

A New Diploid Parthenogenetic Whiptail Lizard from Sonora, Mexico, Is the “Missing Link” in the Evolutionary Transition to Polyploidy

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Submitted September 23, 2020; Accepted February 27, 2021; Electronically published June 4, 2021

Online enhancements: supplemental PDF. Dryad data: <https://doi.org/10.5061/dryad.2z34tmpm2>.

ABSTRACT: Transitions between sexual and unisexual reproductive modes have significant consequences for the evolutionary trajectories of species. These transitions have occurred numerous times in vertebrates and are frequently mediated by hybridization events. Triploid unisexual vertebrates are thought to arise through hybridization between individuals of a diploid unisexual lineage and a sexual species, although additional evidence that confirms this mechanism is needed in numerous groups. North American whiptail lizards (*Aspidoscelis*) are notable for being one of the largest radiations of unisexual vertebrates, and the most diverse group of *Aspidoscelis* includes numerous triploid lineages that have no known diploid unisexual ancestors. This pattern of “missing” ancestors may result from the short evolutionary life span of unisexual lineages or the selective advantages of polyploidy, or it could suggest that alternative mechanisms of triploid formation are operating in nature. We leverage genomic, morphological, and karyotypic data to describe a new diploid unisexual whiptail and show that it is likely the unisexual progenitor of an extant triploid lineage, *A. opatae*. We also resolve patterns of polyploidization within the *A. sexlineatus* species group and test predictions about the phenotypic outcomes of hybridization.

Keywords: hybridization, speciation, *Aspidoscelis*, genomics, phenotypic evolution.

Introduction

Transitions in reproductive mode have extensive consequences for organismal evolution and diversification (Dar-

win 1859; Weismann 1889; Fisher 1922; Mayr 1963; Maynard Smith 1968). Despite the theoretical costs of sexual reproduction (Maynard Smith 1971), the vast majority of eukaryotic organisms reproduce sexually. This is thought to result from the prediction that unisexual populations with clonal inheritance have slower rates of adaptation than sexual populations and are subject to the accumulation of deleterious mutations (because they lack genetic recombination; Fisher 1930; Muller 1964; Bell 1982; Bast et al. 2018). Therefore, even when unisexual species arise, they are expected to be evolutionarily ephemeral owing to high rates of extinction (Vrijenhoek 1989; but see Schwander and Crespi 2009; Janko 2014). Although unisexual vertebrates are numerically rare, they are phylogenetically widespread (Schön et al. 2009). Many of these species reproduce by true parthenogenesis, a form of clonal reproduction in which embryonic development proceeds from an unfertilized egg (Darevsky et al. 1985; for a discussion of related unisexual reproductive modes in vertebrates, see Neaves and Baumann 2011). The evolutionary mechanisms that generate and maintain parthenogenetic diversity are assumed to be largely conserved across vertebrates, particularly in comparison to the diversity of parthenogenetic modes observed in invertebrates (Beukeboom and Vrijenhoek 1998). However, this phenomenon is still poorly studied in many vertebrate groups, and novel parthenogenetic lineages continue to be discovered (Grismer and Grismer 2010; Abdala et al. 2016).

Nearly all obligately unisexual vertebrates are thought to have been formed by hybridization, owing to meiotic disruptions that occur when chromosomes from divergent sexual lineages are brought together in hybrid offspring (Moritz et al. 1989a). Despite a clear association between

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ZooBank ID: [urn:lsid:zoobank.org:pub/924373C2-244F-4401-BD35-EF232B031239](https://zoobank.org/pub/924373C2-244F-4401-BD35-EF232B031239).

Am. Nat. 2021. Vol. 198, pp. 295–309. © 2021 by The University of Chicago. 0003-0147/2021/19802-60226\$15.00. All rights reserved.

DOI: 10.1086/715056

the evolution of unisexual reproduction and hybridization, the mechanisms that underlie these transitions remain poorly understood. In large part, this stems from a dearth of evidence from empirical systems that can be brought to bear in testing theoretical models for this process (Janko et al. 2018). The evolution of unisexual reproduction is thus facilitated by hybridization but is counterbalanced by evolutionary processes that limit the formation and persistence of these lineages over longer timescales (Simon et al. 2003; Kono 2006; Burke and Bonduriansky 2017; Boyer et al. 2021). The hybrid origin of unisexual lineages has further been proposed as an intrinsic explanation for their ecological success. For example, this could be explained by a heterosis-like process due to high heterozygosity that becomes fixed on the transition to clonal reproduction or the evolution of unique phenotypes and ecological roles when novel combinations of genotypes are produced by hybridization (Schultz 1971; Dessauer and Cole 1984; Vrijenhoek and Parker 2009). Polyploid lineages are prevalent in many groups of unisexual vertebrates, and ploidy elevation has also been proposed as a mechanism that compensates for disadvantages of unisexual reproduction (e.g., by providing an additional genetic source of adaptive flexibility; Neaves and Bauman 2011).

The majority of polyploid parthenogenetic vertebrates are thought to be derived through a multistep process (fig. 1a; Bogart 1980; Schultz 1980). The first “primary hybrid” step in the process involves an initial hybridization event between two sexual species that generates a diploid parthenogenetic clone (Schultz 1969; Alves et al. 2001; Avise 2008). In the subsequent “genome addition” step of the process, this “ancestor” produces an unreduced egg containing both parental genomes, which is then fertilized through a backcrossing event with one of the original parental species or a third sexual species (Lowe and Wright 1966; Cole 1979). One alternative theoretical route to polyploidy is a spontaneous origin model in which a nonhybrid female produces an unreduced gamete that is fertilized by sperm from a male of another species (Cimino 1972; Avise 2008). Another potential route is through a genome duplication model, in which suppression of a meiotic equational division in a hybrid female produces a fully homozygous, unreduced gamete that is fertilized by sperm from another species. Because diploid parthenogenetic ancestors of many polyploid vertebrates are unknown, we currently lack confirmatory evidence that mechanisms of parthenogenesis and polyploid formation are conserved across species (Adams et al. 2003; Pellegrino et al. 2003; Fujita et al. 2007). Furthermore, previous studies have found the phenotypic outcomes of hybridization and ploidy elevation to be inconsistent (Kearney and Shine 2004). As a consequence, the ecological and evolutionary mechanisms that mitigate competition between parthenogenetic lineages and their sexual progeni-

tors are poorly understood (Case 1990; Hanley et al. 1994). Additional empirical data are needed to refine the theory that has been developed and better understand the dynamics of these processes.

