

Spontaneous speciation by ploidy elevation: Laboratory synthesis of a new clonal vertebrate

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The transition from diploid (2n) to polyploid (3n, 4n, and so forth), a relatively common process in plants and some animal taxa, represents rapid speciation, as only rarely are hybrids able to form genetically balanced gametes (1). In vertebrates, polyploidy is often associated with asexual reproduction: sperm-dependent (gynogenesis, hybridogenesis, and kleptogenesis) in amphibians and fish, and sperm-independent (parthenogenesis) in squamate reptiles (2). For the latter, approximately 0.6% of known species are parthenogenetic, and, with few exceptions, these arose via hybridization between sexually reproducing progenitors (3). Given the relatively recent origin of most parthenogenetic taxa (2), it should then be possible to recreate parthenogenetic lineages of varying ploidy by hybridizing known progenitors in the laboratory, as has been achieved for some diploid hybrid plant species (4) and unisexual fish and frogs (see ref. 5). However, until now, this has not been achieved for parthenogenetic reptiles (e.g., ref. 6). In this context, the report by Lutes et al. (5) in PNAS is highly significant. Following from an earlier observation of a potentially fertile, field-collected 4n female (7), Lutes et al. (5) crossed parthenogenetic 3n *Aspidoscelis exsanguis* females and a sexual *Aspidoscelis inornata* male to produce three generations of parthenogenetically reproducing 4n female lizards. Parthenogenetic reproduction was confirmed by cytogenetic analysis of female meiosis and multilocus genotyping across multiple generations of the progeny. That a self-sustaining 4n lineage can be produced in the laboratory, but is not observed in nature, raises the question of what constrains development of cascading polyploid series, as seen in some invertebrates (8). Further, is it possible that 3n asexual lineages can form a bridge in the evolution from 2n to 4n sexually reproducing species (1)?

The whiptail lizards of the western deserts of North America are remarkable in having a substantial diversity of both 2n and 3n parthenogenetic lineages, all of which arose via hybridization among sexual species (9) (Fig. 1A). Parthenogenetic lineages are often sympatric with congeneric sexual taxa, resulting in numerous reports of high ploidy hybrids in nature (reviewed in refs. 10 and 11). As *Aspidoscelis* have XY sex determination

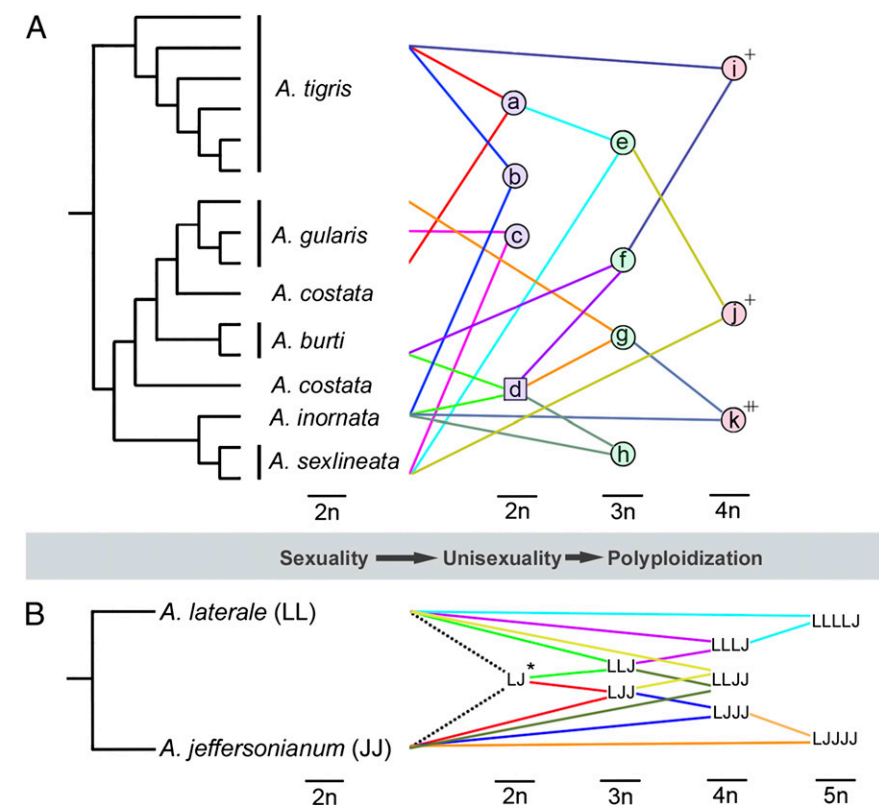


Fig. 1. Phylogeny of *Aspidoscelis* for representative sexuals and unisexuals, based on figure 6 in ref. 9 (A), and a possible route of ploidy elevation of unisexual salamanders in the *Ambystoma laterale*–*jeffersonianum* complex (B) (19). Species in A are as follows: a, *Aspidoscelis tessellata* complex; b, *Aspidoscelis neomexicana*; c, *Aspidoscelis laredoensis* complex; d, intermediate ancestor; e, *Aspidoscelis neotesselata* complex; f, *Aspidoscelis flagellicauda* and *Aspidoscelis sonorensis* complexes; g, *Aspidoscelis exsanguis*; h, *Aspidoscelis opatae*, *Aspidoscelis uniparens*, and *Aspidoscelis velox* complexes; i, 4n by *A. sonorensis* (female) × *Aspidoscelis tigris* (male); references 25, 26, and 28 in ref. 5; j, 4n by *A. neotesselata* (female) × *Aspidoscelis sexlineata* (male); reference 27 in ref. 5; and k, 4n by *A. exsanguis* (female) × *A. inornata* (male) (5, 7). *LJ is not a “true” hybrid directly derived by *A. laterale* × *A. jeffersonianum* (19); †sterile or unknown fertility; ‡fertile (5) or potentially fertile (7).

