BREVIORA

Museum of Comparative Zoology

US ISSN 0006-9698

CAMBRIDGE, MASS. 5 DECEMBER 2014 NUMBER 539

NEAVES' WHIPTAIL LIZARD: THE FIRST KNOWN TETRAPLOID PARTHENOGENETIC TETRAPOD (REPTILIA: SQUAMATA: TEIIDAE)

CHARLES J. COLE,[1] HARRY L. TAYLOR,[2] DIANA P. BAUMANN,[3] AND PETER BAUMANN[4]

ABSTRACT. The first known tetraploid amniote that reproduces through parthenogenetic cloning by individual females is named and described. The species originated through hybridization between *Aspidoscelis exsanguis* (triploid parthenogen) × *Aspidoscelis inornata* (diploid bisexual or gonochoristic species) in the laboratory. We compared multivariate morphological variation between two lineages that arose from separate F_1 hybrid zygotes in one clutch and among several generations in those lineages. The tetraploid species is also compared with its ancestral taxa, with two hybrids of *A. exsanguis* × *A. inornata* that were found in nature at two localities that are 100 km apart in southern New Mexico, and with three laboratory hybrid males. This will facilitate identification of field-caught tetraploids in the future.

KEY WORDS: *Aspidoscelis neavesi*; new species; tetraploid; parthenogenesis; clonal lineages

INTRODUCTION

Among amniotes, true parthenogenesis (initiation and completion of embryogenesis in the absence of spermatozoa) occurs as the normal mode of reproduction in only a few species, all of which are reptiles (several lizards and apparently one snake; reviewed in Dawley and Bogart, 1989; Lutes *et al.*, 2011; Neaves and Baumann, 2011). Many of these are whiptail lizards (*Aspidoscelis*), of which the unisexual (all-female) lineages receive formal recognition as species because they are of unique ancestry (reviewed by Reeder *et al.*, 2002) and individuals reproduce by parthenogenetic cloning (Lutes *et al.*, 2010, 2011). Additionally, rare instances of hybridization and fertilization of a parthenogenetic female's cloned eggs have resulted in triploid clonal species of unique

[1] Division of Vertebrates (Herpetology), American Museum of Natural History, 200 Central Park West, New York, New York 10024, U.S.A.; e-mail: cole@amnh.org.

[2] Department of Biology, Regis University, Denver, Colorado 80221, U.S.A.; e-mail: htwhiptail@gmail.com

[3] Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, Missouri 64110, U.S.A.; e-mail: dpb@stowers.org

[4] Howard Hughes Medical Institute and Stowers Institute for Medical Research, Kansas University Medical Center, Kansas City, Missouri 64110, U.S.A.; e-mail: peb@stowers.org

ancestry (e.g., *Aspidoscelis exsanguis*; reviewed by Reeder *et al*., 2002). Although a few tetraploid hybrid individuals were reported in the past (Lowe *et al*., 1970a; Neaves, 1971), no tetraploid clonal lineages were known until Lutes *et al*. (2011) reported the laboratory origin of the species for which we provide a name, morphological description, and both intraspecific and interspecific comparisons here.

Aspidoscelis is a genus of North American whiptail lizards that includes several unisexual (all-female), parthenogenetic species as well as gonochoristic species (reviewed by Reeder *et al*., 2002). All of the unisexual species ultimately had a hybrid origin, the females clone themselves, and the primary lineages bear formal binomials, although derived clonal lineages with postformational mutations usually do not. Although the International Code of Zoological Nomenclature (ICZN, 1999) prohibits naming hybrids (Article 1.3.3), this restriction applies to individual animals that are hybrids between two species, not self-perpetuating clonal entities that are unique evolutionary lineages. In the case of unisexual species of *Aspidoscelis*, historically, the F_1 hybrid females established continuing lineages by cloning themselves parthenogenetically, and the offspring continued to reproduce as did their mother. This quantum leap in evolution (the switch from spermatozoan-dependent to spermatozoan-independent reproduction) occurred in each case in just one generation (reviewed by Reeder *et al*., 2002). In such lineages, individuals of the F_2 and subsequent generations are not hybrids but clones of their single parent. In recognition of this, ICZN (1999) adopted Article 17.3, validating the naming of parthenogenetic entities of hybrid origin.