The North American whiptail lizards (*Aspidoscelis*) are a classic study system in ecology and evolutionary biology because numerous parthenogenetic lineages coexist with diverse sets of sexual species from which they arose through hybridization (Wright and Vitt 1993; Neaves and Baumann 2011). Ambiguity about the number of hybridization events that have contributed to parthenogenetic whiptail diversity and about which sexual species have been involved has limited our understanding of allopolyploidization, its association with parthenogenetic reproduction, and the outcomes of this process. Both diploid lineages (derived from a single F_1 hybrid female) and triploid lineages of parthenogenetic whiptails are known from nature, and even novel tetraploid lineages have been generated by hybridizing lizards in the laboratory (Lutes et al. 2011; Cole et al. 2014, 2017). The primary hybrid and genome addition processes are known to operate in some of the whiptails, evidence for which includes the existence of diploid parthenogenetic ancestors to certain triploid lineages as well as cytogenetic patterns (Neaves 1969; Parker and Selander 1976; Dessauer and Cole 1989). More data are needed to understand whether these processes are general across *Aspidoscelis*. This is particularly true for triploid parthenogenetic whiptails that lack known diploid ancestors and carry two copies of the nuclear genome and the mitochondrial genome from the same sexual progenitor. This pattern of ancestry can be produced by any of the polyploidization models discussed above, and so the underlying process cannot be inferred without additional information (Avise et al. 1992). Locating ancestral diploid parthenogenetic lineages (if they are still extant) and reconstructing their evolutionary history would help elucidate the mechanistic association between hybridization and the evolution of parthenogenesis, the extent to which mechanisms of polyploidization are conserved across vertebrates, and the number of times these processes have occurred. These lineages could also provide information on the evolutionary life spans of parthenogenetic lineages and advantages of polyploidy in nature.

The most poorly understood group of parthenogenetic whiptails includes four triploid lineages from the southwestern United States and northern Mexico: *A. opatae*, *A. sonorae*, *A. uniparens*, and *A. velox*. Previous work suggests that these lineages are derived from hybridization between two polytypic sexual species complexes (Dessauer and Cole 1989). These complexes include little striped whiptails (*A. inornatus* complex) and large spotted whiptails (*A. burti* complex), each containing numerous described taxa that have poorly characterized species boundaries (Duellman and Zweifel 1962; Wright and Lowe 1993). No diploid

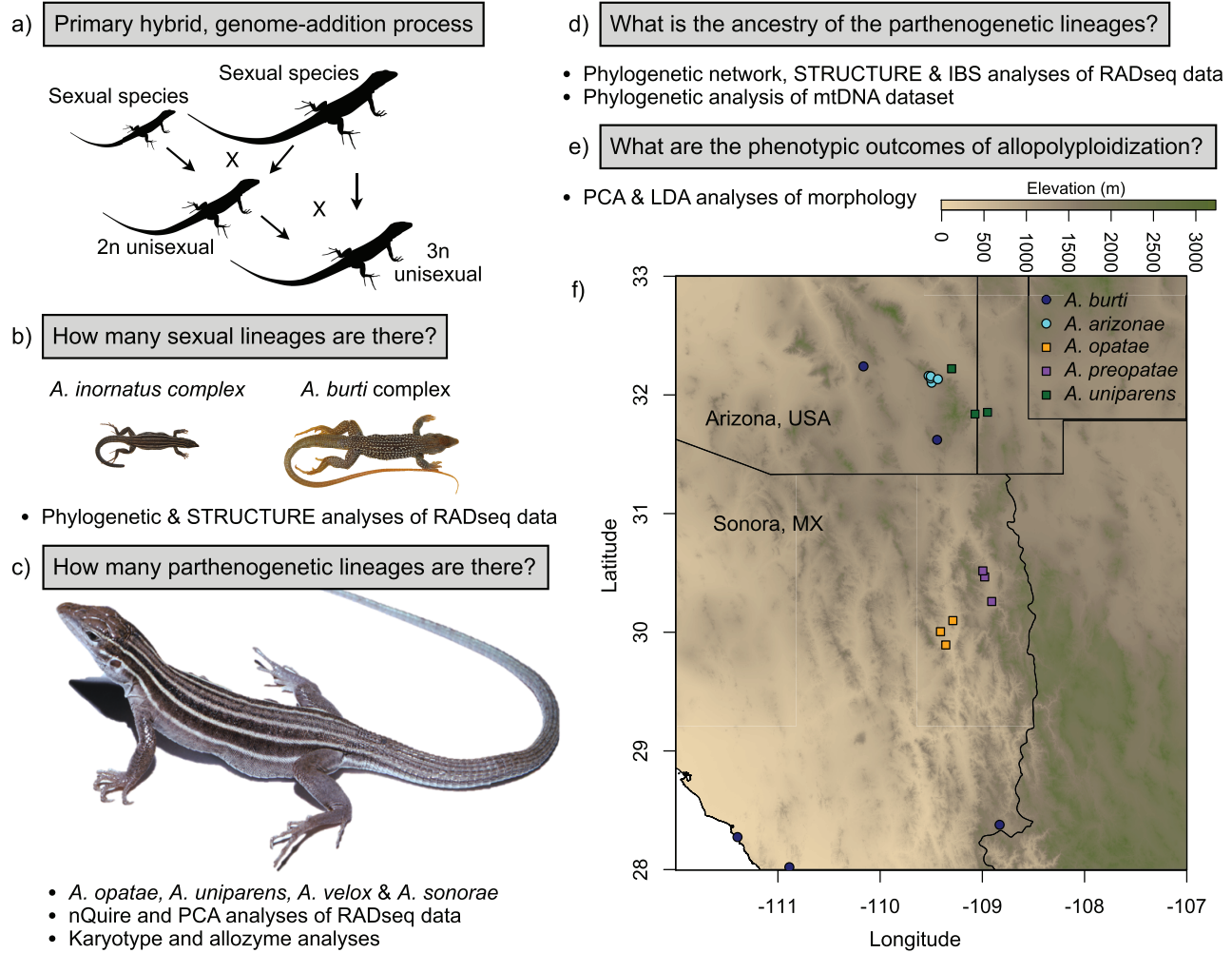


Figure 1: Outline of study. *a* shows a model of the process by which polyploid whiptails are thought to form. *b–e* indicate major questions addressed along with corresponding analyses used to resolve each. *c* lists the described triploid parthenogenetic lineages studied here; the photo is of the *Aspidoscelis opatae* topotype from near Oputo, Sonora (AMNH R-148251; snout-vent length, 72 mm). *f* shows a map of sampling localities for parthenogenetic lineages (squares), including the novel diploid populations identified here (*A. preopatae*), as well as the most closely related populations of their sexual ancestors (circles) from which they are derived by hybridization (*A. burti* and *A. arizonae*). IBS = identity by state; LDA = linear discriminant function analysis; mtDNA = mitochondrial DNA; RADseq = restriction site-associated DNA sequencing; PCA = principal components analysis.

parthenogenetic lineages exhibiting this combination of genetic ancestry are known, despite historical speculation that such populations may remain undiscovered (Wright et al. 1983; Densmore et al. 1989; Wright 1993). Through new fieldwork and studies of natural history museum archives, we assembled large genomic and phenotypic data sets and used them to (1) resolve the evolutionary history of sexual and parthenogenetic lineages in this group and (2) determine whether cryptic, diploid parthenogenetic populations have been overlooked (fig. 1). We identify one such lineage that we describe here as a new species. We then used these results to (3) examine the extent to which the genetic mechanisms that underlie allopolyploidization in parthenogenetic

vertebrates are conserved and (4) elucidate the phenotypic outcomes of hybridization and ploidy elevation across numerous morphological traits and ancestry combinations.

