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Laboratory Hybridization among North American Whiptail Lizards, Including *Aspidoscelis inornata arizonae* × *A. tigris marmorata* (Squamata: Teiidae), Ancestors of Unisexual Clones in Nature

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ABSTRACT

The natural origin of diploid parthenogenesis in whiptail lizards has been through interspecific hybridization. Genomes of the parthenogens indicate that they originated in one generation, as the lizards clone the F_1 hybrid state. In addition, hybridization between diploid parthenogens and males of bisexual species has resulted in triploid parthenogenetic clones in nature. Consequently, the genus *Aspidoscelis* contains numerous gonochoristic (= bisexual) species and numerous unisexual species whose closest relatives are bisexual, and from whom they originated through instantaneous sympatric speciation and an abrupt and dramatic switch in reproductive biology.

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In order to study this phenomenon more closely, with hopes (unfulfilled) to witness the origin of parthenogenetic cloning in one generation, we maintained whiptail lizards in captivity. For more than 29 years, we caged males of bisexual species with females of bisexual and of unisexual species in attempts to obtain laboratory hybrids. Hybrids were raised to adulthood to see whether they would reproduce, but none did. The hybrid status of suspected laboratory hybrids was confirmed by karyotypic, allozyme, and morphological analyses, and histological studies were made on reproductive tissues of the hybrids, which were apparently sterile.

The present paper focuses on the laboratory hybrids of two bisexual species, *A. inornata arizonae* (♀) × *A. tigris marmorata* (♂). These three individuals from one clutch of eggs were the only hybrids between two bisexual species that we obtained. The hybrids had a karyotype, allozymes (21 loci tested), and external morphology that were similar to those of *A. neomexicana,* which is a diploid parthenogen that had a hybrid origin in nature that was the reciprocal cross: *A. t. marmorata* (♀) × *A. inornata* (♂). Histological study showed that the largest and oldest laboratory hybrid raised, which appeared to be a female with inherited X chromosome of *A. t. marmorata,* was an intersex with an enormous adrenal. The other hybrid that reached adult size, a male, was also apparently sterile.

Later, we review and summarize the information on the other laboratory hybrids we obtained over the years. These include two different combinations of hybrids between a male of a bisexual species and females of unisexual species (one diploid, one triploid), producing triploid and tetraploid hybrids, respectively, as a haploid genome from the male was added to the cloned egg. Considering only those specimens whose hybrid status was confirmed with genetic analyses, a total of only five hybrids from three crosses were obtained over 29 years. The effort involved having a total of 74 males of four species caged with 156 females of nine species, where individuals were caged together for at least six months (or less, if mating behavior was observed).

Despite our extensive efforts to provide for their comfort and best health and captive environment, the lizards at times experienced health problems such as metabolic bone disease and a *Salmonella* infection. These definitely had a negative effect on reproduction, the full extent of which is unknown. Nevertheless, we estimate that successful hybridization among whiptail lizards (i.e., which results in healthy offspring capable of reproduction) is much more rare than we previously thought, although, paradoxically, it is far more common among *Aspidoscelis* than among nearly all other genera of lizards in the world, with the possible exception of lacertids.

INTRODUCTION

Whiptail lizards of the genera *Aspidoscelis* and *Cnemidophorus* are of special interest because they include both unisexual (all-female) species and bisexual species (gonochoristic; with separate sexes). Bisexuality is the ancestral state, and unisexuality evolved rather recently, although the potential for this to happen in squamate reptiles apparently has existed for around 200 million years (for a review, see Reeder et al., 2002). The unisexual species normally reproduce by means of parthenogenetic cloning, in the complete absence of spermatozoa. This phenomenon is known to occur as the normal mode of reproduction for certain squamate reptiles but no other vertebrates (e.g., Hardy and Cole, 1981; Hardy et al., 1989; Dessauer and Cole, 1986).

The evolutionary history of unisexual teiid lizards is bizarre in comparison to most other vertebrates. Origin of the species was not a result of the typical historical splitting of an ancestral lineage into two derived forms in allopatry. Instead, they had an instantaneous origin

through hybridization and therefore have a reticulate phylogeny and experienced sympatric speciation (for reviews, see Cole, 1985; Dessauer and Cole, 1989; Reeder et al., 2002). The word "instantaneous" applies because the unisexual females clone the F_1 hybrid combination of alleles from their ancestors, indicating that the historic switch from spermatozoan-based bisexual reproduction to unisexual parthenogenetic cloning occurred in a single generation. In fact, this appears to be the case for nearly all the parthenogenetic reptiles, globally, in addition to at least six separate origins of diploid parthenogenetic cloning in *Aspidoscelis* (for reviews, see Darevsky et al., 1985; Dawley and Bogart, 1989; Wright and Vitt, 1993; Reeder et al., 2002). The only exceptions to the hybrid origins of unisexual vertebrates appear to be the all-female xantusiid lizards of the genus *Lepidophyma,* for which no evidence of a hybrid origin has been found, despite considerable genetic research (Bezy and Sites, 1987; Sinclair et al., 2010).

Evidence for the instantaneous hybrid origin of unisexual species of *Aspidoscelis* is so overwhelming, and its occurrence throughout the genus so widespread, that it is easy to imagine one might hybridize captive individuals of bisexual species and witness the origin of parthenogenetic cloning in the laboratory, unless the percentage of F_1 hybrid females that clone themselves is very small. With this in mind, we often caged together males and females of different species during many of the years that we maintained a laboratory colony of whiptail lizards at the American Museum of Natural History (AMNH; e.g., Townsend and Cole, 1985). In addition, we often caged females of unisexual species (either diploid or triploid) with males of bisexual species in an effort to produce polyploid hybrids and determine whether they would reproduce (e.g., Cole, 1979; Hardy and Cole, 1998). Our success at producing hybrids was very limited, and at the end of this paper we present a summary of all the laboratory hybrids produced at the AMNH. However, William Neaves and collaborators independently are investigating the same and additional questions at the Stowers Institute for Medical Research, Kansas City, Missouri. Consequently, they have established an *Aspidoscelis* colony (see Lutes et al., 2010) and also have produced some laboratory hybrids that are currently under study (Peter Baumann and William Neaves, personal commun.).

The primary focus of this paper concerns three hybrids from one clutch of eggs with a genealogy that closely approximated the hybrid origin in nature of the diploid, unisexual *Aspidoscelis neomexicana.* This species originated as a result of hybridization between *Aspidoscelis tigris marmorata* (♀) × *Aspidoscelis inornata* (♂) (for reviews, see Neaves, 1969; Neaves and Gerald, 1969; Brown and Wright, 1979; Parker and Selander, 1984; Cole et al., 1988; Dessauer and Cole, 1989), for which two comments on the scientific names of the parental forms are pertinent: (1) some authors disagree with applying names of modern taxa to ancestral events, but the ancestral hybridization could have happened only a few hundred or a few thousand years ago and the genetic signature of the ancestors is clear—we have no basis for calling them anything else; and (2) some authors today treat *A. t. marmorata* as a species separate from *A. tigris* (we follow Dessauer et al., 2000) and some treat *A. inornata arizonae* (see below) as a species separate from *A. inornata* (we follow Wright and Lowe, 1993, with respect to considering *arizonae* as part of *inornata*).

Inconsistent with our laboratory hybridization, the natural origin of *A. neomexicana* involved a female of *A. t. marmorata* and a male of *A. inornata* (Brown and Wright, 1979;

Densmore et al., 1989a; Dessauer et al., 1996a), and the hybridization event probably occurred in the Rio Grande Valley (Cole et al., 1988), where the relevant named subspecies of *A. inornata* would probably be either *A. i. llanuras* or *A. i. heptagramma* (if subspecies are accepted). These forms are extremely similar to *A. i. arizonae* and their insufficient diagnoses require additional research (e.g., for Wright and Lowe's, 1993, diagnostic characters, their "Eddy" sample of *heptagramma,* "Edge of Sands" and "Grant" samples of *llanuras,* and "Mountainair" sample of *juniperus* are essentially the same as their samples of *arizonae*). In the laboratory, we used individuals of *A. i. arizonae* for the attempted crosses because these lizards today generally have a larger body than individuals from the Rio Grande Valley and are thus a better match to the large body size of *A. tigris,* for compatibility in mating. *Aspidoscelis i. arizonae* has mitochondrial DNA that closely matches that of the maternal ancestral form of other unisexual species of whiptail lizards (e.g., Densmore et al., 1989b), and for the 47 gene loci tested previously it is similar to population samples of *A. inornata* from the Rio Grande Valley of New Mexico and western Texas (Cole et al., 1988; Dessauer and Cole, 1989), so apparently no experimental potential was lost in using this form. Finally, in the laboratory we caged females of one species with males of the other and we set up cages with the potential for producing reciprocal crosses. Our focus in this paper is on the only clutch of hybrid eggs that was produced by these two species in our laboratory.

MATERIALS AND METHODS

Laboratory Maintenance of Lizards

Lizards were caught in the field, held temporarily at the Southwestern Research Station (SWRS, Portal, Arizona), then flown to New York and maintained in a laboratory colony at the American Museum of Natural History by our usual methods (Cole and Townsend, 1977; Townsend, 1979; Townsend and Cole, 1985). Note, however, that there are problems yet to be resolved in order to maximize success with captive whiptail lizards (Porter et al., 1994).

The following three methods were used to try to obtain hybrids:

(1) We caged one or more females of one species with one or two males of a different species in cages of various sizes at the AMNH. This is the only method that worked, and the caged lizards did it themselves, without artificial insemination.

(2) We housed lizards (*A. inornata* and *A. t. marmorata*) in large outdoor enclosures at the SWRS, again pairing males and females of the different species, with potential reciprocal cross combinations in different enclosures. We suspect that the reason this produced no offspring was because the environment was inadequate. The only land we had for enclosures was on the property of the SWRS, in pine-oak woodland that is more than 300 m higher in elevation than the desert and desert-grassland where the two species occur. Also, the enclosures lacked natural burrow systems, so we designed and built artificial hibernacula; although a few lizards survived the relatively harsh winters, we do not know how much winter mortality there may have been. Enclosures also had ultraviolet lights that switched on at night to attract diverse insects, which were available as food for the lizards each morning; this worked very well. Finally, for those

who may be considering similar enclosures in the future, it was very important to strategically locate wire mesh of various sizes and flashing materials to exclude potential lizard predators (especially snakes, roadrunners, and raptors), and to exclude burrowing rodents from entering the enclosures, thus providing escape tunnels. On one occasion, an adult whipsnake (*Coluber bilineatus*) was found stuck in the wire mesh at the point of a large bulge in its belly, while trying to leave the enclosure.

(3) We tried artificial insemination, for which males were sacrificed, epididimydes and testes were minced in a Ringer's solution, presence of motile spermatozoa were confirmed by microscopy, and a blunt Tom Cat Catheter mounted on a tuberculin syringe was used to insert a sperm suspension into the cloaca of multiple females lined up to receive it one after another. For Ringer's solution used see Sexton (1977). In each of the many instances in which females laid eggs after this treatment, conspecific (not hybrid) hatchlings emerged, suggesting that the eggs were fertilized by spermatozoa acquired by the females prior to capture, even though we did this with females captured in the early spring.

Karyotypes

As reported elsewhere for whiptail lizards (Cole, 1979), we used standard methods for preparing giemsa-stained chromosomes (sodium citrate cell suspension, methanol and glacial acetic acid fixation, flame drying on slides). For *A. inornata,* we have examined 115 bone marrow cells at mitotic metaphase from 21 individuals (including both sexes) from widespread localities representing four subspecies, including seven specimens of *A. i. arizonae* from the vicinity of Willcox, Cochise County, Arizona, all of which were karyotypically identical to each other (e.g., Lowe et al., 1970; Cole et al., 1988). For *A. tigris sensu lato,* we have examined at least 40 specimens also from widespread localities representing various subspecies, all of which are karyotypically identical to each other, excepting for the sex chromosomes that distinguish males and females (e.g., Cole et al., 1969; Lowe et al., 1970; Dessauer et al., 2000; Taylor et al., 2001; Cole et al., 2007). For *A. neomexicana,* which has a diploid karyotype consistent with its hybrid origin of *A. t. marmorata* × *A. inornata,* we have examined about 175 cells at mitotic metaphase from more than 30 individuals from throughout its geographic range (Cole et al., 1988; Manning et al., 2005). One individual of special relevance to this paper and for which chromosomes were examined for the first time is AMNH R-153158, an adult-sized, apparently female laboratory hybrid of *A. i. arizonae* × *A. t. marmorata,* for which we recorded observations from 11 mitotic cells that were all consistent with each other (see Results for details on parentage of this animal).

Allozymes

We determined genotypes at 21 nuclear gene loci, based on phenotypes of tissue protein activities on starch gels following electrophoresis. Specifically for this report, we used one individual each of *A. i. arizonae* (AMNH R-153168) from the vicinity of Willcox, Cochise County, Arizona, and *A. t. marmorata* (AMNH R-153163) from the vicinity of Lordsburg,

Hidalgo County, New Mexico, each from the same locality where the parents of the laboratory hybrids were collected. Circumstances precluded our using the parents for these comparisons, so the above specimens served as their proxy. We also examined one of the laboratory hybrids of *A. i. arizonae* × *A. t. marmorata* (AMNH R-153157, the adult-sized apparent male). These were compared on the same gels with an individual of *A. neomexicana* from New Mexico (AMNH R-151740). Details on each individual are listed in the Results and Specimens Examined (appendix).

We followed Dessauer et al. (1996b) for methods of collecting and storing tissue samples. Methods of preparing homogenates, conducting electrophoresis (except we used vertical gels), localizing specific proteins, and scoring gel phenotypes, as well as the abbreviations for specific gene loci, followed Harris and Hopkinson (1976), Murphy et al. (1996), and, particularly for lizards of the genus *Aspidoscelis,* Dessauer et al. (2000).

The data were interpreted against our background of having analyzed hundreds of specimens of *A. tigris, A. inornata,* and *A. neomexicana* in the course of other studies (Dessauer and Cole, 1984, 1989; Dessauer et al., 2000; Cole et al., 1988, 2007; Taylor et al., 2001; Manning et al., 2005), and the results were consistent with our previous work.

External Morphology and Statistics

Photographs and color notes were recorded prior to preservation of the lizards. Three laboratory hybrids of *A. i. arizonae* (♀) × *A. t. marmorata* (♂), their two parents, 17 additional specimens of *A. i. arizonae,* 19 additional of *A. t. marmorata,* and 22 of *A. neomexicana* collected in the field were scored for snout-vent length (SVL) and seven meristic characters abbreviated and counted as follows: COS (number of circumorbital semicircle scales, total of both sides, counted as per Wright and Lowe, 1967: 19); FP (number of femoral pores, one leg only); GAB (= SAB, number of granules or scales around midbody, counted as per Wright and Lowe, 1967: 15–17); GUL (number of gular scales, counted as per Cole et al., 1988: 5); ILS (number of interlabial scales, total of both sides, as per Cole et al., 1988: 4–5); PSC (number of scales in contact with the outer perimeter of the parietal and interparietal scales, beginning on one side at the suture of the frontoparietal and parietal and counting posteriorly and around to the comparable place on the other side, including the large wedgelike scale, if present, on the anterior end); and SDL (number of fourth toe subdigital lamellae, one toe only, but otherwise as per Cole et al., 1988: 4). We used SPSS Statistics 17.0 software (SPSS, Inc., 2008) for all statistical procedures.

We used two principal components analyses (PCAs) to examine two-dimensional patterns of meristic variation among the three hybrids, their parents, and samples of the three taxa collected in nature. This showed the pattern of variation among the lizards without a priori identification of specimens to group, and most of the variation was depicted clearly in two-dimensional plots. Principal components scores for each specimen were based on loadings derived from a correlation matrix. We also used a canonical variate analysis (CVA) with the samples of *A. i. arizonae, A. t. marmorata,* and *A. neomexicana* serving as three a priori groups. The three hybrids were included in the CVA as unclassified in order to determine the a priori group most closely resembled by each. Canonical variate scores were based on standardized canonical coefficients.

