearly all parthenogenetic lizards (Fujita et al. 2020). Our mitochondrial data are unable to provide insight into whether the Meselson effect can be seen in parthenogenetic lizards, though we expect that any heterozygosity contributed by such divergence will be low compared to that contributed by hybrid origins.

Previous studies have found that even young asexual lineages (from ∼100,000 to ∼500,000 years old) have experienced an increased rate of nonsynonymous substitutions in mitochondrial genes compared to sexual lineages (Johnson and Howard 2007; Neiman et al. 2010; Henry et al. 2012). Our findings are in line with the theory that purifying selection in the mitochondrial genomes acts less effectively in asexual populations than in their sexual counterparts. We carried out several d*N*/d*S* analyses to test for mutation accumulation in protein-coding genes between asexual and sexual whiptail lizards. Using all 13 protein-coding genes, we found that asexual lineages accumulate nonsynonymous substitutions at a faster rate despite their relatively young age. Further, we found that this rate is due to relaxed selection rather than positive selection. While the uniparentally inherited and clonally reproducing mitochondrial genome exhibits no recombination, it can still serve as a valuable marker to investigate the genomic consequences of asexuality. In sexual populations, the nuclear genome and the mitochondrial genome still segregate from each other, while a transition to asexuality will force the nuclear and mitogenome into complete linkage. The tight linkage between the nuclear and mitochondrial genomes in asexuals should reduce their effective population sizes, resulting in less efficient selection and eventually an accumulation of nonsynonymous variants. Additionally, the mitochondrial genomes can be a useful marker to test the genomic consequences of asexuality, since mitochondria DNA evolves at a higher rate than nuclear DNA (Brown et al. 1979; Xia 2012).

Sex facilitates the long-term survival of metazoan lineages by allowing alleles to separate between generations (segregation) and by shuffling genetic variation (crossing over and independent assortment); thus, sex increases both genetic diversity and the efficiency of natural selection, allowing populations to purge deleterious alleles and to bring together beneficial variants. On the other hand, asexuality is often viewed as an evolutionary dead-end because of the long-term consequences of accumulating harmful mutations and the reduced capabilities to adapt to novel environments (Vrijenhoek 1998; Butlin 2002). While some studies have seen possible evidence of adaptive evolution in asexual organisms (Jaron et al. 2021), our study is more consistent with the expectations that reduced effective population sizes (due to asexuality and complete linkage) results in less effective natural selection, leading to the accumulation of mutations.

CONFLICT OF INTEREST

No one involved in this project had any conflict of interest.

AUTHOR CONTRIBUTIONS

JAM and MKF conceived the work. JAM collected and processed the data. JAM and TJF analyzed the data. ASH contributed the majority of the samples. JAM wrote the manuscript with contributions by TJF and MKF.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 A complete list of our samples we sequenced with their locality data.