Material and Methods

Sampling

We sampled populations of taxa in the *Aspidoscelis burti* and *A. inornatus* sexual species complexes of the southwestern United States and Mexico as well as populations of the parthenogenetic lineages thought to be derived from these species (*A. opatae*, *A. sonorae*, *A. uniparens*, and *A. velox*). We

also revisited several historical collection localities for these taxa (see sec. S1 of the supplemental PDF, available online). We collected genomic data using a modified version of a double-digest restriction site–associated DNA sequencing protocol (ddRADseq; Peterson et al. 2012), sequencing the resulting library on the Illumina NovaSeq SP platform using a 100SR/10× protocol as part of a larger sequencing effort. We also sequenced the NADH-ubiquinone oxidoreductase chain 1 mitochondrial gene (*ND1*) for a subset of our samples. Through examination of notes and data archived at the Natural History Museum of Los Angeles County (LACM) and the University of Arizona Museum of Natural History (UAZ), we present novel karyotype data for several parthenogenetic lineages. Most of these data were generated by C. J. Cole in approximately 1968 from lizards collected by J. Wright, using standard methods for preparing Giemsa-stained chromosomes (Cole 1979). We also present a set of 20 allozymes that were developed by H. C. Dessauer for samples collected by C. J. Cole.

Genomic Ancestry

We used the RADseq read count data to estimate sample ploidy using the nQuire (Weiß et al. 2018) statistical framework (supplemental PDF, sec. S2). For genetic analyses of the diploid taxa, we assembled the RADseq data de novo using ipyrad (ver. 0.9.26; Eaton and Overcast 2020) under default parameters after identifying an optimal clustering threshold of 0.9, as described in McCartney-Melstad et al. (2019). For genetic analyses including triploid individuals, we assembled the data de novo using the dDocent pipeline (Puritz et al. 2014) and quality-filtered variants using vcflib (<http://github.com/ekg/vcflib>). In all genetic analyses, we excluded genotypes with <10× (for diploids) or <15× (for triploids) coverage and selected loci that minimized the proportion of missing data based on the included samples.

We estimated phylogenetic relationships among all of the sexual individuals in the concatenated RADseq data set to identify the major genetic groups within the two species complexes. We performed a phylogenetic analysis of the *ND1* data to resolve the mitochondrial genealogical relationships among the parthenogenetic lineages and their maternal ancestors. We used MrBayes (ver. 3.2.6; Ronquist et al. 2012) to run both of these phylogenetic analyses (see sec. S3 of the supplemental PDF for methodological details). We also used the admixture model in STRUCTURE (Pritchard et al. 2000) to identify how finely we could distinguish population genetic structure within the sexual species using the RADseq data (see sec. S3 of the supplemental PDF for methodological details). We then used STRUCTURE to estimate the genetic ancestry of each parthenogenetic lineage. Because this model is sensitive to hierarchical levels of genetic struc-

ture, we were able to estimate ancestry on only a very coarse level (even with the LOCPRIOR model), and we present the results of an analysis for $k = 2$ (to account for the two divergent genetic source pools for the sexual species from which the parthenogenetic lineages are drawn). We more finely quantified ancestry of the parthenogenetic lineages through an identity-by-state (IBS) analysis using the SNPRelate package (Zheng et al. 2012) in R (ver. 3.5.1; R Core Team 2018). After pruning the data set of single-nucleotide polymorphisms (SNPs) that were in linkage disequilibrium (with the snpgdsLDpruning function and a threshold of 0.2), we used the snpgdsIBS function for this analysis, including several representative samples from each sexual population that was identified by STRUCTURE. We evaluated the distinctiveness of the parthenogenetic lineages that had similar patterns of genetic ancestry by performing a principal components analysis (PCA) of the SNP data using the dudi.pca function in the R package ade4 (Dray and Dufour 2007) and by calculating F_{ST} using the Weir and Cockerham (1984) method with the snpgdsFst function in the SNPRelate package. To determine the mechanism by which the triploid parthenogenetic lineages formed, we assessed heterozygosity between their two paired nuclear genomes using the RADseq data and a polyploid genotyping protocol (Blischack et al. 2018; supplemental PDF, sec. S4).

We identified a single diploid parthenogenetic population that we investigated further to more finely estimate its parental ancestry. We calculated concordance factors from biallelic SNPs (selecting a single SNP per RAD locus; Olave and Meyer 2020) and used PhyloNetworks software (Solís-Lemus and Ané 2016) to calculate the maximized negative log pseudo likelihood for each possible network topology. We performed a bootstrap analysis with 100 replicates to estimate support values for nodes in the best phylogenetic network in PhyloNetworks. We also estimated a phylogenetic network in a Bayesian framework using the unlinked biallelic SNP model implemented in PhyloNet (Than et al. 2008; Zhu et al. 2018). We limited the maximum number of reticulations to one, allowed the population mutation rate estimates to vary across branches, ran the analysis for 5 million Markov chain Monte Carlo iterations (sampling every 1,000 iterations following 1 million iterations of burn-in), and assessed convergence using Tracer (ver. 1.7.1), ensuring that the posterior, likelihood, and prior all had effective sample sizes >1,000 (Rambaut et al. 2018).

Morphological Data

We collected morphological data from preserved lizard specimens for a set of traits that typically distinguish species of whiptails, including snout-vent length (SVL),

femur length, head length, head width, the number of dorsal granules in a row around the midbody, the number of femoral pores, and the number of enlarged subdigital lamellae under the fourth toe on the left foot (Walker et al. 2012). Measurements were taken with digital calipers. To visualize how the morphological variation is partitioned among lineages, we performed a PCA (using the `prcomp` function in the `stats` package) and a linear discriminant function analysis (LDA; using the `lda` function in the `MASS` package) of the data in R.

Results

We sampled 255 individual lizards for a combination of RADseq, mitochondrial DNA (mtDNA), allozymes, karyotypes, and morphology (see sec. S1 of the supplemental PDF for species assignments and sampling details). Both the RADseq data and the karyotype data demonstrated that a population of parthenogenetic whiptails from the foothills of the northern Sierra Madre Occidental in Sonora, Mexico, is diploid (which we describe here as *Aspidoscelis preopatae* sp. nov. in app. A), whereas the other populations of parthenogenetic whiptails we sampled correspond to currently named triploid lineages (see the nQuire results in sec. S2 of the supplemental PDF and the karyotype plates in app. B). For each parthenogenetic individual, the allozyme data and the inferred ancestry estimates from the STRUCTURE analysis closely matched those expected given the number of *A. burti* complex and *A. inornatus* complex genomes each lineage was thought to possess (fig. 2b; supplemental PDF, sec. S5). Phylogenetic analysis of *ND1* showed that *A. opatae*, *A. preopatae*, *A. uniparens*, and *A. sonorae* have nearly identical mtDNA haplotypes that are most closely related to haplotypes of *A. a. arizonae*, indicating a recent common origin from a genetically homogenous ancestral population (supplemental PDF, sec. S6). This result is consistent with the IBS analysis, which showed high levels of shared ancestry across the genome between the parthenogenetic lineages, populations of *A. a. arizonae*, and populations of *A. burti* from Arizona/Sonora. Despite having similar ancestry (i.e., two *A. inornatus* complex genomes and one *A. burti* complex genome), the PCA showed that *A. opatae*, *A. uniparens*, and *A. velox* are all distinctive with genome-scale resolution (fig. 2c). Furthermore, the diploid parthenogenetic population appears to be the intermediate ancestor of *A. opatae* (because *A. preopatae* is genetically more closely related to *A. opatae* than the other two species; supplemental PDF, sec. S7). The paired nuclear subgenomes in each triploid parthenogenetic lineage showed similar levels of heterozygosity to sexual individuals, indicating their derivation from a genome addition rather than a genome duplication process (supplemental PDF, sec. S4).