Internal Anatomy and Histology

We examined the reproductive capabilities of the specimens discussed here by histological analyses of the gonads. The histological sample consisted of 4 specimens: 1 adult male *Aspidoscelis t. marmorata* (AMNH R-153156), and 3 laboratory hybrids of *A. i. arizonae* × *A. t. marmorata* (AMNH R-148432, 153157, and 153158). After preservation in 10% formalin and storage in 75% ethanol, the gonads and associated adrenal glands and mesenteries were removed for histological examination. All of the tissue samples were completely serially sectioned, except the abnormally large tissue sample from the adult-sized, apparently female hybrid (AMNH R-153158), which was sectioned through about half of the tissue (the remainder is preserved in the block of embedding medium). All tissues were embedded in 56°–57°C embedding medium (Paraplast by Lancer) using a tertiary-butyl alcohol process (Weesner, 1960), for the large, apparently female hybrid (AMNH R-153158), or a standard ethanol process for the other three specimens. All were sectioned at 8 μm. Odd-numbered slides were stained with Ehrlich's hematoxylin and eosin (progressive method). Even-numbered slides were stained with the Mallory triple connective tissue technique (Pantin method; Presnell and Schreibman, 1997) combined with Ehrlich's hematoxylin. Histological and anatomical terminology follows Hardy and Cole (1981).

Abbreviations Used in Histology Figures

A	adrenal gland	ML	mesonephros, large tubules
AC	adrenal cells	MS	mesonephros, small tubules
BV	blood vessel	O	ovary
CO	cortex of ovary	Og	oogonia of germinal epithelium
Cy	cytoplasmic strand	Oop	ooplasm
Dod	distal oviduct	SMB	smooth muscle band
E	epididymis	Sp	spermatozoa
I	interrenal cells	Ss	secondary spermatocyte
Lip	lip of germinal epithelium of ovary	ST	seminiferous tubule
M	mesonephros	T	testis
MD	mesonephric duct	V	vas deferens
Met	metanephros	Y	yolk granule of oocyte

RESULTS AND DISCUSSION

The Laboratory Hybrids of *A. i. arizonae* (♀) × *A. t. marmorata* (♂)

Reproduction of the Parents in the Laboratory

The following details pertain to the clutch of three eggs and laboratory hybrids on which we focus for most of this paper, from a cross of *A. inornata arizonae* (♀) × *A. t. marmorata* (♂). The female *arizonae* was AMNH R-148431 and the male *marmorata* was AMNH R-153156 (see appendix, Specimens Examined, for locality data).

The female lived in captivity for nearly six years, from 30 July 1994 to 21 June 2000, and the male also for nearly six years, from 13 July 1996 to 8 May 2002. During the four years that they were living in the laboratory simultaneously, they were caged with each other for only part of the time. The female was probably a yearling when captured; she was first measured five months later at 60 mm snout-vent length.

The female produced 10 clutches of eggs in captivity in the absence of conspecific males, with a total of more than 28 eggs (1–4 per clutch). The absolute total number of eggs is unknown because one clutch was eaten by a cagemate before we found the eggs. Only 7 of the 28 eggs hatched, from two full clutches. The first successful clutch was of 3 eggs and these were the 3 hybrids on which this paper focuses. The second was a clutch of 4 eggs, all of which hatched into abnormal offspring with unusually short snouts and 2 of which also had unusually large eyes; all of these were weak and died within 17 days of hatching.

The successful clutch of three eggs that produced the hybrids described here was laid on 19 May 1999, when the mother had a snout-vent length of 70 mm and weight of 7.8 g. The clutch weighed 1.8 g, or 23% of the mother's weight. Egg dimensions were as follows (in mm): 8.9 × 13.5; 9.1 × 13.6; and 8.1 × 15.9. At 11:00 A.M., when the spent mother emerged from her damp burrow, the presumed father mated with her. Following incubation at room temperature, one egg hatched in 69 days, on 26 July 1999, and the other two in 70 days, on 27 July 1999. All three offspring appeared to be normal, and the light spots on their sides suggested immediately that they were hybrids, as hatchlings of the maternal species do not have spots on the body.

During her tenure in captivity, at various times the mother had been caged with other females of *A. i. arizonae* and with at least five different males of *A. t. marmorata,* but the only male with which she was observed mating was the one we refer to as the presumed father. From March through June 1999 the father was mating frequently with the only female in his cage, but that was not the mother, which had been housed alone in a different cage beginning 18 August 1998. However, because of this male's frequent mating activity at this time, we added the mother to the cage, on 11 May 1999, eight days before oviposition of the hybrid clutch, and apparently just in time.

The three hybrid offspring were the following: (1) AMNH R-148432, which hatched on 26 July 1999; its sex was not apparent while it was alive, and it died under the water dish in its cage on 13–15 November 1999, apparently from an accident when a caretaker (not in Acknowledgments) returned the cleaned dish to the cage; (2) AMNH R-153157, which hatched on 27 July 1999, which appeared to be a male, and had attained a snout-vent length of 78 mm when sacrificed for study on 15 August 2002 at more than three years of age; and (3) AMNH R-153158, which appeared to be a female and had attained a snout-vent length of 81 mm when sacrificed for study on 13 August 2003 at more than four years of age.

Upon hatching, the three hybrids were caged together but with no other lizards for about three months, until 1 November 1999, at which time they were maintained separated thereafter to ensure that no mating could occur. None of these lizards ever laid eggs, but the apparent female (AMNH R-153158) developed a large mass in the abdomen, the histology of which is described below.

Identification of the Hybrids: Genetics

KARYOTYPES: Clearly resolved karyotypes of most of the species in all the species groups of *Aspidoscelis* were published previously (e.g., Lowe et al., 1970; Cole et al., 1988), including the species discussed in this paper. Each species group has a diagnostically distinct karyotype, and *A. inornata* belongs to the *A. sexlineata* species group while *A. tigris* belongs to the *tigris* group (for phylogenetic relationships, see Reeder et al., 2002).

The haploid set of *A. inornata* (including *A. i. arizonae, n* = 23) consists of a single large Set I metacentric chromosome (with a slightly elongate satellite on one arm set off by a subterminal secondary constriction) + 12 smaller Set II intermediate-sized telocentric or subtelocentric macrochromosomes + 10 Set III microchromosomes. Sex chromosomes are not morphologically recognizable.

The haploid set of *A. tigris* (including *A. t. marmorata*; *n* = 23) consists of three large Set I biarmed (metacentric or submetacentric) macrochromosomes + eight smaller Set II biarmed (submetacentric) intermediate-sized macrochromosomes + 12 Set III microchromosomes. The second largest chromosome in Set I has a dotlike satellite on one arm set off by a nearly terminal secondary constriction, which often is difficult or impossible to see with standard microscopy. The third largest chromosome is the sex chromosome (XX female, XY male; Cole et al., 1969; Bull, 1978), the X and Y differing in centromere position, although both are submetacentric. In both *A. inornata* and *A. tigris* the secondary constrictions on large macrochromosomes are the nucleolar organizer regions (Ward and Cole, 1986).

As expected, the karyotype of the laboratory hybrid examined (AMNH R-153158) was diploid (2*n* = 46), consisting of one normal haploid complement each from the maternal *A. inornata* and the paternal *A. tigris* (fig. 1). The individual examined was the adult-sized apparent female, and, as expected, had the X sex chromosome of *A. t. marmorata.* The karyotype confirmed that this laboratory-hatched lizard was indeed a hybrid, probably between the

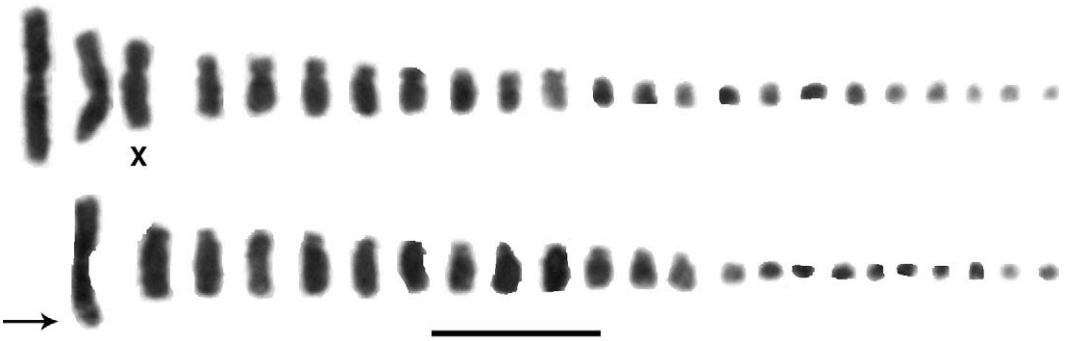

FIGURE 1. Karyotype of laboratory hybrid of *A. i. arizonae* (♀) × *A. t. marmorata* (♂), AMNH R-153158, adult-sized intersex individual that superficially resembled a female. Upper row represents the haploid complement of *A. t. marmorata* (with 3 large Set I metacentric and submetacentric macrochromosomes including the X + 8 biarmed Set II macrochromosomes + 12 Set III microchromosomes). Lower row represents the haploid complement of *A. i. arizonae* (with 1 Set I macrochromosome including its characteristic NOR and satellite [arrow] + 12 subtelocentric Set II macrochromosomes + 10 Set III microchromosomes). Line represents 10 microns.

A. inornata arizonae and *A. tigris marmorata* adults in whose cage the clutch of eggs appeared. This karyotype also is identical to that reported previously for one of the unisexual, parthenogenetic, and clonal species, *A. neomexicana* (see review in Cole et al., 1988), which had an origin in nature resulting from hybridization between *A. t. marmorata* (♀) × *A. inornata* (♂).

Allozymes: Protein electrophoresis of allele products representing 21 nuclear gene loci, with emphasis on those that specifically demonstrated the hybrid origin of *A. neomexicana,* provided additional evidence on the parentage of the laboratory hybrid examined. For these analyses, on each gel we compared one specimen each of *A. i. arizonae* from the maternal parent's population (AMNH R-153168), one of *A. t. marmorata* from the paternal parent's population (AMNH R-153163), one of *A. neomexicana* from New Mexico (AMNH R-151740), and one of the laboratory hybrids (AMNH R-153157), the adult-sized apparent male.

TABLE 1. Genotypes[a] at 21 Gene Loci[b] in Samples of *Aspidoscelis*[c]

Locus	INO	I×M	NEO	MAR
Oxidoreductases				
IDDH	bb	bb	bb	ab
LDH1	bb	ab	ab	aa
sIDH	bb	ab	ab	bb
sSOD	bb	ab	ab	aa
Transferases				
sAAT	bb	ab	ab	aa
mAAT	aa	ab	ab	bb
Hydrolases				
ESTD	aa	aa	ab	aa
PEPA	aa	ab	ac	bb
PEPB	bb	ab	ac	aa
PEPD	aa	aa	ab	aa
ADA	aa	ab	ab	bb
Isomerases				
MPI	bb	ab	ab	aa
GPI	bb	ab	bb	bb

[a] Alleles are designated in alphabetical order according to decreasing anodal migration of their allozymes. For multilocus systems, loci are numbered in order of decreasing anodal migration of their isozymes; s, cytosolic enzyme; m, mitochondrial enzyme.

[b] Abbreviations for loci are as follows: IDDH, L-iditol dehydrogenase; LDH, L-lactate dehydrogenase; IDH, isocitrate dehydrogenase; SOD, superoxide dismutase; AAT, aspartate aminotransferase; EST, esterase; PEP, peptidase; ADA, adenosine deaminase; MPI, mannose-6-phosphate isomerase; and GPI, glucose-6-phosphate isomerase. The following 8 loci were invariant (i.e., all lizards had one and the same allele in the homozygous state): G3PDH (glycerol-3-phosphate dehydrogenase); LDH2; s and mMDH (malate dehydrogenase); sMDHP (malate enzyme); mIDH; mSOD; and PGM2 (phosphoglucomutase).

[c] Abbreviations for species and individuals (columns) are as follows: INO, *A. inornata arizonae*; I×M, laboratory hybrid of *A. i. arizonae* × *A. t. marmorata*; NEO, *A. neomexicana*; and MAR, *A. t. marmorata.* See appendix (Specimens Examined) for details on each individual.

The allozyme data (table 1; fig. 2) confirm the differences previously reported for *A. inornata* versus *A. tigris* and the hybrid nature of both the unisexual *A. neomexicana* and the laboratory hybrid. For 8 loci (footnote b, table 1), all individuals examined were homozygous for the same allele (i.e., no variation observed), as expected for these lizards. Of the 13 loci showing variation (table 1), 6 were simply as expected, needing no further discussion, showing the normal allelic differences between *A. inornata* and *A. tigris,* with both the laboratory hybrid and *A. neomexicana* being heterozygous for the two respective parental alleles (LDH1, sSod, sAAT: fig. 2A; mAAT, ADA, and MPI: fig. 2B). The same is basically true for the remaining 7 loci, which are consistent with expectations based on Cole et al. (1988) and Dessauer et al. (2000), but the allozymes observed in these individuals merit further discussion, as follows.

IDDH (previously referred to as Sord): One would predict that all individuals compared here would be homozygotes for one and the same allele (no variation), because *A. t. marmorata*

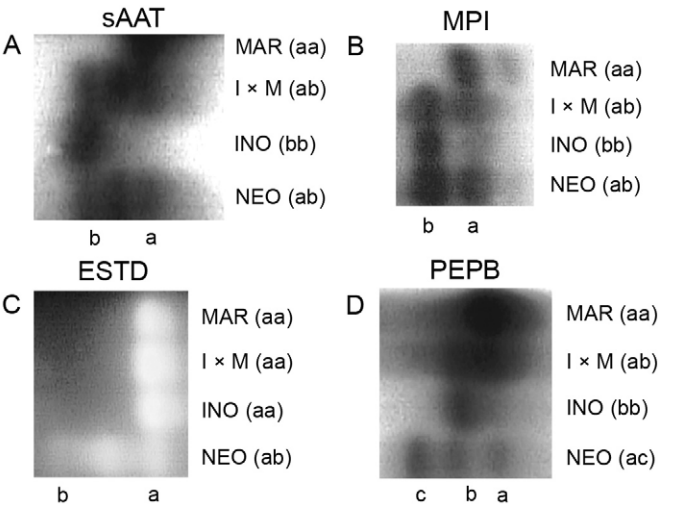

FIGURE 2. Electrophoretic phenotypes representing products of four gene loci of four individuals of *Aspidoscelis.* **A.** sAAT, a dimeric enzyme, from skeletal muscle homogenates. **B.** MPI, a monomeric enzyme, from erythrocyte hemolysates. **C.** ESTD, a dimeric enzyme, from skeletal muscle homogenates. **D.** PEPB, a dimeric enzyme, from kidney homogenates. Letters below gels identify allozymes based on alleles present (table 1). Lanes for individual lizards are labeled beside each gel (with genotype) as follows: MAR, *A. t. marmorata*; I × M, laboratory hybrid of *A. i. arizonae* × *A. t. marmorata*; INO, *A. i. arizonae*; and NEO, *A. neomexicana.* Anode for each is to the right.

is usually homozygous for the b-allele at this locus. However, an alternative a-allele of lower frequency does occur in *A. t. marmorata* (see Dessauer et al., 2000), and the individual examined for this report happened to be a heterozygote having both the a-allele and b-allele.

sIDH (previously referred to as Icd-1): One would predict the results obtained (table 1) except that the *A. inornata* would have the a-allele, as found in the heterozygous state in *A. neomexicana* and laboratory hybrid. However, *A. inornata* is known to have alternative alleles of lower frequency (Cole et al., 1988), including the b-allele seen here.

ESTD (previously referred to as Es-D): *A. inornata* and *A. tigris* were known to normally have the same allele, consistent with the laboratory hybrid being homozygous for their common allele (fig. 2C). The alternative allele seen in the heterozygous state in the field sample of *A. neomexicana* is its orphan allele at this locus, known to characterize *A. neomexicana,* but having never been observed in either of its parental species.

PEPA: The a-allele observed is typical for *A. inornata,* as is the b-allele for *A. tigris* from the Lordsburg area, and as found in the heterozygous laboratory hybrid. The c-allele, which is typical for *A. neomexicana,* is found at times in *A. tigris* from the Rio Grande Valley, perhaps the area of origin of *A. neomexicana* (see Cole et al., 1988).

PEPB: All alleles observed fit the prediction (fig. 2D). The c-allele seen in the heterozygous state in the field sample of *A. neomexicana* is its orphan allele at this locus, known to characterize this species, but which has not been observed in either of its parental species.