Some clonal lineages of hybrid origin are morphologically cryptic species, but many have distinctive morphologies in size, color pattern, and/or scalation. Consequently, several were diagnosed, described, and named well before scientists knew that unisexual, clonal lizards exist (e.g., *Ameiva tesselata* [Say *in* James, 1823:50–51], known today as *Aspidoscelis tesselata*). Each clone with a distinctive ploidy and distinctive combination of ancestral genomes has been named, reflecting its unique historical origin (reviewed in Reeder *et al*., 2002). Additionally, some clones with distinctive scalation and/or coloration derived as a consequence of postformational mutations have been named even though they share a common hybrid origin with other clones (e.g., *Aspidoscelis maslini* Fritts, 1969, versus other clones of the *Aspidoscelis cozumela* complex; Taylor *et al*., 2005). Coauthors of the present paper differ in philosophy and practice on this point, but in general, we do not favor naming postformational clones but prefer to treat them as a complex of derivative forms under one specific epithet (e.g., the *A. tesselata* complex), which clearly reflects their relationships. This is similar to recognizing that extensive genetic variation occurs within named species of gonochoristic taxa.

In this paper we name, diagnose, and describe a unique unisexual species of hybrid origin. It is the first tetraploid vertebrate known to clone itself parthenogenetically and it is reproductively isolated from all other species (Lutes *et al*., 2011). The name, given below, will provide for efficient, effective, and unambiguous communication, particularly for tracking data among various publications, as this model organism is being used for considerable research on basic biological processes (e.g., molecular aspects of meiosis; Lutes *et al*., 2010, 2011) and DNA sequencing, which involves listing in on-line data information services such as GenBank, RefSeq, and UniProt.

Although the presently known reproducing lineages of this tetraploid species originated in the laboratory by hybridization

between the triploid parthenogenetic *A. exsanguis* (♀) and *Aspidoscelis inornata* (♂; a diploid bisexual species), this same combination of chromosomes was found in a tetraploid female (MCZ 101991) from Alamogordo, Otero County, New Mexico, in 1967 by William B. Neaves, and the female laid eggs in captivity (Neaves, 1971). At the time, however, the female oviposited in dry sand, and the eggs became desiccated and were discarded, although otherwise they looked normal. A second relevant specimen (UCM 29196) was found near Mesilla, Dona Ana County, New Mexico (Taylor *et al*., 1967). This one was a male and was identified as representing the all-female *A. exsanguis*. However, we hypothesize that this specimen is also a tetraploid of hybrid origin between *A. exsanguis* and *A. inornata*, because eggs of whiptail lizards that receive a Y-bearing spermatozoan produce males, regardless of ploidy level (Cole *et al*., 1969; Lowe *et al*., 1970a; Taylor *et al*., 2001). We borrowed and examined both of these specimens from New Mexico and included them in our comparisons below.

In addition to describing the unique tetraploid species in this paper, we present morphological data for individuals of several lineages. These were cloned from F_1 zygotes of a single pair of parents (*A. exsanguis* × *A. inornata*). We also compare samples of two well-represented lineages with each other (including generation-to-generation comparisons) and compare the tetraploids to samples of their parental species and specimens of similar hybrid ancestry found in nature.

MATERIALS AND METHODS

Specimens. Methods for maintaining the lizards in a captive colony were described by Lutes *et al*. (2010, 2011). Identity of individuals is tracked as follows: the individual mother of each egg clutch is noted (as is the father, if applicable), hatchlings are noted as to clutch, and throughout life, individuals are photographed periodically and tracked as to which enclosures they occupy at any time. Lineage membership is confirmed using microsatellite DNA analysis (e.g., Lutes *et al*., 2011).

Morphological Characters Examined. These are listed in Appendix 1, and specimens examined are in Appendix 2. Nomenclature for epidermal scales follows Smith (1946). Sex was determined by dissection and examination of primary sexual characters, a history of oviposition, or examination of external secondary characters.

Museum abbreviations for specimens examined: AMNH, American Museum of Natural History, New York, New York; MCZ, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts; MSB, Museum of Southwestern Biology, University of New Mexico, Albuquerque, New Mexico; SIMR, Stowers Institute for Medical Research, Kansas City, Missouri; UCM, University of Colorado Museum, Boulder, Colorado.

Multivariate Statistical Analyses. Although we had 177 specimens available for this study, we could use only specimens with complete data for the suite of 10 meristic characters analyzed. Therefore, as required by the procedures, 65 specimens were excluded because of one or more damaged or missing characters. This left us with the following numbers of specimens for principal components analyses (PCAs) and canonical variate analyses (CVAs): tetraploids (90 of 130); *A. exsanguis* (10 of 25); *A. inornata* (10 of 20); field-caught, presumed or known 4n hybrids (1 of 2); and laboratory F_1 tetraploid hybrid males (1 of 3).

We based our comparisons of lineages, generations, and taxa on PCAs of meristic characters (Appendix 1) followed by CVAs

of the principal components. As an alternative to using the raw meristic data for CVAs, the benefits and rationale for using a PCA intermediary were detailed by Jombart *et al.* (2010). We used samples of the species as a priori classified groups and stepwise selection of principal components for inclusion in CVA models. Principal components were added if F-to-enter probabilities were <0.05 and did not exceed 0.06 when other components were included in the model. Statistical procedures and tests were performed with SPSS© and NCSS© software.