Phylogenetic analyses for the *A. burti* and *A. inornatus* complex sexual species illustrate that taxa in these groups exhibit a wide range of genetic divergence that contrasts with earlier morphological studies (see sec. S3 of the supplemental PDF for results of phylogenetic analyses and STRUCTURE analyses). In several cases described taxa were not readily distinguishable using the genetic data, whereas in others they clearly represent distinct species (app. A and sec. S3 of the supplemental PDF contain several taxonomic recommendations we adopt here). Analyses using the pseudo-likelihood framework implemented in PhyloNetworks support a hybridization event between a female *A. a. arizonae* (*A. inornatus* species complex) and a male *A. b. stictogrammus* (*A. burti* species complex) as the most likely origin of *A. preopatae* (supplemental PDF, sec. S8; Densmore et al. 1989). This ancestry estimate for *A. preopatae* was generally supported by the bootstrapping analysis in PhyloNetworks and the Bayesian analysis in PhyloNet (fig. 2d), although some uncertainty remains regarding precisely which population gave rise to *A. preopatae* because of the extreme genetic similarity between *A. b. barrancorum* and *A. b. stictogrammus* (e.g., their IBS proportions differ by <1%; fig. 2a; supplemental PDF, sec. S8).

Several morphological traits clearly distinguish *A. burti* and *A. arizonae* from their parthenogenetic descendants (supplemental PDF, sec. S9). Both PCA and LDA illustrated that the parthenogenetic lineages are generally intermediate in morphology compared with their sexual progenitors (fig. 3), although patterns differed substantially across traits (supplemental PDF, sec. S9), suggesting that genetic architecture, polyploidy, and hybridization interact in complex ways to determine phenotypes of parthenogenetic lineages. LDA performed well (i.e., 93% successful classification rate) in distinguishing the diploid *A. preopatae* from its triploid descendant *A. opatae* using the morphological data.

Discussion

Whiptail lizards are a model system for understanding the association between hybridization and the evolution of parthenogenetic reproduction due to the large number of times reproductive transitions have been documented in this clade. The evolutionary history of the *Aspidoscelis burti* and *A. inornatus* sexual species complexes and their parthenogenetic descendants has been a subject of intensive previous study but has remained intractable because of its complexity. Results of the analyses presented here provide high-resolution insights into the histories of these groups and identify the first diploid parthenogenetic population of whiptails from Sonora, Mexico (figs. 1, 2). In addition to clarifying the tempo and mode of evolution of parthenogenesis, these results also contribute to our