PEPD: This locus has not been analyzed before in *A. neomexicana,* in which we observed heterozygosity for ab, but the other results are not surprising. Considering that this locus is the most variable known in *A. tigris* (see Dessauer et al., 2000), it would not be surprising if the ancestral *A. t. marmorata* had contributed the slower b-allele observed here in *A. neomexicana.*

GPI: The a-allele and b-allele both occur as variants in both *A. inornata* and *A. t. marmorata* (Dessauer and Cole, 1989), and the a-allele is known in *marmorata* from the Lordsburg area (Dessauer et al., 2000), so it is not surprising that it occurred in the laboratory hybrid.

In summary, the allozyme data are fully consistent with the karyotype data and indicate that the laboratory hybrid did have the *A. inornata* × *A. tigris* hybrid origin predicted, even though this hybrid differed from *A. neomexicana* in allele combinations at 5 loci analyzed, including the 2 orphan alleles previously known for *neomexicana.*

Identification of the Hybrids: External Morphology

Color and Pattern: The variation in colors and patterns of the whiptail lizards discussed here is difficult to quantify, but *A. i. arizonae* and *A. t. marmorata* are so different they can be readily identified even while running on the ground. Consequently, we use general descriptions based on field notes and specimens noted in life. The *A. i. arizonae* are from the vicinity of Willcox, Cochise County, Arizona; the *A. t. marmorata* from the vicinity of Lordsburg, Hidalgo County, New Mexico; and the clonal *A. neomexicana* from throughout its range.

Aspidoscelis inornata arizonae (Maternal Species, fig. 3): The dorsum of this lizard is dark gray to brown (bluish gray atop head) usually with seven conspicuous light yellow stripes (but the vertebral stripe may be less conspicuous than the others and may be broken). There are no spots or bars on the body. The arms are uniform grayish brown, but there may be a light beige

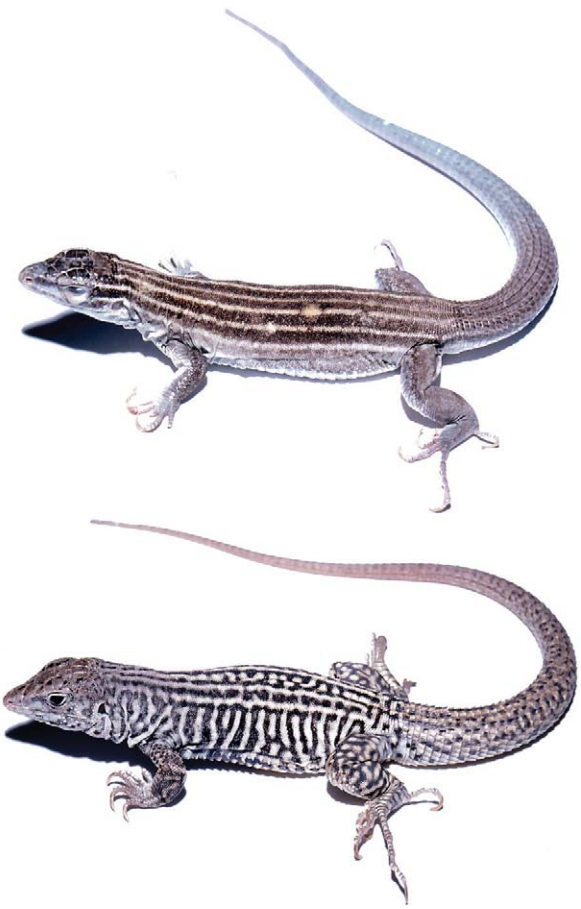

FIGURE 3. Parents of the *Aspidoscelis* hybrids shown in fig. 4. **Upper.** *A. i. arizonae,* as a very old female, AMNH R-148431, SVL = 69 mm. **Lower.** *A. t. marmorata,* male, AMNH R-153156, SVL = 82 mm.

line on the outer edge of the lower arm. The thighs are brown with a trace of a light yellowish beige reticulation, the lower legs grayish brown. The tail is basically tan at the base, with a rapid transition to bright blue. The ventral surfaces are pale blue to nearly white (in females) or bright blue (males, especially in the breeding season).

Aspidoscelis tigris marmorata (Paternal Species, fig. 3): The dorsum often is "unstriped (a few light yellow stripes or portions or traces thereof may be visible), with a dark brown to black

FIGURE 4. Three *Aspidoscelis* of hybrid origin. **Upper.** Laboratory hybrid, intersex, AMNH R-153158, SVL = 81 mm. **Middle.** Laboratory hybrid, male, AMNH R-153157, SVL = 78 mm. **Lower.** *A. neomexicana* from nature, AMNH R-122946, SVL = 77 mm. Parents of upper two are in fig. 3. Ancestry of lower one was of similar parents but the reciprocal cross.

ground color and a pattern (reticulate, marbled, or, especially laterally, cross-barred) of light yellow or beige, including some light spots; some individuals have more prominent wavy vertebral and paravertebral stripes" (Cole et al., 1988: 13). Moreover,

> The dorsal surfaces of the hind legs are dark brown to black with numerous light yellow to beige spots. . . . The dorsal surfaces of the arms are similar to the legs, although in the largest individuals they may become covered with a gray wash. . . . The anterior third of the tail is checkered with dark brown and yellow to beige; posteriorly, the tail is essentially uniform brown with occasional darker brown (or black) flecks. . . . The ventral surfaces are as follows: throat with black spots on an orange or gray wash; chest checkered with black, orange, and a few cream spots; abdomen, hind legs, and tail yellow (Cole et al., 1988: 14).

Aspidoscelis neomexicana and Laboratory Hybrid, Adult-sized Apparently Female, AMNH R-153158 (fig. 4): The laboratory hybrid noted in detail and typical *Aspidoscelis neomexicana* were so similar that the notes for both are included here. The extract below quotes a description of the color notes of *A. neomexicana*; these apply equally to the laboratory hybrid of *A. i. arizonae* × *A. t. marmorata,* except where differences of the hybrid are noted in brackets:

> A dark brown [medium brown] ground color but it consistently has both light spots (beige) and seven light stripes; the ventralmost stripe is cream and the others are pale yellow [vertebral stripe very pale tan]. The two ventralmost stripes are essentially straight [less wavy than the others] but the vertebral and paravertebral stripes are, respectively, quite wavy (zigzag) [somewhat less so] and somewhat wavy posterior to the shoulder region. The beige spots are most evident in the two lateral dark fields, tending to be in a row within the field . . . the arms usually are brown with several beige spots or stripes . . . the hind legs are brown with a . . . conspicuous beige reticulation . . . the posterior three-quarters of the tail . . . is grayish green [blue upon hatching] . . . the ventral surfaces are . . . unmarked and generally pale blue or gray (abdomen essentially cream) [pale bluish gray including the abdomen]; underside of most of the tail is gray (Cole et al., 1988: 14–15).

In addition, the hybrid, unlike *A. neomexicana* but somewhat like its paternal parent had a very pale orange wash across the entire throat on which there were many dark gray to pale black dots or small spots.

What is most striking about the colors and patterns of the laboratory hybrids is that they appeared much more similar to *A. neomexicana* than to either of their parents (figs. 3, 4). This is no surprise, given that *A. neomexicana* had a hybrid origin of *A. t. marmorata* × *A. inornata.*

Scalation and Multivariate Statistics: The maternal parent (*A. i. arizonae*) of the laboratory hybrids had lower scores than the paternal parent (*A. t. marmorata*) for all seven meristic characters (table 2). Hybrids were either intermediate to their parents or they equaled one of the parents in GAB, FP, COS, SDL, and PSC scores (see Materials and Methods and table 2 for details on recording the characters abbreviated here with capital letters). All three hybrids had smaller GUL scores than those expressed by the maternal parent, as did two

TABLE 2. Counts of meristic characters and SVL for three laboratory hybrids and their maternal (*Aspidoscelis inornata arizonae*) and paternal (*A. tigris marmorata*) parents and descriptive statistics for samples of *A. inornata arizonae, A. tigris marmorata,* and the related *A. neomexicana.* Means ± SE are subtended by sample size and (range limits); M = maternal parent of hybrids; P = paternal parent of hybrids.

	Individuals and Samples							
Character[a]	*arizonae* M[b]	Hybrid 1[c]	Hybrid 2[d]	Hybrid 3[e]	*marmorata* P[f]	*arizonae*[g]	*marmorata*[h]	*neomexicana*[h]
GAB	70	74	79	72	89	65.3 ± 0.79 18 (59–71)	91.5 ± 1.13 20 (84–102)	80.9 ± 0.68 22 (75–87)
FP	14	15	17	15	18	15.2 ± 0.34 18 (13–19)	21.3 ± 0.36 20 (18–24)	20.0 ± 0.18 22 (18–21)
COS	10	13	16	16	23	9.5 ± 0.38 18 (7–12)	21.6 ± 0.72 20 (14–27)	23.7 ± 0.39 21 (20–27)
ILS	17	13	18	7	24	16.1 ± 0.90 18 (11–29)	32.2 ± 1.48 20 (16–41)	22.6 ± 0.77 12 (18–28)
SDL	25	32	29	30	32	27.3 ± 0.58 18 (23–33)	32.6 ± 0.37 20 (30–36)	32.9 ± 0.26 21 (30–35)
GUL	21	17	20	19	26	18.6 ± 0.51 18 (15–22)	22.8 ± 0.41 18 (21–26)	18.8 ± 0.68 12 (15–21)
PSC	15	20	20	19	20	18.0 ± 0.42 18 (15–21)	19.8 ± 0.49 20 (14–24)	18.2 ± 0.36 20 (16–22)
SVL	69	78	81	49	82	64.1 ± 1.25 18 (55–72)	85.8 ± 0.97 20 (76–93)	69.4 ± 0.95 22 (62–78)

[a] Characters are as follows: GAB, number of granules (scales) around midbody; FP, number of femoral pores on one thigh (left was chosen unless damaged); COS, total number of circumorbital scales; PSC, number of scales contacting the outer perimeter of parietal and interparietal scales; ILS, total number of interlabial scales: GUL, number of gular scales; SDL, number of subdigital lamellae on fourth toe of one foot (left was chosen unless damaged); SVL, length of body from snout to vent.

[b] AMNH R-148431.

[c] AMNH R-153157.

[d] AMNH R-153158.

[e] AMNH R-148432.

[f] AMNH R-153156.

[g] Sample from Willcox vicinity, Cochise County, Arizona.

[h] Samples from Lordsburg vicinity, Hidalgo County, New Mexico. Specimen identities are provided in Specimens Examined (appendix).

hybrids for ILS. The two hybrids that attained adult size resembled the larger, paternal parent (*A. t. marmorata*) in SVL (table 2).

We used two principal components analyses (PCAs) to provide unbiased representations of meristic variation. PCA does not use a priori information on specific affiliations (i.e., specimen identification is not recognized), and we coupled PC scores to particular specimens only after the analyses were completed. As determined from character loadings (table 3), both PCAs were identical in having the greatest contributions to PC1 made by GAB, FP, COS, ILS, SDL and GUL, while PC2 was based primarily on the PSC character.

Our first PCA model (PCA1; $N = 39$ specimens) addressed the question of how the laboratory hybrids compared with each other, with their parents, and with field samples of their

TABLE 3. Principal component loadings and standardized canonical variate coefficients used in multivariate analyses of three laboratory hybrids, their *Aspidoscelis tigris marmorata* and *A. inornata arizonae* parents, geographically representative samples of the parental species, and the related *A. neomexicana.*

	PCA1[a]		PCA2[b]		CVA[c]	
Character	PC1	PC2	PC1	PC2	CV1	CV2
GAB	0.962	−0.080	0.955	−0.054	0.553	0.396
FP	0.938	−0.050	0.937	−0.065	0.400	−0.016
COS	0.930	−0.131	0.866	−0.255	0.579	−0.896
ILS	0.866	−0.068	0.838	0.040	−0.105	0.730
SDL	0.831	−0.055	0.829	−0.240	0.047	−0.152
GUL	0.755	−0.054	0.598	0.340	0.275	0.687
PSC	0.434	0.901	0.346	0.857	0.170	−0.089
Eigenvalue	4.868	0.848	4.407	0.982	17.283	3.228
Total variance explained (%)	69.5	12.1	63.0	14.0	84.3	15.7

[a] PCA1 = a principal components analysis of three hybrids, parents of the hybrids, and representative samples of *A. inornata arizonae* and *A. tigris marmorata.*

[b] PCA2 = a principal components analysis of three hybrids, parents of the hybrids, and representative samples of *A. inornata arizonae, A. tigris marmorata,* and *A. neomexicana* (see appendix, Specimens Examined).

[c] CVA = a canonical variate analysis using samples of *A. inornata arizonae, A. tigris marmorata,* and *A. neomexicana* as a priori groups (parental specimens included in appropriate groups) and the three hybrids included in the CVA model as unassigned for classification to a priori group.

parental taxa from the same localities where their parents were collected. Although the three hybrids were intermediate to their individual parental specimens on the first principal component axis (PC1), where 69.5% of the variation was summarized, the hybrids most closely resembled their paternal parent, *A. t. marmorata* on PC2, where 12.1% of the variation was summarized (fig. 5). Considering all samples rather than just the individual parents, however, the laboratory hybrids were most similar to *A. i. arizonae,* perhaps expressing matriclinous inheritance.

Our second PCA model (PCA2; $N = 50$ specimens) was identical to PCA1 except that a geographically relevant sample of *A. neomexicana* was added to the mix in order to determine whether the laboratory hybrids resembled this parthenogenetic species of similar hybrid origin. Because the laboratory hybrids originated from a reciprocal cross between the same parental species that gave origin to *A. neomexicana* (evidence summarized in Cole et al., 1988), this permitted us to assess differences in the pattern of multivariate variation between *A. i. arizonae* (♀) × *A. t. marmorata* (♂), the source of the laboratory hybrids, and *A. t. marmorata* (♀) × *A. inornata* (♂), the source of the lineage represented by contemporary *A. neomexicana.* The first principal component summarized 63.0% of the meristic variation in this analysis, with PC2 summarizing 14% of the remaining variation (table 3). The three hybrids were intermediate to their individual parental specimens on both PC1 and PC2 (fig. 6; table 4). The tendency of the laboratory hybrids and a hybridization-derived species to exhibit matriclinous resemblances was demonstrated by the products of the two reciprocal crosses—the laboratory hybrids most closely resembled their maternal *A. i. arizonae,* and the *A. neomexicana* most closely resembled their maternal *A. t. marmorata* on PC1 (fig. 6).

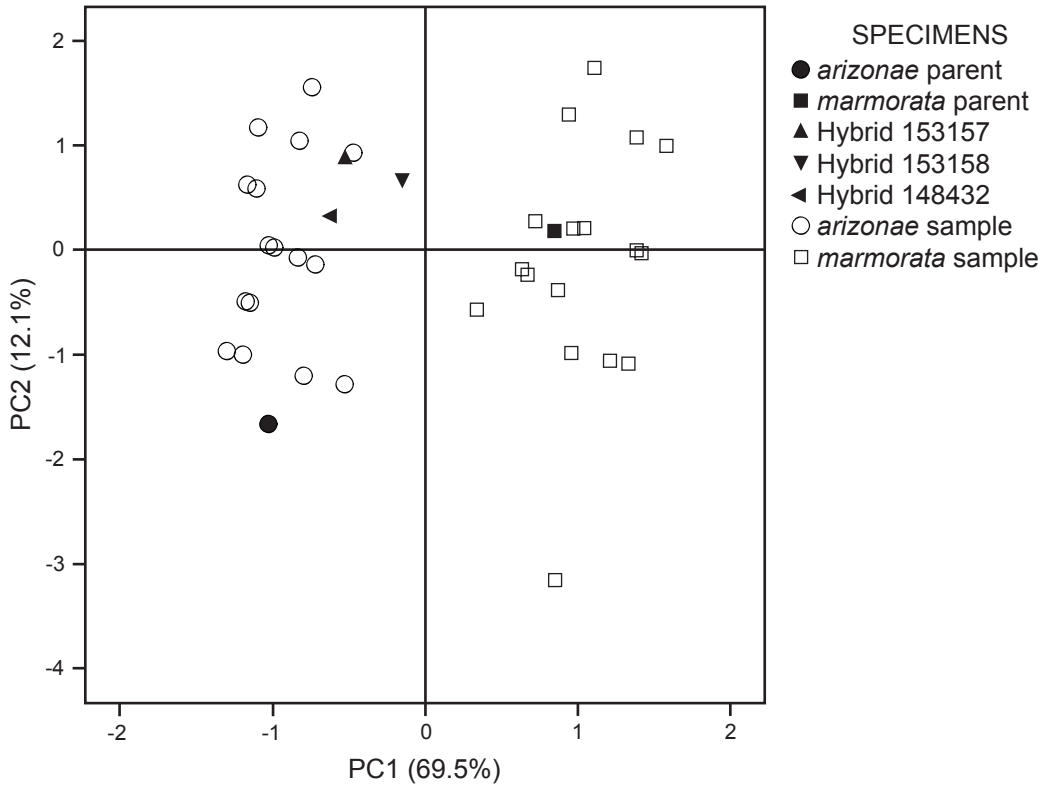

FIGURE 5. Pattern of morphological variation expressed by the distribution of scores on the first two principal components extracted from a correlation matrix of seven meristic characters of three laboratory hybrids, 18 specimens of *A. i. arizonae* (including the maternal parent of the hybrids), and 18 specimens of *A. t. marmorata* (including the paternal parent of the hybrids). All samples represent populations in the vicinities of those from which the parents of the hybrids were collected. Note that the three hybrids are intermediate to their individual parents on PC1.