with slightly heteromorphic sex chromosomes, both male and female hybrids are observed, although these are sterile or of unknown fertility (10). This is the case for previously reported and cytogenetically confirmed 4n hybrids between 3n parthenogens and 2n sexual males (10, 11). Both these and the hybrids generated by Lutes et al. (5) have unbalanced (i.e., AABC) chromosome sets (Fig. 1A), so why the latter should be fertile but former not (or not known to be) is a mystery.

In other sexual–parthenogenetic complexes of reptiles, higher ploidy hybrids are also typically sterile. For example, Caucasian rock lizards of the genus *Darevskia* contain several diploid parthenogenetic lineages of hybrid ancestry. Triploids, as

commonly found in nature where 2n parthenogens and sexuals coexist, are typically sterile (ref. 10; but see ref. 12). This has been attributed to disruption of sex determination, as these species have moderately to highly heteromorphic ZW sex chromosomes (13). However, this does not explain sterility of such hybrids in *Heteronotia* and *Lepidodactylus* geckos, which lack heteromorphic sex chromosomes (14, 15). In contrast, cascading

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polyploidy elevation readily occurs in some groups of amphibians [e.g., *Bufo viridis* group (16)] and fish (17, 18). For example, North American unisexual mole salamanders of the genus *Ambystoma* occasionally incorporate sperm from congeneric sexual males in syntopy to generate natural lineages with increasing ploidy (19) (Fig. 1B).

Why are there no such examples in reptiles? One simple possibility is that successful elevation to higher ploidy is a rare event and is facilitated in unisexual amphibians and fish because of their continuing dependence on sperm to initiate the onset of development. In unisexual fish, for example, this often leads to variable amount of paternal leakage that ranges from microchromosomes to a whole genome set (17). However, in some circumstances, hybrids between parthenogenetic and sexual reptiles are very common [e.g., as high as 50% (12)], suggesting that this alone is not the whole story. Another possibility is that such transitions depend on intense selection for rare balanced gametes and, thus, are more probable in taxa with high female fecundity (1).

A related question is whether unisexual triploidy is a bridge to formation of sexually reproducing tetraploids (1). Such transitions have been observed in fish (18), but how most sexual polyploid vertebrates (e.g., amphibians and fish) arose is poorly understood. In general, 4n sexual reproduction is most likely with matched chromosome sets (AABB), allowing bal-

anced pairing between homologous (A–A and B–B) rather than homeologous (A–B) chromosomes. As yet, all known 4n hybrids of *Aspidoscelis*, including those generated by Lutes et al. (5), have unmatched chromosome sets (Fig. 1A). Therefore, and notwithstanding previous efforts (6), it would be fascinating to

Lutes et al. demonstrate the potential to generate higher-ploidy parthenogenetic lineages of reptiles in the laboratory.

extend these experiments to generate 4n hybrids with balanced chromosome sets (e.g., *A. sonorae* × *A. inornata*, or *A. uniparens* × *Aspidoscelis burti*, Fig. 1A).

The results from Lutes et al. (5) demonstrate the potential to generate higher-ploidy parthenogenetic lineages of reptiles in the laboratory. So, given that these particular taxa, *A. exsanguis* and *A. inornata*, are known to occur in sympatry (10), why do we not see a self-sustaining 4n lineage in nature? One possibility might be that very specific genomic combinations of particular parental individuals are needed to initiate or to maintain unisexuality (2). For example, populations

of unisexual salamanders are characterized by complex ploidy and genome combinations, but all share a single origin, and no recurrent hybridization between known sexual sperm donors has generated new unisexual lineages (19). Lutes et al. (5) find that the laboratory-produced 4n hybrids are perfectly viable with no competitive disadvantage in prey capture when housed with their progenitors. However, as Lutes et al. (5) are aware, this does not ensure that these newly formed 4n parthenogens are capable of persisting under natural conditions, and in the face of competitive pressure from both sexual and unisexual progenitors in a constantly changing environment.

The ability to produce self-sustaining, higher-ploidy hybrids between parthenogenetic and sexual lineages in the laboratory, but their absence in nature, raises many questions and also creates opportunities for further research. The relatively recent ability to develop genomic resources for such systems and, therefore, to examine patterns of gene expression and intergenomic interactions in sterile versus fertile hybrids relative to their parthenogenetic and sexual progenitors offers a new window into often proposed, but still poorly understood, genetic constraints on the evolution of parthenogenesis and polyploidy (20).

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