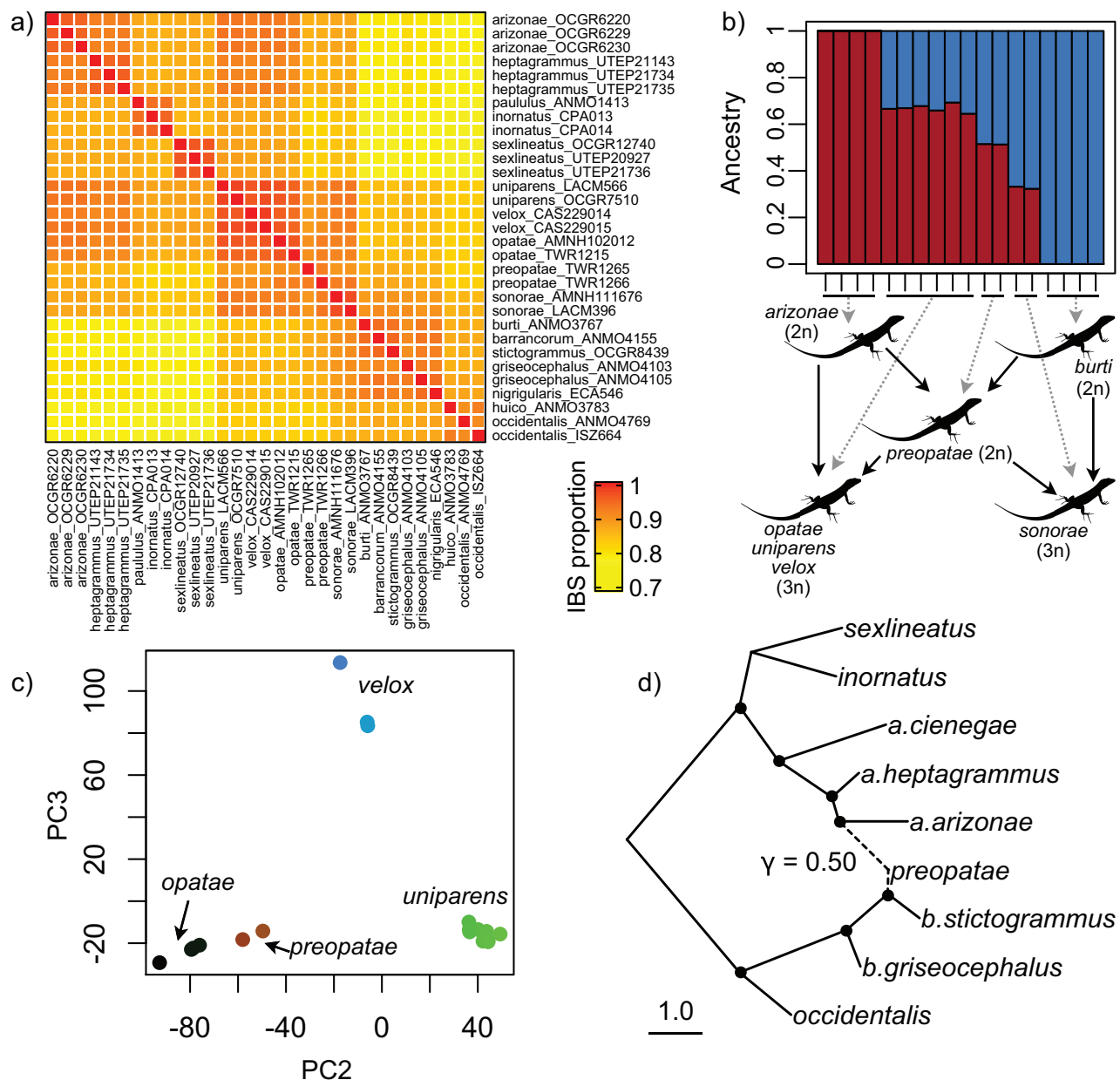


Figure 2: Results of genetic analyses. *a*, Identity-by-state (IBS) analysis for 42,967 single-nucleotide polymorphisms (SNPs) indicating relatedness of parthenogenetic lineages and their sexual progenitors. Sampling for the sexual taxa is based on subsampling several individuals from each population between which we detected genetic differentiation (supplemental PDF, sec. S3). *b*, STRUCTURE plot illustrating genomic ancestry estimates for $k = 2$ (analysis based on 14,590 SNPs). Parthenogenetic individuals have mixed ancestry proportions that match the number of subgenomes they inherited from each sexual species via the patterns of hybridization illustrated below the plot. *c*, Plot of principal components analysis of SNP data for parthenogenetic lineages with similar ancestry (based on 8,708 SNPs). *d*, Maximum pseudo-likelihood network estimate from PhyloNetworks illustrating the ancestry of *Aspidoscelis preopatae* (based on 25,065 SNPs). All nodes have bootstrap proportions of 1.0, and nodes with circles have a posterior probability of 1.0 in Bayesian network analysis in PhyloNet. Branch lengths are in coalescent units; γ represents the inheritance probability estimate.

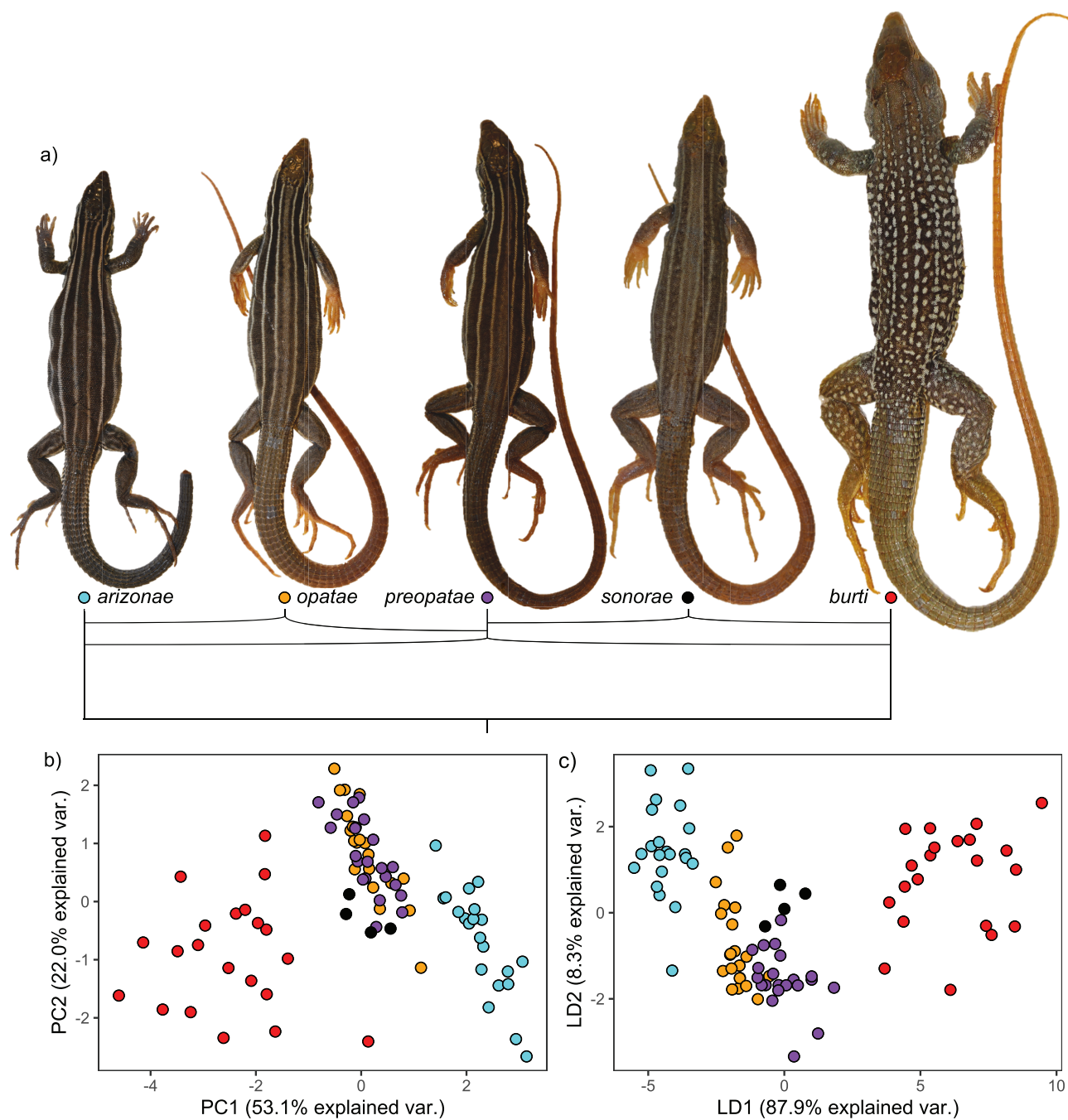


Figure 3: Morphological differentiation between the sexual species *Aspidoscelis arizonae* and *A. burti* and their parthenogenetic descendants. *a*, Specimen snout-vent lengths are as follows: for *A. arizonae* (RCT 940), 60 mm; for *A. opatae* (LACM 109283), 67 mm; for *A. preopatae* (LACM 114783), 65 mm; for *A. sonoreae* (LACM 134727), 80 mm; and for *A. burti* (LACM 123417), 114 mm. The evolutionary network illustrates genealogical relationships among taxa. *b*, *c*, Results of principal components analysis (*b*) and linear discriminate function analysis (*c*) of morphological data.

understanding of both the mechanism by which parthenogenetic vertebrates form and the phenotypic outcomes of this process.

Patterns of Allopolyploidization and Evolution

A major source of confusion regarding the evolution of parthenogenesis in these lizards is derived from ambiguity in species boundaries between the sexual lineages. Phylogenetic and population genetic analyses suggest that within both the *A. inornatus* complex and the *A. burti* complex there are northern and southern groups of populations that likely represent distinct species. The two northern lineages (to which the species names *A. burti* and *A. arizonae* are applied) appear to be the primary ancestors of the triploids *A. opatae*, *A. uniparens*, *A. velox*, and *A. sonorae* (fig. 2; supplemental PDF, sec. S3). Both *A. burti* and *A. arizonae* contain morphologically distinctive populations that are highly genetically similar even from a genome-scale perspective, and this is consistent with the fact that morphologically and genetically intermediate populations are known in many cases (fig. 2a; supplemental PDF, sec. S3; Duellman and Zweifel 1962; Wright and Lowe 1993). The most likely ancestry estimate for the diploid *A. preopatae* accords well with the biogeography of the region, as *A. b. stictogrammus*/*A. b. barrancorum* and *A. a. arizonae* are the most geographically proximate populations to *A. preopatae* within their respective complexes (fig. 1f; supplemental PDF, sec. S3; Dessauer and Cole 1989).