We also wondered how these laboratory hybrid specimens might be identified if they had been found in the field by a collector who prepared no material for genetic analyses. Because the three laboratory hybrids had the basic colors and pattern of *A. neomexicana,* their true identity might not have been recognized in the field. However, meristic counts would have aroused suspicion. Scores for laboratory hybrids were below the lower range limits in our reference sample of *A. neomexicana* (table 2) for the following characters: GAB (two hybrids); FP (all three hybrids); COS (all three hybrids); and ILS (two hybrids). These low counts presumably reflect genetic effects that had been derived from *A. i. arizonae,* their maternal parent. Therefore, we did a canonical variate analysis (CVA), with the hybrids included in the model as unknowns. The reference samples of *A. i. arizonae, A. t. marmorata,* and *A. neomexicana* were used as a priori groups. Although not uniformly obvious from the CV coefficients in table 3, GAB, COS, FP, SDL, and ILS had higher correlations (communalities) with CV1 while GUL and PSC were most highly correlated with CV2. The CVA assigned hybrid AMNH R-153157 to the *A. i. arizonae* a priori group ($P = 1.0$) and hybrids AMNH R-153158 and 148432 to the

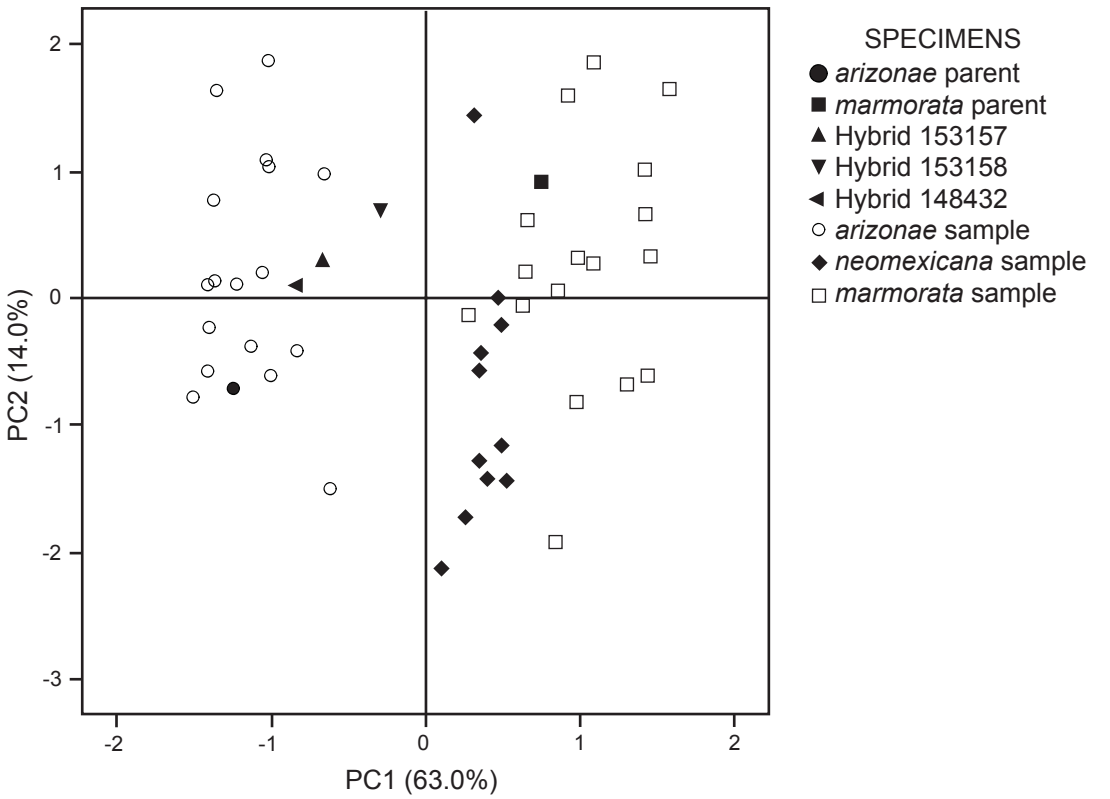

FIGURE 6. Pattern of morphological variation expressed by the distribution of scores on the first two principal components extracted from a correlation matrix of seven meristic characters of three hybrids, 18 specimens of *A. i. arizonae* (including the maternal parent of the hybrids), 18 specimens of *A. t. marmorata* (including the paternal parent of the hybrids), and 11 specimens of the unisexual *A. neomexicana.* All samples represent populations in the vicinities of those from which the parents of the hybrids were collected. Note that the three hybrids are intermediate to their individual parents and that *A. neomexicana* most closely resembles its maternal progenitor species, *A. t. marmorata* on PC1.

A. neomexicana a priori group ($P = 0.88$ and $P = 0.54$, respectively). Nevertheless, collectively, the hybrids appeared to occupy a position intermediate to their two parental taxa and *A. neomexicana* on CV1 (fig. 7). Consequently, a field collector in the absence of genetic data might have identified these hybrids incorrectly, as hybrids between *A. neomexicana* (♀) × *A. i. arizonae* (♂), considering both color pattern and meristics.

As a check on the distinctiveness of the laboratory hybrids as an independently recognizable entity, we included them as a fourth a priori group in a follow-up CVA (plot not reproduced here). The a priori hybrid group was as distinctive as the *A. i. arizonae, A. t. marmorata,* and *A. neomexicana* a priori groups, as there was 100% classification success for the members of each group, and each hybrid had an assignment probability of 1.0. Finally, we compared the list of characters of our laboratory hybrids (table 2) with the same characters for the type specimen of "*Cnemidophorus perplexus*" (USNM 3060) as reported by Wright and Lowe (1967). The specimens are quite similar, differing primarily in appearance of the vertebral light

TABLE 4. Multivariate statistics for three laboratory hybrids and their maternal (*Aspidoscelis inornata arizonae*) and paternal (*A. tigris marmorata*) parents and descriptive multivariate statistics for samples of *A. inornata arizonae, A. tigris marmorata,* and the related *A. neomexicana.* Means ± SE are subtended by range limits; M = maternal parent of hybrids; P = paternal parent of hybrids.

	Individuals and Samples							
Analysis[a]	*arizonae* M[b]	Hybrid 1[c]	Hybrid 2[d]	Hybrid 3[e]	*marmorata* P[f]	*arizonae*[g]	*marmorata*[h]	*neomexicana*[h]
PCA 1						*N* = 18	*N* = 18	
PC1	−1.03	−0.53	−0.15	−0.62	0.85	−0.94 ± 0.05	1.02 ± 0.08	—
						−1.29 to −0.48	0.34 to 1.58	—
PC2	−1.65	0.88	0.69	0.33	0.19	−0.01 ± 0.23	−0.09 ± 0.26	—
						−1.65 to 1.56	−3.16 to 1.74	—
PCA 2						*N* = 18	*N* = 18	*N* = 11
PC1	−1.25	−0.69	−0.30	−0.83	0.75	−1.15 ± 0.06	1.02 ± 0.09	0.37 ± 0.04
						−1.51 to −0.63	0.28 to 1.59	0.10 to 0.52
PC2	−0.71	0.27	0.71	0.10	0.91	0.15 ± 0.21	0.29 ± 0.22	−0.81 ± 0.30
						−1.50 to 1.87	−1.91 to 1.85	−2.12 to 1.44
CVA						*N* = 18	*N* = 18	*N* = 11
CV1	−4.65	−2.93	−0.71	−2.20	3.40	−5.04 ± 0.19	3.75 ± 0.31	2.11 ± 0.19
						−6.73 to −3.89	1.13 to 5.69	1.13 to 2.94
CV2	1.85	−1.60	−0.34	−2.95	0.57	0.34 ± 0.23	1.50 ± 0.25	−3.01 ± 0.30
						−1.03 to 1.95	−0.40 to 3.40	−4.75 to −1.41

[a] PCA1 = a principal-components analysis of three hybrids, parents of the hybrids, and representative samples of *A. inornata arizonae* and *A. tigris marmorata.* PCA2 = a principal-components analysis of three hybrids, parents of the hybrids, and representative samples of *A. inornata arizonae, A. tigris marmorata,* and *A. neomexicana.* CVA = a canonical-variate analysis using samples of *A. inornata arizonae, A. tigris marmorata,* and *A. neomexicana* as a priori groups, and parental specimens included as members of the appropriate a priori group. The three hybrid specimens were included in the CVA model as unassigned for classification to group. All three analyses used the following meristic characters: GAB, number of granules (scales) around midbody; FP, number of femoral pores on one thigh (left was chosen unless damaged); COS, total number of circumorbital scales; PSC, number of scales contacting the outer perimeter of parietal and interparietal scales; ILS, total number of interlabial scales; GUL, number of gular scales; SDL, number of subdigital lamellae on fourth toe of one foot (left was chosen unless damaged).

[b] AMNH R-148431.

[c] AMNH R-153157.

[d] AMNH R-153158.

[e] AMNH R-148432.

[f] AMNH R-153156.

[g] Sample from Willcox vicinity, Cochise County, Arizona.

[h] Samples from Lordsburg vicinity, Hidalgo County, New Mexico. Specimens are listed in Specimens Examined (appendix).

stripe and ILS, the latter of which could reflect different methods of counting by different investigators. This illustrates once again the difficulties of accurately identifying perplexing individuals of whiptail lizards in the absence of genetic data. For example, Taylor and Walker (1996) and Walker (1997) suggested that USNM 3060 is an unusually large individual of *A. neomexicana,* rather than a hybrid as suggested by Wright and Lowe (1967), all in the absence of genetic data.

As discussed above, *Aspidoscelis neomexicana* shows matriclinous inheritance, in resembling *A. t. marmorata.* This stronger multivariate resemblance of *A. neomexicana* to *A. t. marmorata*

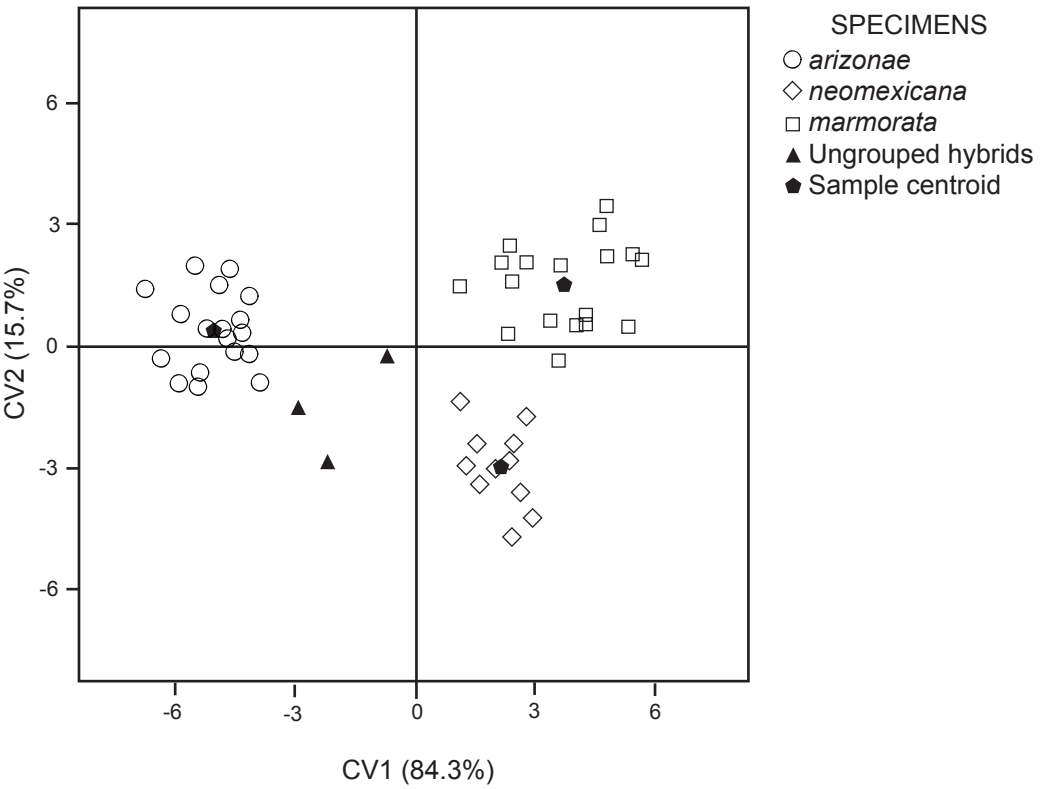

FIGURE 7. Pattern of morphological distinctiveness expressed by the distribution of canonical variate scores derived from a canonical variate analysis of seven meristic characters of three a priori groups: 18 specimens of *A. i. arizonae* (including the maternal parent of the laboratory hybrids), 18 specimens of *A. t. marmorata* (including the paternal parent of the hybrids), and 11 specimens of *A. neomexicana.* All samples represent populations in the vicinities of those from which the parents of the hybrids were collected. The three laboratory hybrids were included in the CVA as unassigned, for classification to the a priori group that each most closely resembled. Note the position of the hybrid group intermediate to *A. i. arizonae, A. t. marmorata,* and *A. neomexicana* clusters. This suggested that the hybrid group itself is distinctive, which was verified by a follow-up CVA (not illustrated, but see text).

was also evident in a previous analysis (Cole et al., 1988: fig. 5). That analysis differed from the present one in two respects, neither of which should negate the conclusion of matriclinous inheritance: (1) their reference sample for *inornata* was of *A. i. llanuras* Wright and Lowe, 1993, from the vicinity of Lordsburg, Hidalgo County, New Mexico, rather than *A. i. arizonae*; and (2) in addition to the sample of *A. neomexicana* from the Lordsburg area (the same sample we used here), they also used a sample of *A. neomexicana* from the northern periphery of its range in the Rio Grande Valley, New Mexico. In fact, the morphological data analyzed for the two subspecies of *A. inornata* (*arizonae* vs. *llanuras*) are very similar (compare Cole et al., 1988; Wright and Lowe, 1993; and table 2 here).