It is clear that the formation of all of the extant parthenogenetic lineages in this group occurred recently, rapidly, and from a relatively genetically homogeneous ancestral population (fig. 2a; supplemental PDF, sec. S6). Despite exhibiting similar patterns of ancestry, *A. opatae* and *A. uniparens* can be readily distinguished from each other at the genomic level (fig. 2b, 2c; supplemental PDF, sec. S7). This is consistent with them being derived from independent hybridization events given that *A. velox* (which shows a similar level of genetic differentiation from both) is derived from a different diploid ancestor that was the product of a reciprocal hybrid cross between a male *A. arizonae* and a female *A. burti* (supplemental PDF, sec. S7; Moritz et al. 1989b). The biogeographic distributions of these parthenogenetic taxa are strikingly different, which is generally consistent with theory suggesting that ecological differences may explain the relative success of parthenogenetic lineages (Kearney et al. 2009). That being said, the observed differences are not directly correlated with ploidy or patterns of ancestry. For example, *A. opatae* and *A. preopatae* appear to be restricted to adjacent closed basins along the Rio Bavispe in the foothills of the northern Sierra Madre Occidental in Sonora (where they occur at much lower

densities than the sympatric, broadly distributed congener *A. sonorae*; Wright 1967). By contrast, *A. uniparens* and *A. velox* have broad geographic distributions across the southwestern United States and/or northern Mexico (Jones and Lovich 2009). Given that cryptic diploid lineages continue to be discovered in nature (Laguna et al. 2010), the dynamics that explain differences in patterns of distribution between parthenogenetic lineages warrant further study. For example, additional empirical data are needed to confirm the extent to which polyploid parthenogenetic lineages are more common in nature as well as to distinguish between alternative mechanisms that could explain such a pattern. These mechanisms include the relative life spans of parthenogenetic lineages, selective advantages of polyploidy, lineage stabilization through ploidy elevation, or neutral processes such as clonal turnover (Bell 1982; Janko 2014; Dedukh et al. 2020).

Our results also confirm that all of the triploid parthenogenetic lineages in the *A. sexlineatus* group are derived from independent, multistep, primary hybridization and subsequent genome addition processes (fig. 1a; supplemental PDF, sec. S4). This was previously known to be the case for other triploid whiptails with different combinations of ancestry (Dessauer and Cole 1989) and suggests that these processes are likely ubiquitous across *Aspidoscelis*. The pattern of having paired nuclear subgenomes that match the mitochondrial genome is the less common version of the primary hybrid model for triploid formation (Avisé 1992, 2008). However, our results suggest that this occurred multiple times in the evolutionary history of whiptails, as *A. opatae* and *A. uniparens* exhibit similar patterns of ancestry and heterozygosity within their two subgenomes that are derived from *A. arizonae* (supplemental PDF, sec. S4). These mechanisms of polyploid formation appear to be rather conserved across independent transitions to true parthenogenesis in vertebrates more broadly, particularly when compared with parthenogenetic invertebrates and other unisexual vertebrates that reproduce by the related reproductive modes of gynogenesis or hybridogenesis (Schön et al. 2009). Interestingly, in the only known instance of vertebrate parthenogenesis evolution that was not induced by hybridization (in the lizard genus *Lepidophyma*), mechanisms of reproduction were recently shown to be much more labile than was previously understood (Kratochvíl et al. 2020). Several postformational mutations are clear in the chromosomes of *A. opatae* and *A. preopatae* (app. B). This rapid accumulation of postformational karyotypic changes is congruent with data from other parthenogenetic whiptail populations and stands in notable contrast to the temporal stability of karyotypes in sexual whiptail species (Lowe et al. 1970a; Cole 1979). Presumably similar sublethal mutations arise in sexual species but are mildly deleterious and efficiently eliminated through

selection in sexually reproducing populations (Cole et al. 2019).

Morphological Outcomes of Hybridization

Hybridization combines alleles from divergent populations and can lead to phenotypic and ecological novelties. This provides a potential mechanism for the ecological success and subsequent diversification of hybridization-derived lineages (Gilbert 2003; Rieseberg et al. 2007; Richards et al. 2018). The evolutionary trajectories of hybrid lineages and the mechanisms by which natural selection can act on genetic variation will also be shaped by changes in reproductive mode and ploidy that can co-occur with hybridization (Neaves and Bauman 2011; Monnahan and Brandvain 2020). A large set of hypotheses have been proposed that predict how hybridization, ploidy elevation, and transitions to unisexuality will influence the success of evolutionary lineages (Parker et al. 1977; Vrijenhoek 1979; Bulger and Schultz 1982; Lynch 1984; Wetherington et al. 1987; Cullum 1997; Soltis and Soltis 2000). Because hybridity, unisexuality, and polyploidy are frequently associated with each other in vertebrates, disentangling their effects on phenotype is often challenging (Kearney and Shine 2004). The existence of *A. preopatae* expands the potential to do this using the most diverse group of parthenogenetic whiptails. These lizards represent the only known whiptail complex that includes extant diploid parthenogenetic populations, populations of their sexual progenitors, and multiple triploid parthenogenetic populations derived from both types of reciprocal backcrossing events (fig. 2b).

Hybrid-derived lineages exhibit diverse patterns with respect to the phenotypes of their parental lineages. These include closely matching one of the parental phenotypes, being largely intermediate between the two parents, or falling outside the range of parental phenotypic variation (e.g., owing to heterosis or transgressive segregation; Parker 1979; Thompson et al. 2019). These different outcomes may reflect variation in the underlying genetic architecture of a particular trait as well as the level of divergence that has accumulated among the alleles that hybridization brings together (Stelkens et al. 2009). For example, if heritability is mostly additive, hybrids should exhibit intermediate trait values (Merót et al. 2020). Some hybridization-derived whiptails have been shown to exhibit intermediate phenotypes. However, patterns in which hybrid traits more closely match one of the parental phenotypes or exhibit heterosis have also been documented (Wright and Lowe 1967; Lowe et al. 1970b; Cole et al. 1988; Walker et al. 1989, 2012; Taylor et al. 2001; Cole et al. 2007, 2020).