Internal Anatomy: The maternal parent of the laboratory hybrids (AMNH R-148431) was of normal size and external morphology for an individual of *A. i. arizonae.* The left and right oviducts were swollen and of normal appearance for a reproductive female, although

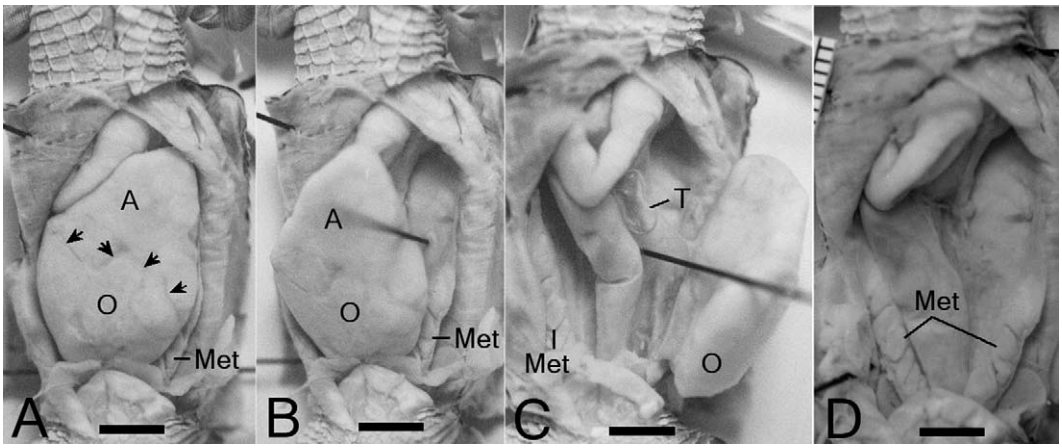

FIGURE 8. Gross morphology of the adult-sized, apparently female (but intersex) laboratory hybrid (AMNH R-153158) of *A. i. arizonae* × *A. t. marmorata.* **A.** Ventral view of the viscera through the opened body wall; the organs have not been disturbed. The arrows indicate a boundary between the left adrenal gland and the left ovary. **B.** The left kidney and dorsal body wall visible with the adrenal/ovary mass displaced to the right. **C.** Adrenal/ovary mass displaced to the left. **D.** Remaining viscera after removal of the adrenal/ovary mass and a suspected testis. Scale bars: 5 mm. Abbreviations in figures 8–17 are explained in Materials and Methods.

neither contained eggs. The left ovary contained one yellowish ovum 3.4 mm in diameter that appeared to be undergoing vitellogenesis; all the other ova that were visible macroscopically in this ovary were white and the largest one was 1.5 mm in diameter. The right ovary contained three yellowish ova that were undergoing vitellogenesis and were 3.5, 3.7, and 3.3 mm in diameter (from anteriormost to posteriormost). The next largest ovum in the right ovary was white and 1.7 mm in diameter. Both ovaries appeared normal. The stomach was full and there was no evidence of any internal abnormalities. This animal was reproductive (she had produced offspring in the laboratory) and appeared normal in every respect; therefore, no organs were removed for histological study.

The paternal parent (AMNH R-153156) contained testes and epididymydes of normal appearance on both sides, as expected for an individual of *A. t. marmorata.* The anterior end of the left testis was 4 mm posterior to the posterior end of the right testis, and seminiferous tubules were visible macroscopically in both. The left testis and epdidymus were removed for histological study.

The adult-sized, apparently female hybrid (AMNH R-153158) appeared to be in good health when sacrificed and preserved. Examination of the abdominal viscera via incisions in the ventral abdominal wall revealed a greatly enlarged mass (23 × 16 mm) that dominated the abdominal cavity (fig. 8A). The mass, when displaced to the right, but still intact, revealed the left metanephric kidney against the dorsal body wall (fig. 8B). When the mass was displaced to the left the right metanephric kidney could be seen (fig. 8C). Also visible on the left side, approximately adjacent to the anterior end of the left metanephric kidney, was a small oval structure to be identified later as having malelike structures (discussed below as specimen AMNH R-153158B;

fig. 8C). Consequently, histological examination revealed that this individual was an intersex. The large mass was removed also (discussed below as specimen AMNH R-153158A); no other gonadlike structures were visible in the body cavity (fig. 8D).

The smallest hybrid offspring (AMNH R-148432) was in bad condition, as it had died in its cage and was partly decomposed when found. The stomach was thin-walled and full of cricket parts, but the intestine distal to the pyloric sphincter was empty. Two small structures (provisionally thought to be possibly a gonad and adrenal gland) on the right side were removed for histological study.

The remaining adult-sized hybird offspring (AMNH R-153157) appeared to be a male with paired testes, epididymides, and vasa deferentia. The vas deferens and epididymus were neither enlarged nor convoluted, so this individual did not appear to be reproductively functional at the time of preservation. There was no evidence macroscopically of any ovary, oviduct, or uterus. A testis and epididymus were removed for histological study.

Reproductive Histology

Paternal Parent: An adult male of *Aspidoscelis t. marmorata,* AMNH R-153156 was the probable father of the laboratory hybrids discussed here. His testis appeared normal with well-defined seminiferous tubules (fig. 9A). The seminiferous tubules were thin-walled and appeared similar in structure (fig. 9B) to those studied earlier in other specimens of this taxon (Taylor et al., 2001: fig. 19D–F). The tubules of this specimen contained cellular and noncellular debris, few spermatocytes, and sperm only in a few peripheral tubules (figs. 9A, 10A, 10B). However, the vas deferens was packed with sperm (figs. 9C, 9D, 10C). The testis was adjacent to a normal-appearing adrenal gland with adrenal (chromaffin) cells and interrenal cells (fig. 10A), and the edge away from the testis contained both small and large mesonephric tubules (figs. 9A, 10C), representing the epididymus. This specimen was not undergoing additional spermatogenesis when preserved but was reproductive with a sperm-packed epididymus that had not been completely evacuated.

Adult-sized, Apparently Female Hybrid (AMNH R-153158): The smaller tissue sample sectioned (AMNH R-153158B) consisted of 20 slides. The major structures in this sample included part of the mesonephros and adrenal gland (fig. 11A) and a small piece of liver. The adrenal gland was predominantly composed of interrenal cells with small, scattered clusters of chromaffin or adrenal cells (fig. 11B). The largest tubules visible were the mesonephric duct concentrated near the posteriomedial portion of the adrenal (fig. 11C). Mesonephric tubules formed a layer on the lateral and posteriolateral edge of the adrenal and consisted of larger tubules toward the outer surface and smaller tubules closer to the adrenal gland (fig. 11D). The physical relationship of the mesonephric tubules to the adrenal was consistent with that seen in other species of lizards studied previously (Hardy et al., 1989). There was no evidence of any gonadal tissue in this sample; however, the relationship of the mesonephros and adrenal gland was similar to that seen in male hybrids of *A. tesselata* × *A. t. marmorata* (Taylor et al., 2001: fig. 19G) and the mesonephros even resembled an epididymus in AMNH R-153158. This particular structure is more malelike than femalelike in this hybrid.

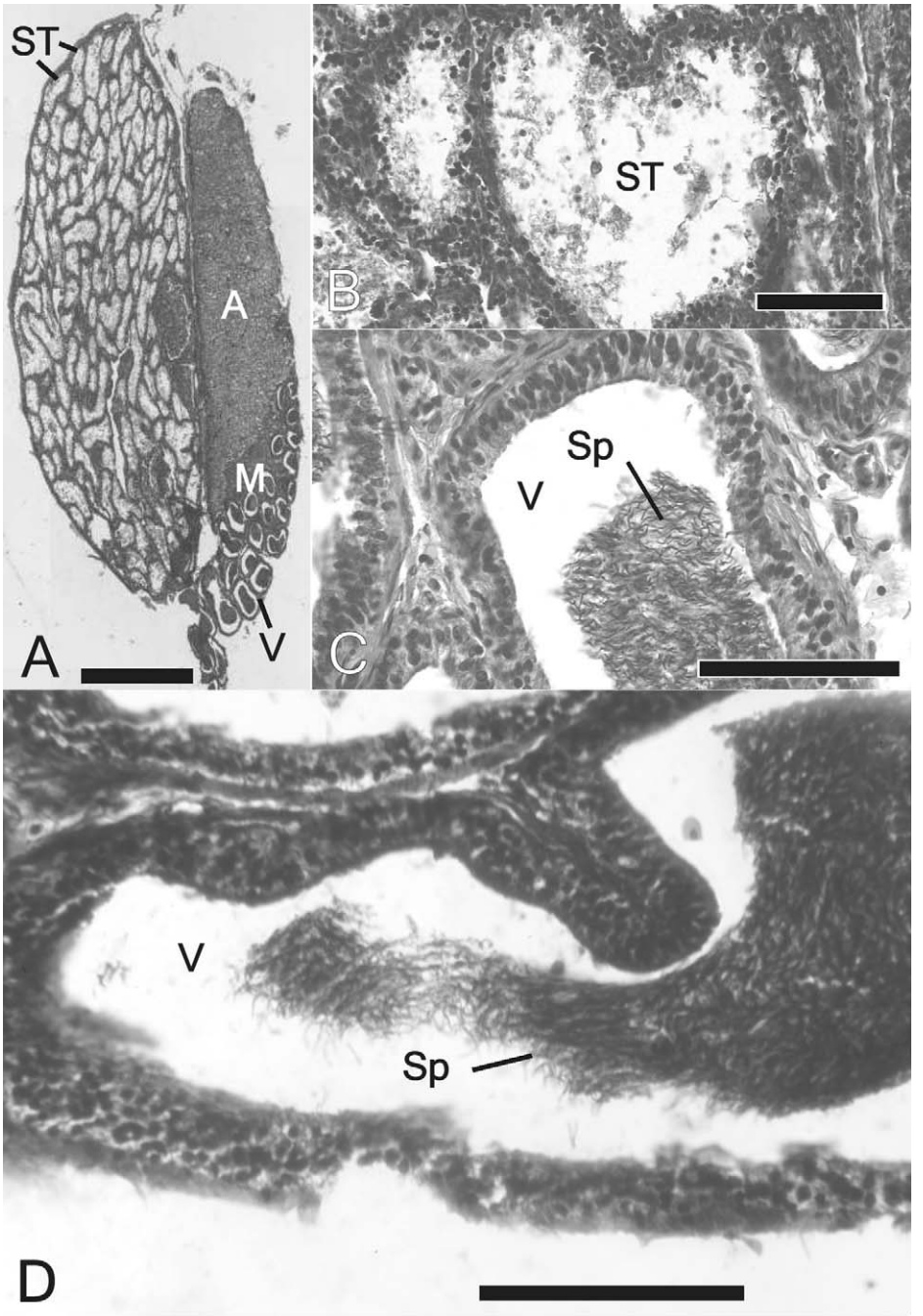

FIGURE 9. Testis, adrenal gland, mesonephros, and vas deferens of *A. t. marmorata,* the father of the hybrids. **A.** Testis, adrenal gland, mesonephros, and vas deferens (AMNH R-153156, slide 8, row 1, section 2). **B.** Seminiferous tubule (AMNH R-153156, slide 8, row 1, section 2). **C.** Vas deferens containing mature spermatozoa (AMNH R-153156, slide 9, row 1, section 6). **D.** Vas deferens with mature spermatozoa (AMNH R-153156, slide 6, row 1, section 2, Mallory Triple, Pantin method). Scale bar: 0.1 mm except for A, 1.0 mm.

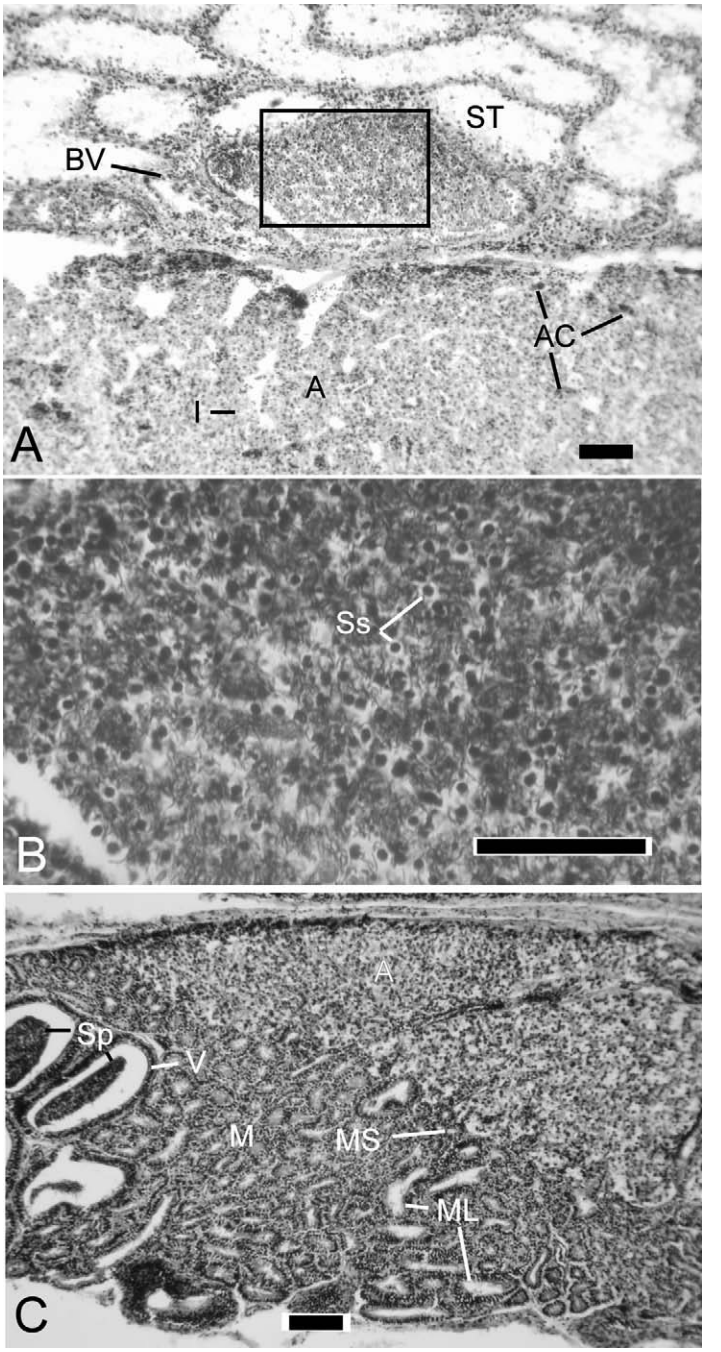

FIGURE 10. Testis, adrenal gland, mesonephros, and vas deferens of *A. t. marmorata,* the father of the hybrids. **A.** Testis and adrenal gland (AMNH R-153156, slide 9, row 1, section 7). Area outlined by the black rectangle indicates an active seminiferous tubule adjacent to the adrenal gland and is enlarged in figure 10B. Note that the surrounding seminiferous tubules were empty. **B.** A productive seminiferous tubule (AMNH R-153156, slide 9, row 1, section 7). **C.** Adrenal gland, mesonephros, and vas deferens (AMNH R-153156, slide 7, row 1, section 6). Note the small and large tubules of the mesonephros and the vas deferens, all of which comprise the epididymus. Scale bars: 0.1 mm.

The larger tissue sample sectioned from the same lizard (AMNH R-153158A) consisted of 197 slides. The tissue had some clear organization; approximately the anterior half was composed of adrenal (fig. 12A–D) and the posterior half was a disorganized mass of stromalike material (fig. 12A, C–E). As the left adrenal gland is normally anterior to the left ovary and the

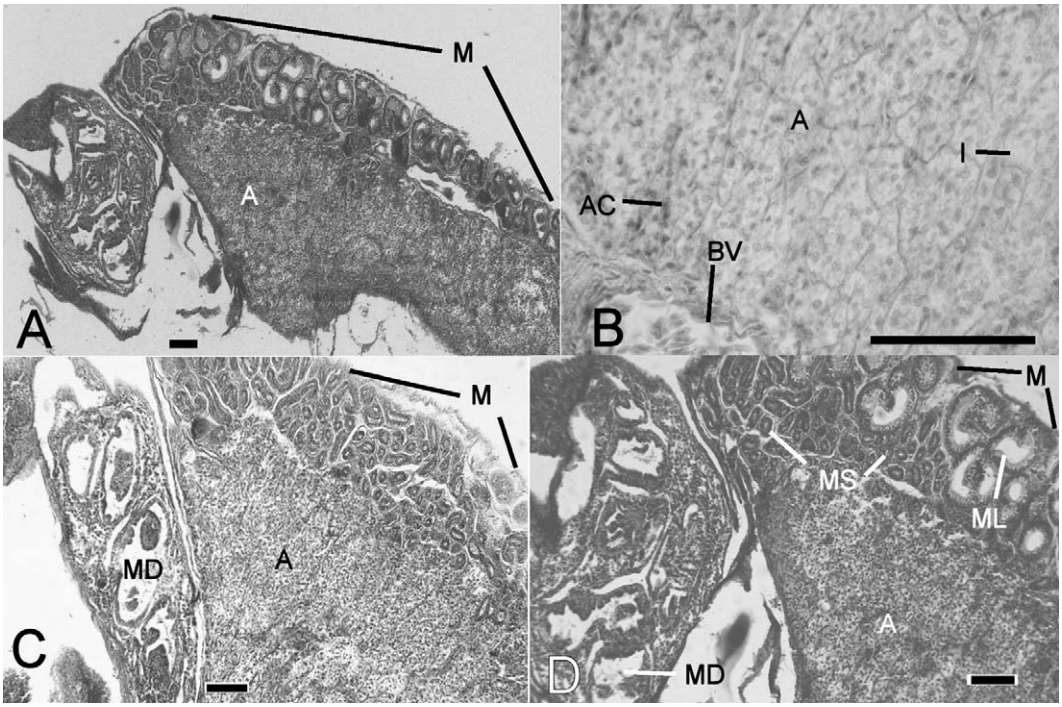

FIGURE 11. The small tissue sample from the adult-sized, apparently female (but intersex) laboratory hybrid (AMNH R-153158). **A.** Mesonephros and adrenal gland (AMNH R-153158B, slide 6, row 2, section 1). **B.** Adrenal gland (AMNH R-153158B, slide 3, row 1, section 10). **C.** Mesonephros and adrenal gland (AMNH R-153158B, slide 5, row 1, section 6). **D.** Mesonephros and adrenal gland (AMNH R-153158B, slide 6, row 2, section 1). Scale bars: 0.1 mm.

right adrenal gland is normally posterior to the right ovary in other species of unisexual lizards (Hardy et al., 1989), the anterior position of the identifiable adrenal gland in this specimen suggests that it was the left adrenal gland. Consequently, the tissue posterior to the adrenal gland (figs. 12, 13) is identified here as the left ovary. The apparent division between the adrenal portion and the ovarian portion is evident macroscopically (arrows in fig. 8). The adrenal gland contained interrenal cells and scattered adrenal (chromaffin) cells (figs. 12B, 13B, 13C). Even though the anterior-posterior organization was still evident (fig. 13) deeper in the tissue, the whole structure was poorly organized. The adrenal was recognizable (fig. 13B, C) and the transition between the adrenal and the ovary could be seen (fig. 13D, E). The tissue of the ovary adjacent to the transition zone (fig. 13G) had the same composition as that seen more posteriorly (fig. 13F, H, I) and as that part of the ovary visible in the transition zone (fig. 13D, E). The ovary seemed to be composed of irregularly shaped stroma (fig. 13F–I) with spaces that were lined with an irregular cuboidal epithelium and which contained loose cells and amorphous material. The ends of individual trabeculae of the ovary sometimes resembled Bowman's capsules with vascularization; however, no glomeruli, Bowman's capsules, or any other indication of the mesonephros were present.