The sexual parents of the parthenogenetic lineages studied here (*A. burti* and *A. arizonae*) are two of the most ecologically and phenotypically divergent whiptails (fig. 3;

Jones and Lovich 2009). Morphological traits for the parthenogenetic taxa derived from these two species show patterns that are both consistent and inconsistent with dosage-dependent additive genetic effects (fig. 3; supplemental PDF, sec. S9). Triploid parthenogens with two copies of *A. burti* chromosomes (*A. sonora*) are larger bodied (with maximum SVL being closer to that of *A. burti*) and have the characteristic light spots in the dorsolateral dark fields (Lowe and Wright 1964). Triploid lineages with two copies of *A. arizonae* chromosomes (e.g., *A. opatae*, *A. uniparens*, and *A. velox*) are smaller and lack light spots (Wright and Lowe 1965). The diploid *A. preopatae* also lacks these light spots in dark fields, and although our sample sizes are small, the largest individual that has been collected is slightly larger than the largest *A. opatae* specimen. The mean number of dorsal granules that surround the body matches expectations under additivity nearly perfectly for *A. opatae* and *A. preopatae* (supplemental PDF, sec. S9). Thus, it is interesting that the trait data for *A. sonora* do not also match this expectation (with the mean being smaller than expected, given its genetic ancestry). Alternatively, several other scalation traits in these parthenogenetic taxa closely resemble those seen in *A. burti*, where values are typically larger than in the other sexual parent *A. arizonae*. The presence of both genome dosage effects and nonadditive effects in parthenogenetic whiptails (and parthenogenetic squamates more broadly) suggests that complex evolutionary mechanisms and ecological dynamics may underlie geographic distribution patterns in these systems (Wright and Lowe 1968; Parker 1979; Kearney and Shine 2004). Additional data are thus needed to fully understand the processes that predominate in nature.

Conclusions

Our results clarify long-standing uncertainty regarding the nature and number of hybridization events that have generated the most diverse group of triploid parthenogenetic whiptail lizards. We identify a previously unknown diploid parthenogenetic lineage that is the “missing link” in a multistep hybridization and genome addition process. We also demonstrate that this process has likely been ubiquitous in the formation of polyploid whiptails. This diploid lineage is the likely progenitor of the triploid *A. opatae*, but our results also suggest that all of the triploid whiptails in this group were derived from a genetically homogeneous population of diploid ancestors over a short period of time. Analysis of morphological data shows that phenotypic outcomes of hybridization and ploidy elevation in whiptails are consistent with multiple underlying genetic mechanisms beyond simple additivity and that these differences are not clearly correlated with divergent types of traits (e.g., scalation, size, or coloration). The

diversity and divergent success of lineages in this group provide a compelling system for understanding the ecological and evolutionary trajectories of parthenogenetic lineages in nature.

Acknowledgments

We acknowledge John Wright (deceased), whose unpublished notes and data were invaluable to the completion of this study. We thank G. Pauly, A. Clause, and N. Camacho for access to specimen loans and data archives at the Natural History Museum of Los Angeles County and G. Bradley and M. Bucci for access to the chromosome files at the University of Arizona Museum of Natural History. We also thank L. Scheinberg and C. Spencer for access to specimens at the California Academy of Sciences and the Museum of Vertebrate Zoology, respectively. We thank U. García-Vázquez, N. Manriquez-Morán, D. Lara-Tufiño, A. Esquivel-Ramírez, A. Peralta, L. Bell, A. Leaché, T. Devitt, B. Hollingsworth, P. Heimes, P. Escalante, and C. Townsend for assistance with fieldwork and specimen collection. J. A. Cole provided the collection permit for the Navajo Nation. We thank the University of Texas El Paso Biodiversity Collections, the Louisiana State University Museum of Natural Sciences, the Museum of Southwestern Biology, and the American Museum of Natural History Department of Herpetology for loans of tissues samples. Some analyses for this article were run using the University of Hawai'i Information Technology Services high-performance computing cluster. The research was supported by grants from the National Science Foundation's Division of Environmental Biology (DEB-1754350 and DEB-1754675) and Extreme Science and Engineering Discovery Environment (DEB-190017), as well as a grant from Dirección General de Asuntos del Personal Académico (DGAPA), Universidad Nacional Autónoma de México (UNAM; Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica [PAPIIT] IN217818).

Statement of Authorship

All authors contributed to project conceptualization, experimental design, review and editing of the manuscript, and field sampling and data collection. A.J.B., T.W.R., R.C.T., and A.N.-M.O. were responsible for funding acquisition. A.J.B. was responsible for data analysis, validation, and writing the manuscript.

Data and Code Availability

The *ND1* DNA sequences have been deposited in GenBank under accession numbers MW689302–MW689235. DNA sequence alignments and phylogenetic trees are available on

TreeBase (accession no. 27850). Demultiplexed ddRADseq reads have been deposited in the National Center for Biotechnology Information Sequence Read Archive (SRA PRJNA707030). All additional data associated with the manuscript are available in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.2z34tmpm2>; Barley et al. 2021).

APPENDIX A

Systematic Account

Aspidoscelis preopatae sp. nov.

Aspidoscelis “sp. P” Densmore et al. 1989:970.

Holotype. LACM 114783, collected by John W. Wright (J.W.W.) 18 July 1974, along the Rio Bavispe, 5.6 miles south (by road) of Bacerac, Sonora, Mexico.

Paratypes. Six specimens, all from along the Rio Bavispe, Sonora, Mexico. TWR 1265, 1266, collected by TWR, ANMO, 13 June 2001, 11 km north of Huachinera at 1,047 m (30.2833, –108.9345); LACM 137195–137197, collected by J.W.W., near Huachinera; UAZ 21642, collected by J.W.W., J. L. Patton, 3 June 1967, near Bavispe.

Referred specimens. Twelve specimens, all from along the Rio Bavispe, Sonora, Mexico. At Huachinera: LACM 109275, 109278, 109280, 131724. 5.6 miles south of Bacerac (by road): LACM 114763, 114766, 114772, 114783, 121634, 121665. At Bacerac: LACM 134732. 11 km north of Huachinera: TWR 1262–1264. Just north of Bavispe: TWR 1285, 1286.

Diagnosis. A species of *Aspidoscelis* distinguished by the following combination of characters: (1) a diploid parthenogenetic member of the *sexlineatus* species group; (2) adult body size small (SVL typically <75 mm); (3) dorsal granules around midbody 81–95; (4) femoral pores 35–40; (5) supraocular scales 4; (6) subdigital lamellae under fourth toe 26–32; (7) postantibrachial scales granular; (8) mesoptychial scales slightly enlarged; (9) circumorbital semicircle scales 4–7, terminating short of the frontal-frontoparietal suture; (10) enlarged parietal scales 3 (or 4 if interparietal split); (11) frontoparietal scales 2; (12) dorsal pattern a series of six paired light stripes, plus a seventh, partially split middorsal stripe separated by dark fields that lack light spots; (13) adult ventral coloration white. *Aspidoscelis preopatae* can be distinguished from its sexual ancestral species and other parthenogenetic lineages derived from them through hybridization by a combination of the above morphological traits (see sec. S9 of the supplemental PDF for a summary) and its unique karyotype (fig. B1).

Description of holotype. A large female (SVL 63.3), body robust (axilla – groin distance/SVL = 0.5), limbs well developed (femur length/SVL = 0.2), tail long (SVL/tail length = 0.5), head robust (head length/SVL = 0.2),

longer than wide (head width/head length = 0.7). Snout tapered, rounded at tip. Dorsal cephalic scales include one rostral scale, paired nasal scales in contact, pierced by naris, two loreal scales, first in contact with frontonasal, second in contact with paired prefrontals. Frontal longer than wide, tapering posteriorly, in contact with prefrontals anteriorly, first three supraoculars laterally, and frontoparietals posteriorly. Four supraoculars, two frontoparietals, two parietals, and two interparietals, surrounded posteriorly by four enlarged occipital scales. Circumorbital semicircle scales between supraoculars and medial cephalic scales seven. Supraciliary scales six, supralabial scales seven, infralabial scales seven, interlabial scales six, subocular scales four, third elongate. Single mental and postmental scales, first pair of chin shields in contact anteriorly. Mesoptychial scales slightly enlarged, postantebrachial scales granular. Dorsal scales granular, 81 scales in row around dorsum at midbody. Three enlarged preanal scales, femoral pore scales 18 (on each side). Relative finger lengths with number of enlarged subdigital lamellae in parentheses (left/right): IV(13/12) = III(12/13) > II(11/12) > V(10/9) > I(9/9). Hind limbs larger than forelimbs, relative toe length with lamellae (left/right) in parentheses: IV(29/30) > III(19/18) > V(14/16) > II(15/16) > I(9/9). Venter white, immaculate, scales wider than long. Dorsal pattern of seven light stripes with middorsal stripe partially divided, dark field between stripes unspotted.

Distribution. This species appears to have a restricted distribution, being known only from several localities along the Rio Bavispe in northeast Sonora, Mexico (generally between Bavispe and Huachinera).

Habitat and natural history. No formal studies of the natural history of this species have been conducted. This diurnally active lizard is known only from desert scrub habitat along the Rio Bavispe in the foothills of the northern Sierra Madre Occidental (~1,000–1,100 m). Presumably its habits are similar to those of *A. opatae* (which is closely related and exhibits a similarly restricted distribution nearby; Wright 1967) and other parthenogenetic lineages in the *A. sexlineatus* group.

Etymology. The species name is derived from notes left by John Wright, deceased curator of herpetology at the LACM, who discovered populations of this species in the 1960s and planned to describe this taxon before his retirement in the 1990s. It is in reference to the hypothesis that this parthenogenetic lineage is the progenitor of the triploid lineage, *A. opatae*, originally named for the Ópata indigenous group native to the Rio Yaqui valley in Sonora, Mexico (Wright 1967).

Further taxonomic recommendations. On the basis of analyses of the data presented here, we make and adopt several taxonomic recommendations for the sexual species complexes from which *A. preopatae* originated through

hybridization (supplemental PDF, sec. S3). This includes reassigning the specific epithet of *A. arizonae* to several subspecies within the *A. inornatus* species complex and reassigning some populations from the United States and Mexico under the specific epithet *A. burti*.

APPENDIX B

Karyotype Data

Aspidoscelis opatae (triploid) and *A. preopatae* (diploid) are the only parthenogenetic lineages in the *A. sexlineatus* group for which karyotype data have not been published. *Aspidoscelis burti* and *A. arizonae* (the sexual species from which they are derived through hybridization) have 23 pairs of chromosomes that can be grouped into three sets based on size (Lowe et al. 1970a: one pair of large, metacentric chromosomes in set I; 12 smaller pairs of telocentric or subtelocentric chromosomes in set II; 10 pairs of microchromosomes in set III). Novel chromosome spreads were examined for three specimens of the triploid *A. opatae* (fig. B1): UAZ 21642 (five cells), UAZ 30269 (three cells), and UAZ 25480 (13 cells). All cells for *A. preopatae* UAZ 21642 (from Bavispe, Sonora) were clearly diploid ($2n = 47$) with two set I, 24 set II, and 21 set III chromosomes (which differ based on size, from largest to smallest) and one nucleolus organizer region (NOR). This is consistent with other members of the *A. sexlineatus* group (Lowe et al. 1970a) in which the large metacentric chromosomes usually have a NOR (Porter et al. 2019) with the exception of the extra microchromosome. Presumably this represents a postformational mutation (possibly a duplication). The cells of UAZ 30269 (from south of Oputo, Sonora) were all $3n = 68$ ($4 + 34 + 30$ chromosomes) with two chromosomes having NORs. The deviation from expectation in this karyotype appears to have resulted from a postformational centric fusion of two different set II chromosomes, which led to the formation of a fourth set I chromosome that is slightly shorter than the others. Cells from UAZ 25480 (from south of Presa Angostura, Sonora) are similar to those from UAZ 30269, with the exception that some show only one NOR. We also recently examined chromosomes on slides prepared at the AMNH about 30 years ago for several field-collected specimens and offspring they produced in the laboratory. The stain is fading on these now, so some details, especially on microchromosomes, are not clear, but in a series of 34 cells examined from 12 individuals, the following was clear. They were all triploids, with four large set I chromosomes, one of which was the large submetacentric apparently formed by the postformational centric fusion of two set II chromosomes. Five of these specimens were F_1 generation offspring and their two P_1 generation mothers, providing evidence that

the karyotype is cloned. AMNH R-148253 and R-148254 are the P₁ topotypes that were collected in the field, and AMNH R-148280, R-148281, R-148283, R-148325, and R-148327 are the F₁ offspring that were cloned in the laboratory.

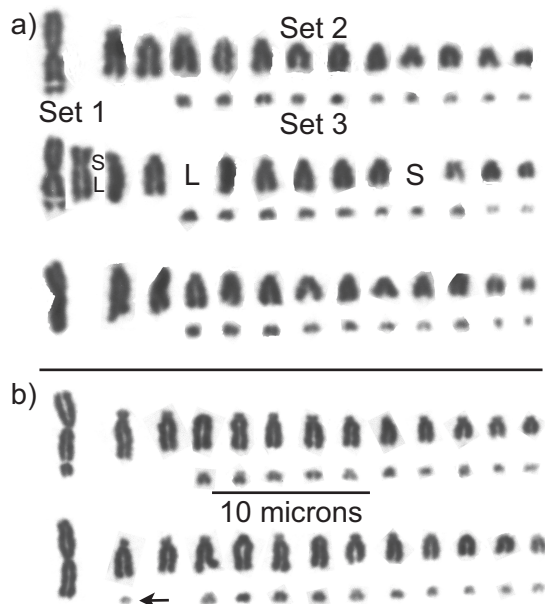


Figure B1: Karyotypes of two parthenogenetic species of *Aspidoscelis* from Sonora, Mexico: *a*, *A. opatae* triploid topotype from near Oputo (UAZ 30269); *b*, *A. preopatae*, diploid paratype from Bavispe (UAZ 21642). Chromosomes are arranged to illustrate the similarities of the haploid complements received through two steps of hybridization among the sexual ancestors of these species. *a* illustrates a clone of a triploid hybrid, which was formed when a diploid ovum of *A. preopatae* was fertilized by a sexual relative. L and S signify two telocentric set II chromosomes that hypothetically underwent centric fusion, resulting in the unique larger submetacentric chromosome in *A. opatae*, presumably after the origin of this species. *b* illustrates a clone of the original diploid hybrid. The arrow indicates the extra set III microchromosome, perhaps from a duplication after the origin of this species.

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