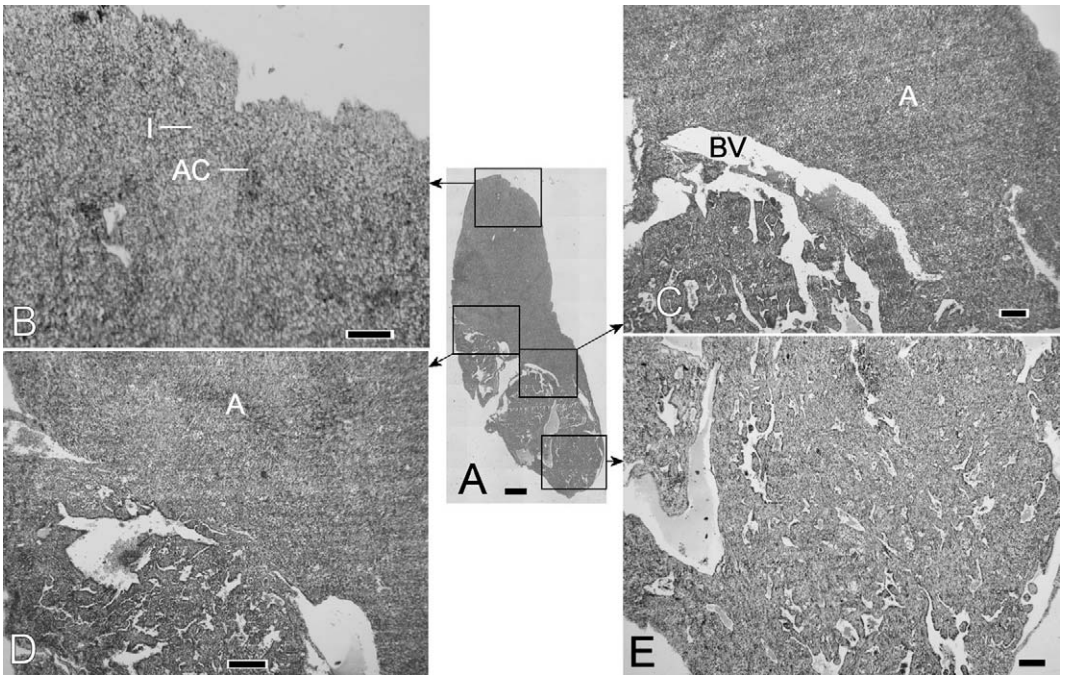

FIGURE 12. The large tissue sample from the adult-sized, apparently female (but intersex) laboratory hybrid (AMNH R-153158). **A.** An entire section (AMNH R-153158A, slide 1, section 3; scale bar: 1 mm). Rectangles identify enlarged views in B–E. **B.** Adrenal gland (AMNH R-153158A, slide 1, section 3). **C** and **D.** Adrenal gland and transition to adjacent ovary (AMNH R-153158A, slide 1, section 3). **E.** Ovary (AMNH R-153158A, slide 1, section 3). Scale bars for B–E: 0.1 mm.

The ovary contained irregular spaces (lacunae; fig. 13H–I). Small groups of yolk granules (fig. 13I) varied in diameter, stained yellowish or orange in Mallory Triple, and had the same appearance as the yolk granules in female hybrids of *A. tesselata* × *A. t. marmorata* illustrated in Taylor et al. (2001, their fig. 16F). A von Kossa test (Sheehan and Hrapchak, 1987) for calcium was negative, thus confirming that the yolk granules were not dystrophic calcium concretions. The ovarian stroma was composed of papillary structures that resembled a benign tumor; however, given the enlarged adrenal gland, we believe this pattern represented secondary hyperplasia produced in response to sex hormone production by the enlarged adrenal cortex rather than a neoplastic process. If the adrenal gland was actually enlarged, this could represent a case of adrenocorticohyperplasia. To test for this, measurements of the adrenal gland were made on several specimens of *Aspidoscelis* that were used in previous studies (table 5). The volume of the adrenal gland in this laboratory hybrid female was significantly larger (more than 50 times) than that of any of the other specimens studied, confirming adrenocorticohyperplasia, and thus would suggest other manifestations such as secondary masculinization. Additional corroboration that this lizard was an intersex came from the histology of the small tissue sample (AMNH R-153158B), which showed a malelike mesonephros with striking resemblance to an epididymus (see above).

Nine of the 11 taxa examined (table 5) had adrenal volumes less than 0.7 mm^3 except *A. inornata* and the *A. inornata* × *A. t. marmorata* hybrid, which had 1.88 and 108 mm^3,

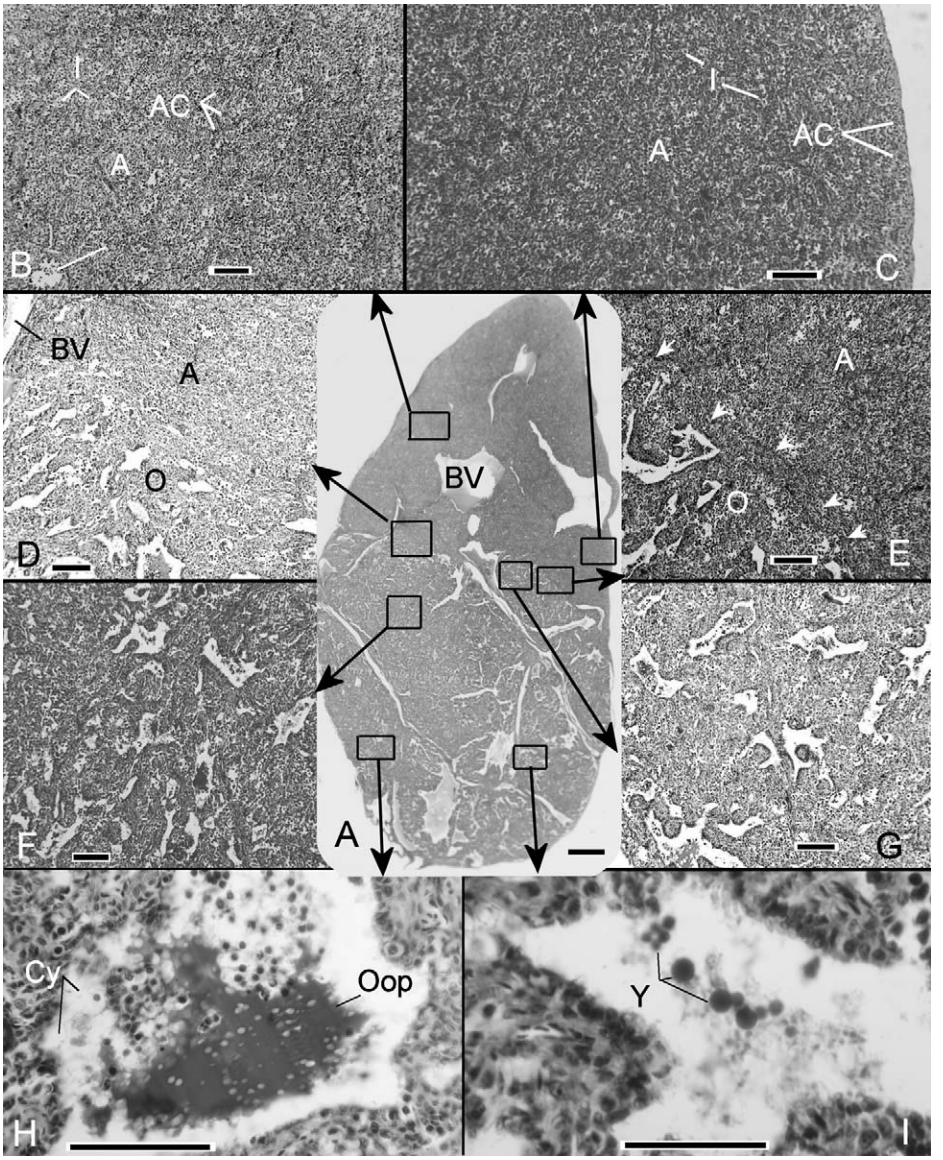

FIGURE 13. The large tissue sample from the adult-sized, apparently female (but intersex) laboratory hybrid (AMNH R-153158). **A.** An entire section (AMNH R-153158A, slide 23, section 1; scale bar: 1 mm). Rectangles identify enlarged views in B–I. **B–C.** Adrenal gland (AMNH R-153158A, slide 23, section 1). **D–E.** Adrenal gland and ovary (AMNH R-153158A, slide 23, section 1). **F–I.** Ovary (AMNH R-153158A, slide 23, section 1). **H.** An enlargement from slide 23, section 2 showing an atretic follicle in the ovary. **I.** An enlargement from slide 23, section 2, showing yolk granules. Scale bars for B–I: 0.1 mm.

respectively (fig. 14). The *A. sonorae* × *A. t. marmorata* hybrid was closer to *A. sonorae* (fig. 14) and contained only one *marmorata* genome. The *A. tesselata* × *A. t. marmorata* hybrid was closer to *A. t. marmorata* (fig. 14) and contained two complements of *marmorata* in its ancestral genomes. The *A. inornata* × *A. t. marmorata* laboratory hybrid was closer to *A. inornata*

TABLE 5. Comparison of adrenal gland sizes in several species of *Aspidoscelis*. Museum number refers to the American Museum of Natural History (AMNH) or Museum of Life Sciences, Louisiana State University, Shreveport (LSUS). Heavy lines separate three hybrids above, seven specimens representing six unisexual species in the middle, then four specimens of bisexual species below, including the mother of the hybrids (*A. i. arizonae*).

			Adrenal Gland			
Species	SVL (mm)	Museum Number	Length[a] (mm)	Width (mm)	Volume[b] (mm)	Adrenal Vol./ SVL
A. inornata × *A. t. marmorata*	80	R-153158	7.3	7.5	108.00	1.350
A. sonorae × *A. t. marmorata*	85	R-122989	2.5	1.0	0.65	0.008
A. tesselata × *A. t. marmorata*	71	R-146694	1.8	0.8	0.30	0.004
A. sonorae	79	R-117812	2.3	1.0	0.60	0.008
A. tesselata	89	R-145144	1.9	0.25	0.24	0.003
A. tesselata	97	R-145142	1.3	0.73	0.18	0.002
A. uniparens	69	R-122991	1.3	0.4	0.05	0.001
A. neomexicana	80	R-122933	2.4	0.8	0.40	0.005
A. velox	76	R-115953	1.8	0.63	0.19	0.003
A. exsanguis	76	R-113356	3.8	0.8	0.64	0.008
A. inornata	68	R-148431[c]	2.8	1.6	1.88	0.028
A. t. marmorata	92	R-146653	1.9	0.6	0.18	0.002
A. t. marmorata	63	R-146652	2.8	0.8	0.47	0.008
A. t. marmorata	85	LSUS 971	2.0	0.9	0.42	0.005

[a] Length is the longitudinal distance through the middle of the adrenal.

[b] Volume is calculated as though the roughly triangular adrenal is a three-dimensional cone. All calculations were made from histological sections near the center of the organ, except for the mother of the hybrids.

[c] Length and width were measured from the preserved specimen.

(fig. 14) and contained only one *marmorata* genome. All the *A. t. marmorata* and the other hybrids involving *marmorata* were clustered below 0.7 mm^3 (fig. 14). The *A. inornata* and the *A. inornata* × *A. t. marmorata* laboratory hybrid had large adrenals. Both *A. velox* and *A. uniparens* have two complements of *A. inornata* in their ancestral genomes plus a complement of the *A. burti* genome (Reeder et al., 2002).

One could speculate that two copies of the *A. inornata* genome alone or with a third genome from another species causes enlarged adrenal growth; however, the *burti* genome with two complements of the *inornata* genome in the triploid *A. velox* and *A. uniparens* has no effect on adrenal growth. Nor is there a problem in typical specimens of *A. neomexicana,* which also has one genome each from *A. inornata* and *A. t. marmorata,* as in this laboratory hybrid (but the reciprocal cross; Brown and Wright, 1979). Perhaps the species represented by the maternal genome makes a difference with respect to abnormal adrenal enlargement.

Some of the lacunae (fig. 13H) of the large, apparently female hybrid contained disorganized clumps of cells and debris. The cellular debris, including nuclei and cytoplasmic strands, was derived from the matrix (= granulosa?) of the trabeculae. Remnants of ooplasm had a stellate surface and contained clear vacuoles, often oblong in shape (fig. 13H). The degenerating ooplasm was stained more intensely than was the acellular material in other lacunae and appeared to be abnormal atretic material very similar to that illustrated by Betz (1963, his fig.

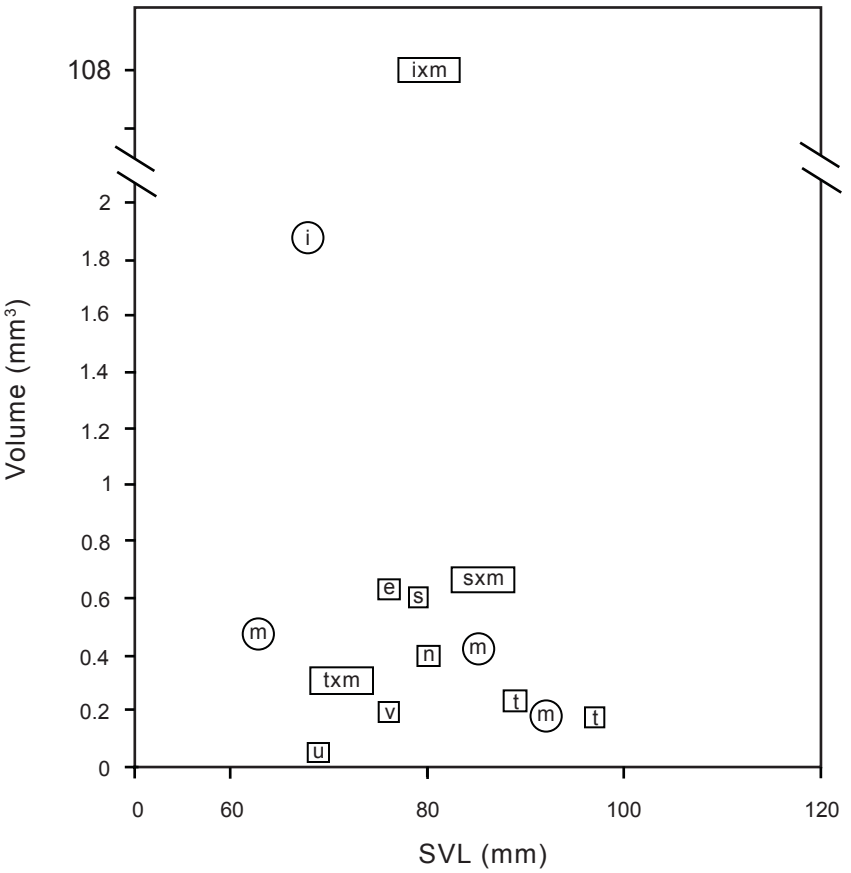

FIGURE 14. Volume of the adrenal gland compared to the snout-vent length (SVL) of the specimens listed in table 5. Hybrids are depicted by rectangles (i × m = *A. i. arizonae* × *A. t. marmorata*; s × m = *A. sonorae* × *A. t. marmorata*; t × m = *A. tesselata* × *A. t. marmorata*), parthenogens by squares (e = *A. exsanguis*; s = *A. sonorae*; n = *A. neomexicana*; v = *A. velox*; u = *A. uniparens*; t = *A. tesselata*), and bisexual species by circles (i = *A. i. arizonae*; m = *A. t. marmorata*).

9). The presence of several scattered yolk granules and isolated masses of degenerated ooplasm that resembled atretic follicle material suggests that the ovary contained some normal cells that possibly could have produced follicles, yolk, and atretic follicles. However, the disorganization of the ovary resulted in atresia in some areas and vitellogenesis in other isolated places rather than normal oogenesis, and this hybrid never laid eggs although it was more than four years old when sacrificed. Follicles had not developed but some vittelogenesis had occurred (i.e., the isolated yolk granules) and follicle cells that could not mature became atretic in the lacunae, which might represent the cavities of abnormal follicles. The adrenal gland might have hypertrophied owing to the lack of physiological feedback that would be coming from a normally developing ovary and the resulting ovulation. This large structure is a hybrid sex organ, containing few characteristics of a normal ovary or of a normal testis. This hybrid individual, in final analysis, appeared to be a sterile intersex.

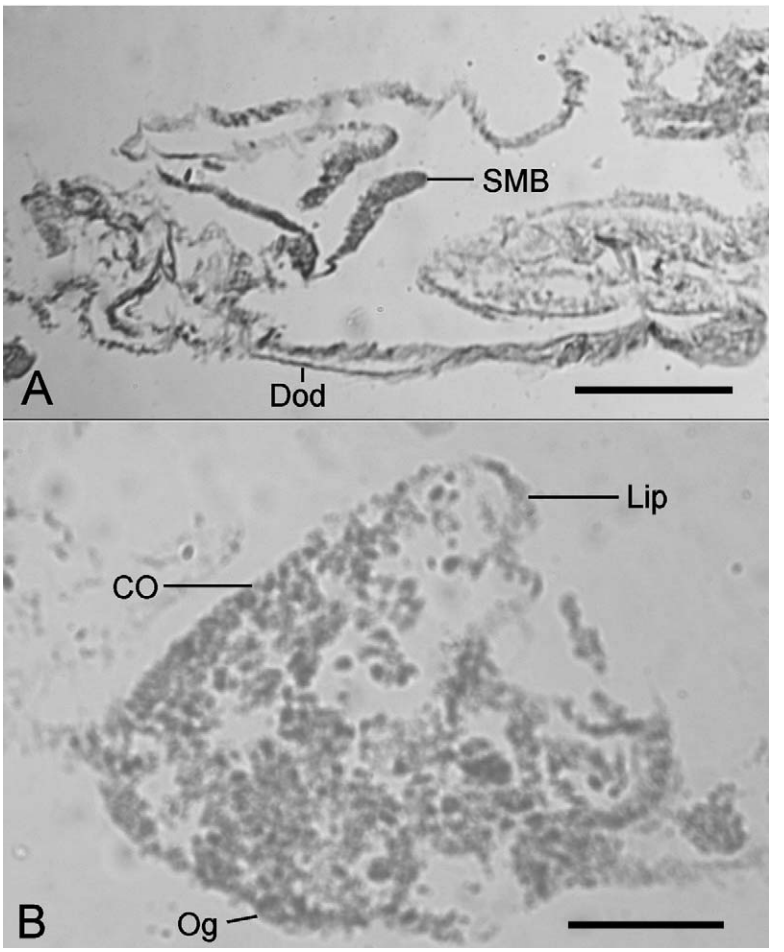

FIGURE 15. The oviduct (part) and ovary of laboratory hybrid AMNH R-148432, which was partly decomposed. **A.** Smooth muscle band and the distal oviduct (AMNH R-148432, slide 1, row 3, section 13). **B.** Ovary (AMNH R-148432, slide 5, row 2, section 3). Scale bars: 0.1 mm.

Smallest Laboratory Hybrid (AMNH R-148432): There were very few histological characteristics about this specimen that could verify the sex. However, the distal oviduct and associated smooth muscle band seen in other species (Hardy and Cole 1981: fig. 10; Hardy et al., 1989: figs. 11, 12) were visible (fig. 15A). The ovary was tiny, poorly developed, and showed the lip and a few oogonia (fig. 15B). This specimen was nonreproductive and appeared to be an immature and infertile female.

Adult-sized, Apparently Male Hybrid (AMNH R-153157): The well-vascularized testis of the male contained seminiferous tubules with possible spermatogonia in the tubule walls and cellular material in the lumena (fig. 16A). The material in the lumena consisted of cells and noncellular debris, but neither spermatozoa nor meiotic figures were seen (fig. 16B). The mesonephros (fig. 16C) contained large and small mesonephric tubules adjacent to the adre-

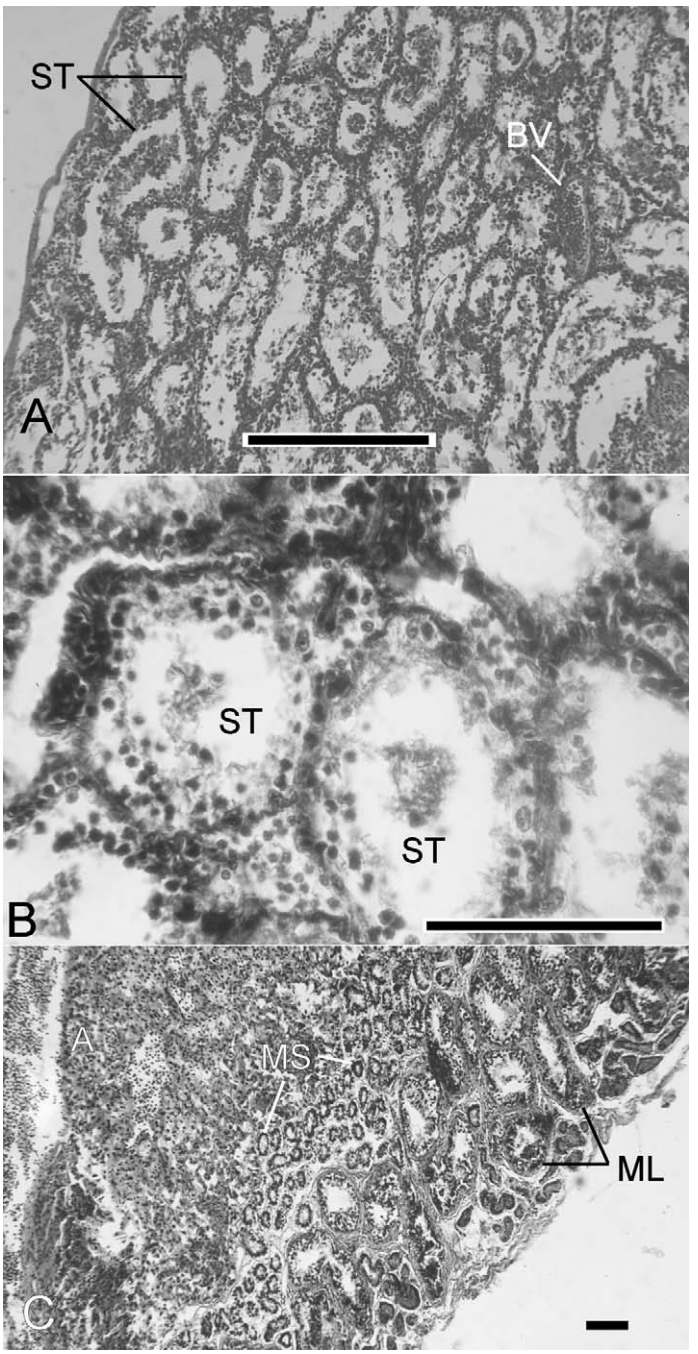

FIGURE 16. The testis of laboratory hybrid AMNH R-153157. **A.** Testis with seminiferous tubules. **B.** Seminiferous tubules containing debris and some cellular material. **C.** Mesonephros with small and large mesonephric tubules. Scale bars: 0.1 mm.

nal gland anteriorly and the testis posteriorly (the latter not visible in fig. 16C). The larger mesonephric tubules contained loosely organized or packed material (fig. 17A); however, the contents were debris and cells that were not spermatozoa, spermatids, or secondary spermatocytes (fig. 17B). A portion of the mesonephros contained large coiled tubules (the

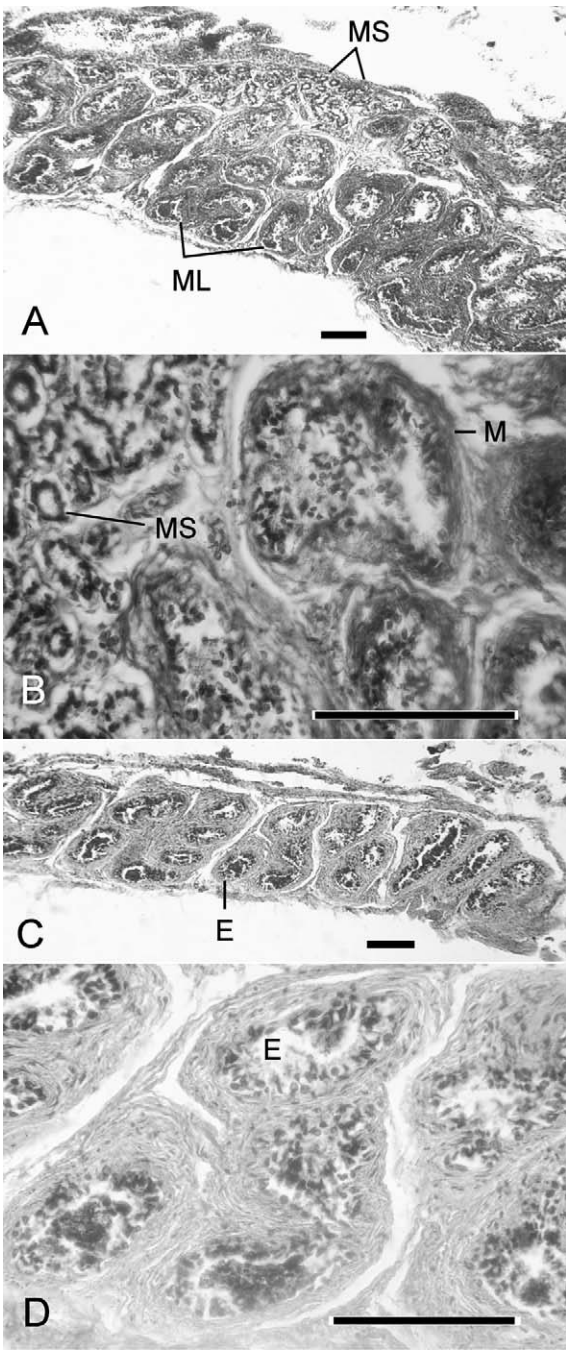

FIGURE 17. Mesonephros of laboratory hybrid AMNH R-153157. **A.** Small and large mesonephric tubules with loosely organized material. **B.** Detail of a large mesonephric tubule. **C.** Large coiled tubules of the mesonephros (= epididymus); note the lack of small tubules in this region. **D.** Detail of large tubules of the epididymus containing debris and cellular material but not spermatozoa.

epididymus; fig. 17C) that contained packed material having the superficial appearance of packed sperm. However, the packed material consisted only of cells or cellular debris; neither spermatozoa nor spermatids were present (fig. 17D). This male was not reproductive at the time of sacrifice.

Summary of All Laboratory Hybrids Produced

The Hybrids

The following is a conservative estimate of the hybrid offspring that we think the lizards produced in the laboratory at the AMNH, listed chronologically with the reasons for including them on the list. Our uncertainty is based on the fact that we do not have genetic evidence bearing on all possible hybrid individuals, and many hatchlings (not included on this list) died after a short time without evidence that they were hybrids, even if their mother mated with a male of a different species; sometimes important characters of coloration do not emerge until after several months of life (Hardy and Cole, 1998).

(1) *A. sonorae* (♀) × *A. t. marmorata* (♂): AMNH R-122989 was a tetraploid hybrid between a triploid parthenogen (*sonorae,* AMNH R-117812) and a male (*marmorata,* probably AMNH R-117811). Genetic confirmation of the hybrid status of the specimen was confirmed by analyses of karyotypes (comparing the mother, father, tetraploid hybrid, and three nonhybrid siblings; Cole, 1979) and allozymes (Dessauer and Cole, 1984). In addition, morphology was described (coloration, scalation, size, and reproductive tissue histology) for this sterile hybrid, including comparisons with the mother, father, and siblings (Hardy and Cole, 1998). Photographs of the mother, father, and hybrid were presented in black and white (Cole, 1979: 97) and in color (Hardy and Cole, 1998: 6). We were not certain that this individual was a hybrid until about five months after it hatched, by which time a dorsal spotting pattern inherited from the father became clear.

(2) *A. neomexicana* (♀) × *A. sexlineata* (♂): AMNH R-125575 was a triploid hybrid between a diploid parthenogen (*neomexicana,* AMNH R-125565) and a male (*sexlineata,* either AMNH R-119498 or 119499). Genetic confirmation of the hybrid status of the specimen was confirmed by analyses of the karyotype and allozymes (Dessauer and Cole, 1984; in which the hybrid specimen cited inadvertently was its sibling, AMNH R-125574). Coloration on the day of hatching indicated the possible hybrid status of this lizard (AMNH R-125575) and a sibling (which was unhealthy and sacrificed on the day of hatching). As noted (colony journal records, p. 138) for the sibling (AMNH R-125574), there were an unusually black dorsal ground color, few to no light spots, and a thin, not very wavy and broken vertebral light stripe.

(3) *A. inornata arizonae* (♀) × *A. t. marmorata* (♂): These are the three diploid hybrids between two bisexual species on which we have focused in the present paper.

There were several other lizards hatched in the laboratory that appeared as if they might be hybrids but for which we have no genetic evidence to confirm or reject their hybrid status. We decided not to list them because this might be misleading, as indicated by two pertinent experiences: (1) one morphologically aberrant specimen hatched in the laboratory from an egg deposited by a normal *A. neomexicana* appeared to be a hybrid with *A. t. marmorata,* but genetic data showed she was not; and (2) a similarly morphologically aberrant specimen was caught in the field and allowed to reproduce in the laboratory; she produced normal offspring and genetic data showed that she and her offspring were not hybrids. This was discussed and illustrated by Dessauer and Cole (1989: 50–51), who cautioned that such aberrations may be

caused by environmental or regulatory phenomena, rather than hybridization. Although hybridization occurs more often among whiptail lizards than nearly all other lizards, we should exercise caution in deciding which individuals are hybrids and which are not.

Effort Expended to Obtain the Hybrids

How readily do species of *Aspidoscelis* hybridize? How readily do males of bisexual species mate with females of unisexual species? How readily are new clones of parthenogenetic species created (whether through interbreeding either between two bisexual species or between a male and parthenogenetic female of either a diploid or triploid species)? Definitive answers to these questions are difficult to get, but at least we can present some observations and discuss issues.

Phylogenetic analyses (Reeder et al., 2002: 26) coupled with analyses of mitochondrial DNA (= mtDNA, which identifies the female in hybridization events; Brown and Wright, 1979; Densmore et al., 1989a, 1989b; Moritz et al., 1989) provided a low-end number of at least six hybridization events between two ancestral bisexual species to explain the origins of the diploid parthenogenetic species of *Aspidoscelis.* At least five additional hybridization events between males and diploid parthenogenetic females would be necessary to produce the founders of the triploid clones, for a total of at least 11 founder hybridization events over hundreds or thousands of years. Owing to the very low level of mtDNA variation in parthenogens, it appeared as if their hybrid origins were very rare as well as recent (Densmore et al., 1989a, 1989b; Moritz et al., 1989).

Alternatively, considerably more frequent hybrid origins of clonal diversity were suggested by relatively high levels of variation in allozymes and external morphology within some parthenogenetic species, especially *A. tesselata* (e.g., Parker and Selander, 1976; Parker, 1979; Dessauer and Cole, 1989). This alternative is not supported by the most recent data. The various forms of *A. tesselata* are now known to be histocompatible (i.e., they do not reject skin transplants from each other), suggesting that they all originated from a single F_1 hybrid individual (Cordes and Walker, 2006), and that the diverse clones recognized in morphology and allozymes are probably results of postformational mutations (Taylor et al., 2003). Another recent study showed that although there is frequent hybridization between the bisexual *A. tigris marmorata* and unisexual *A. tesselata* in the vicinity of Roswell, Chaves County, New Mexico, none of the triploid hybrids studied to date were able to clone themselves or reproduce by backcrossing (Taylor et al., 2001). It now appears as if reproductively competent hybrids appear to be rare, except when hybridization involves close relatives (e.g., *A. t. marmorata* × *A. t. punctilinealis*; Dessauer et al., 2000), and these hybrids are bisexual, not parthenogens.

Our few successes in obtaining hybrids among various species of *Aspidoscelis* in captivity is consistent with the above, particularly considering that apparently none was fertile. For more than 29 years, from 18 June 1973–5 August 2002 (349 months), we had at least one male and one female of different species caged together in hopes that they would reproduce. Throughout this period of time, we had a total of 74 males of four species caged with 156 females of nine

species (table 6) and obtained only the five genetically confirmed hybrid lizards from three clutches of eggs (see above). In most instances, we caged only one or two males per cage with one to four females. Before discussing the results in table 6, however, it is appropriate to discuss the following general caveats:

(1) We excluded from table 6 instances in which males and females were caged together for less than six months, except in cases where mating or attempted mating was observed. Also, we counted attempted mating and actual mating as one category for the table, as attempts illustrated that the male was reproductively active, the cages were not monitored all day long, and undoubtedly some successful mating occurred that was not witnessed by us.

(2) Not all males and females caged together were equal. In some cases there were significant differences in body size, which may have affected physical aspects of mating compatibility. Less conspicuous was the fact that the health for some individuals varied during captivity, and this affected their behavior and viability of their eggs, as some contracted an infection for a while (e.g., respiratory; Townsend, 1979), some developed metabolic bone disease (e.g., Townsend and Cole, 1985), and others had complications from unknown factors that affected reproduction (e.g., Porter et al., 1994). Consequently, in the laboratory, failure of a clutch of eggs to hatch was not necessarily because they contained hybrid embryos.

(3) Individuals of some species adjusted to captivity better than others, in general, and all species exhibited individual variation in acclimation. Size of the area in which lizards were confined also affected acclimation, and the number of lizards in the space, and dominance interactions, including aggression, would be expected to negatively affect feeding and reproduction of subordinate individuals. For example, in some instances caging a large female of *A. t. marmorata* with a small male of *A. inornata* ended disastrously: if he disturbed her too frequently with his mating attempts, the harassed female would grab the little male's head in her jaws and crush his skull. Budgetary and space constraints restricted us to using whatever cages we could find to fit into our limited room.

(4) We had neither facilities nor personnel to experiment significantly with manipulating onset of male reproduction. Use of artificial hibernation or hormones might have encouraged mating activity. We just cared for the lizards as best we could and tried not to disturb them too much. Some males were never seen in a reproductive mode, others became extremely active. Once an active male was known to have mated with his cagemates, we would move him to a different cage where the resident male was inactive; after the reproductive male mated with those new acquaintances, we would pass him on. Consequently, there was a great deal of variation in how long individual males and individual females were cagemates.

With the above caveats in mind, we now discuss the results shown in table 6. These include attempts to have hybridization between two bisexual species (*inornata, gularis, sexlineata,* and *tigris*), between a bisexual species and diploid parthenogen (*laredoensis, neomexicana,* and *tesselata*), and between a bisexual species and triploid parthenogen (*exsanguis* and *sonorae* [which included a few individuals that some herpetologists would refer to *A. flagellicauda*]).

Our greatest efforts (table 6) were to recreate the hybrid origins of *A. neomexicana* (*A. t. marmorata* × *A. inornata* and the reciprocal cross) and *A. laredoensis* (*A. gularis* × *A. sexlineata*

TABLE 6. Combinations of lizards[a] caged together for possible hybridization[b]

Males	Females									
	INO	GUL	SEX	TIG	LAR	NEO	TES	EXS	SON	Total
INO	—	2	10	26	—	14	—	3	4	59
26	—	100.0	90.0	34.6	—	64.3	—	0.0	25.0	
	—	4–7	1–32	6–26	—	8–29	—	12–16	7–12	
GUL	3	—	16	—	—	—	—	—	—	19
10	0.0	—	6.2	—	—	—	—	—	—	
	17–24	—	6–24	—	—	—	—	—	—	
SEX	—	17	—	1	5	10	8	—	2	43
20	—	23.5	—	0.0	60.0	70.0	50.0	—	0.0	
	—	4–29	—	8	6–27	2–12	7–21	—	10	
TIG	19	—	—	—	—	4	3	4	5	35
18	31.6	—	—	—	—	75.0	66.7	25.0	60.0	
	7–42	—	—	—	—	11–21	11–17	12–14	7–32	
Total										Total
74										156

[a] The species are as follows: INO, *Aspidoscelis inornata*; GUL, *A. gularis*; SEX, *A. sexlineata*; TIG, *A. tigris marmorata*; LAR, *A. laredoensis*; NEO, *A. neomexicana*; TES, *A. tesselata*; EXS, *A. exsanguis*; and SON, *A. sonorae.*

[b] For males, the number of individuals is presented. For females, the number of individuals is followed by the percent that mated with a male or was seen to receive attempted matings, followed by the range of number of months the males and females were caged together.

[see Wright et al., 1983] and the reciprocal cross). Only the reciprocal of the first of these crosses produced laboratory hybrids, and all were from a single clutch of eggs.

When a total sample of 10 or more females was involved, the highest frequency of mating or attempted mating (90.0%) involved males of *inornata* and females of *sexlineata,* the latter of which included individuals from population samples ranging from New Mexico to Georgia. Considering the bisexual species included in the phylogenetic analysis (Reeder et al., 2002), which included all of these species, *inornata* and *sexlineata* are among the closest relatives analyzed. Nevertheless, only a single clutch of eggs resulted in hatchlings from these couplings (see above), and the hatchlings all died within a matter of weeks.

When a sample of 10 or more females was involved, the second highest frequency of mating or attempted mating (70.0%) involved males of *sexlineata* and females of *neomexicana,* and only one hybrid was produced from these couplings (see above). In case it is pertinent, *inornata* was one of the ancestors of *neomexicana,* and as mentioned above, *sexlineata* is a close relative. Nevertheless, captive males in most cases attempted to mate with their female cagemates, regardless of distance of relationship. Captive males also frequently mated with males of their own species.

Despite the considerable caveats, we now conclude that hybrid origins of unisexual species of whiptails are rather rare events, although the evidence indicates that all unisexual whiptails had a hybrid origin (with postformational mutations creating clonal diversity).

SUMMARY AND CONCLUSIONS

(1) With hopes (unfulfilled) of witnessing the switch from spermatozoan-based reproduction to parthenogenesis in a single generation, we attempted to produce laboratory hybrids among various species of whiptail lizards (*Aspidoscelis*). We also attempted to produce new clonal polyploid forms by crossing males of gonochoristic (= bisexual) species with parthenogenetic females.

(2) The only successful method for producing hybrids was to cage males of one species together with females of other species, but few genetically confirmed viable hybrids were obtained over a period of 29 years.

(3) The effort involved a total of 74 males of four species caged with 156 females of nine species, where individuals were caged together for at least six months (or fewer, if mating was observed in a shorter period of time). The females represented four bisexual species, three diploid unisexual species, and two triploid unisexual species.

(4) Considering only those specimens whose hybrid status was confirmed with genetic analyses (karyotypes, allozymes), a total of only five hybrids from three crosses (and three clutches of eggs) were obtained over 29 years.

(5) No new clonal parthenogens resulted. All the laboratory hybrids either were sterile or they died before attaining adult size.

(6) Our laboratory hybrids included offspring from the following three combinations: (A) *A. sonorae* (♀) × *A. t. marmorata* (♂); (B) *A. neomexicana* (♀) × *A. sexlineata* (♂); and (C) *A. inornata arizonae* (♀) × *A. t. marmorata* (♂). The first two of these were reported on previously, so focus of the present paper was on the last.

(7) There were three hybrids of *A. i. arizonae* × *A. t. marmorata,* all from one clutch of eggs. From hatching, color pattern, similar in all, indicated that these were hybrids because they were extremely similar to *A. neomexicana,* which arose in nature from the reciprocal cross (but a different subspecies of *A. inornata*).

(8) The one hybrid karyotyped had chromosomes identical to those of *A. neomexicana.* A different individual analyzed biochemically showed the F_1 hybrid state for the 21 allozyme loci tested; it was like *A. neomexicana* but lacked its orphan alleles.

(9) Univariate statistical analyses of seven meristic characters showed that each hybrid was either the same as one of its parents or intermediate to the two parents in each character.

(10) Multivariate statistical analyses (PCAs and CVAs) of the same characters showed the following: (A) the hybrids were more or less intermediate between their parents, but more like *A. i. arizonae* than *A. t. marmorata*; (B) the hybrids clustered as a distinctive group, as did all others, when compared with the parental taxa and with *A. neomexicana*; (C) the hybrids and *A. neomexicana* both seem to demonstrate matriclinous inheritance; and (D) if they had been collected in the field in the absence of genetic data, one might have erroneously identified the three as hybrids of *A. neomexicana* × *A. inornata,* based only on morphological data.

(11) Histological research on the hybrid that became the oldest and largest, which externally appeared to be a female (and inherited the *tigris* X chromosome), revealed that this

individual was a sterile intersex. The specimen had an abnormal ovary, a mesonephros that resembled an epididymus, and an enormous adrenal that was more than 50 times larger than any other observed in an *Aspidoscelis.* This suggested adrenocorticohyperplasia and secondary masculinization.

(12) Histological research on the smallest hybrid, which externally appeared to be a female, revealed structures of an infertile or immature female, but details were lacking because the specimen had begun to decompose before it was found dead in the cage (inadvertently killed in a laboratory accident).

(13) Histological research on the remaining hybrid, which appeared externally to be a male and grew to adult size and age, revealed male structures only, including a testis, but no meiotic cells, spermatozoa, spermatids, or secondary spermatocytes.

(14) Overall, none of the laboratory hybrids we obtained reproduced. Although there were complicating factors in our lizard colony at times (e.g., pathogens and metabolic bone disease), it appears to us that successful hybridization (i.e., producing viable and reproductively capable offspring) is not of frequent occurrence in *Aspidoscelis,* although phylogenetic analyses demonstrate that it is of greater frequency in these lizards than in any others in the world, with the possible exception of certain lacertids.

(15) We conclude that the clonal parthenogens of *Aspidoscelis,* all of which had hybrid origins, are products of a minority of the number of hybrids that have been produced in nature.

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APPENDIX

Specimens Examined

Catalog numbers beginning with AMNH are in the herpetological collections of the American Museum of Natural History, while that beginning with LSUS is in the collections of the Museum of Life Sciences, Louisiana State University in Shreveport.

Laboratory Hybrids, *Aspidoscelis i. arizonae* × *A. t. marmorata*

The three hybrids were from one and the same clutch of eggs, laid on 19 May 1999. AMNH R-148432 hatched on 26 July 1999 and appeared healthy but died in a laboratory accident on 13–15 November 1999; external morphological data and internal histology were analyzed. AMNH R-153157 hatched on 27 July 1999 and was sacrificed and processed on 15 August 2002; external morphological data, allozymes, and internal histology were analyzed. AMNH R-153158 hatched on 27 July 1999 and was sacrificed and processed on 13 August 2003; external morphological data, the karyotype, and internal histology were analyzed.

Parents of the Hybrids Mentioned Above

The maternal parent was *A. i. arizonae* AMNH R-148431 from: Arizona: Cochise County; 6.4 km (by hwy 186) SE Willcox; reproduction in the laboratory and external morphological data were used. The paternal parent was *A. t. marmorata* AMNH R-153156 from: New Mexico: Hidalgo County; 11.3 km WSW Lordsburg; reproduction in the laboratory, external morphological data, and internal histology were analyzed.

Specimens Tested for Allozymes

The laboratory hybrid AMNH R-153157 was compared with the following: *A. i. arizonae* AMNH R-153168 from Arizona: Cochise County; 6.4 km (by hwy 186) SE Willcox (same population as the maternal parent of the hybrids); *A. t. marmorata* AMNH R-153163 from New Mexico: Hidalgo County; 11.3 km WSW Lordsburg (same population as the paternal parent of the hybrids); and *A. neomexicana* AMNH R-151740 from New Mexico: San Miguel County; Conchas Lake State Park, North Area Recreation Area, Cove Campground (the same clone as occurs commonly throughout its range, based on the allozymes tested).

Specimens Used for External Morphology and Multivariate Statistics

The three laboratory hybrids and their parents were used (see above). In addition, we used the following *A. i. arizonae* from Arizona: Cochise County; 3.5 km (by hwy 186) SE Willcox (AMNH R-135020 and 135033); and 6.4 km (by hwy 186) SE Willcox (AMNH R-148211–148212, 148215–148216, 148221–148222, 148231–148237, 148240–148241, 148431, and 153168). In addition, we used the following *A. t. marmorata*: from several sites between 11.6–16.3 km (via hwy 70) NW Lordsburg, Hidalgo County, New Mexico (see Cole et al. [1988: 9] for specific details for each specimen; AMNH R-84842, 125534, 131085–131091, and 131093–131102). In addition, we used the following *A. neomexicana* from several sites between 11.6–28.0 km (by hwy 70) NW Lordsburg, Hidalgo County, New Mexico (see Cole et al. [1988: 9] for specific details for each specimen; AMNH R-86987–86990, 86992–86993, 112846–112848, 114222–114225, 114227, 115998, 120675, 125546–125547, 125550, 125565, and 131066–131067).

Specimens Used for Adrenal Measurements

We list these in the order in which they are listed in table 5.

Laboratory hybrid of *A. i. arizonae* × *A. t. marmorata,* AMNH R-153158 (see above for details).
Laboratory hybrid of *A. sonorae* × *A. t. marmorata,* AMNH R-122989 (see Hardy and Cole, 1998: 3–4 for details).

Natural hybrid of *A. tesselata* × *A. t. marmorata,* AMNH R-146694 (see Taylor et al., 2001: 64 for details).

A. sonorae, AMNH R-117812 (see Hardy and Cole, 1998: 3 for details).

A. tesselata, AMNH R-145144 and 145142 (see Taylor et al., 2001: 63 for details).

A. uniparens, AMNH R-122991, a laboratory offspring from AMNH R-122990 from New Mexico: Hidalgo County; 27.7 km (by hwy 70) NW Lordsburg.

A. neomexicana, AMNH R-122933 and R-122946 (fig. 4) from New Mexico: Sandoval County; at Rio Grande crossing and Cochiti Dam, 5.3 km (by hwy 22) N Pena Blanca.

A. velox, AMNH R-115953, a laboratory offspring from AMNH R-115952 from Arizona: Navajo County; along Silver Creek, 12.7 km (by dirt rd to Woodruff) N Snowflake, then 0.6 km (gravel rd) W.

A. exsanguis, AMNH R-113356, a laboratory offspring from AMNH R-113352 from New Mexico: Hidalgo County; 4.8 km W and 12.1 km N Cloverdale.

A. i. arizonae, AMNH R-148431 (see above, Morphology specimens).

A. t. marmorata, AMNH R-146652–146653 (see Taylor et al., 2001: 63 for details) and LSUS 971 (see Hardy and Cole, 1998: 4 for details).

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