We used pairwise Mahalanobis D^2 distances to quantify the relative meristic resemblances among the tetraploids and progenitor species *A. exsanguis* and *A. inornata*. Distances were calculated from $D^2 = [q(N - g)(Na + Nb)]/[(N - q - g + 1)NaNb] \times Fab$. In this equation (Neff and Marcus, 1980), a and b indicate the two groups being compared, q is the number of predictors (characters), N is the total number of individuals across all groups, g is the total number of groups, Na is the number of individuals in group a, Nb is the number of individuals in group b, and Fab is the F-statistic comparing groups a and b. F-values were from the matrix of F-values and probabilities generated when the last qualifying principal component had been included in the CVA model by stepwise variable selection (provided as output by SPSS and SYSTAT© statistical software).

Mahalanobis D^2 distances are sensitive to multivariate outliers (Tabachnick and Fidell, 2013). Therefore, we checked each a priori group for multivariate outliers by evaluating D^2 distances from each specimen to the centroid of the remaining cases in that group (provided as output by SPSS). Specimens with D^2 values exceeding a critical chi-square value at $P = 0.001$ and degrees of freedom defined by the number of principal components included in the CVA model would be identified as outliers (Tabachnick and Fidell, 2013), but none was found in this study.

We used t tests to test tetraploid lineages of AMNH R-176077–176148 (= SIMR 4921 lineage) and the lineage of MCZ R-192209 (= SIMR 4919) for significant differences and one-way analyses of variance (ANOVAs) to check for differences among lineage/generation combinations of tetraploids. We also used one-way ANOVAs to check for differences among samples of tetraploids, *A. exsanguis*, and *A. inornata*, including canonical variates 1 and 2 (CV1 and CV2). We followed ANOVAs that indicated the presence of significant differences with Tukey multiple comparison tests to identify the significantly different groups.

THE NEW SPECIES

Aspidoscelis neavesi, new species

Neaves' Whiptail Lizard

Figures 1, 2

Holotype. MCZ R-192219 (= SIMR 8093), a cloned adult female of the F_2 laboratory-reared generation that also cloned herself at the SIMR. She hatched on August 13, 2008, and her mother was MCZ R-192209 (= SIMR 4919). See Appendix 2 for a partial list of her offspring.

Paratypes. See Appendix 2, Specimens Examined. Each individual of *A. neavesi* other than the holotype is a paratype, except for two that are still alive.

Diagnosis. A species of the *Aspidoscelis sexlineata* species group as reviewed by Lowe *et al.* (1970b). The species is distinguished from all others in the genus by the following combination of characters: abruptly enlarged mesoptychials; enlarged postantebrachials; 2 frontoparietals; usually 3 parietals; 4 supraoculars each side; unisexual (only females exist); body with 6 longitudinal pale stripes that fade and can disappear in large adults; hatchlings basically unspotted but adults

Figure 1. Upper. Adult holotype of *A. neavesi* (MCZ R-192219 [= SIMR 8093]) in life, January 11, 2011, SVL = 77 mm. Lower. Adult *A. exsanguis* (SIMR 13209) of Alamogordo, Otero County, New Mexico, stock, SVL = 76 mm.

Figure 2. Ontogenetic changes in colors and pattern for two young individuals of *A. neavesi* for comparison with Fig. 1, upper. Upper. MCZ R-192243 (= SIMR 10400), SVL = 36 mm. Lower. SIMR 9575, SVL = 62 mm. Both photographed on January 11, 2011.

with pale dots and spots on body; maximum snout-vent length (SVL) about 80 mm; tetraploid number of chromosomes about 91, with 4 haploid sets of the *sexlineata* species group, including the slightly modified triploid karyotype of some *A. exsanguis* from Alamogordo (Lutes *et al*., 2011), and the 3 largest chromosomes being metacentric.

Description of Holotype. Paired data presented in the form x-y are for scale counts on the left-right sides of the body. SVL, 78 mm; rostral large, visible from above, wider than high; nostril low, posterior to center of nasal; nasals with a long median suture behind the rostral; frontonasal hexagonal; pair of irregularly hexagonal prefrontals with a long median suture; frontal hexagonal, longer than wide, wider anteriorly than posteriorly; pair of irregularly pentagonal frontoparietals with a long median suture; 3 irregular-sized and irregular-shaped parietals in a transverse series, the medial one basically heptagonal; irregular-sized and -shaped occipitals, posterior to but in contact with parietals, much smaller than parietals, much larger than dorsal granules. Scales in contact with outer perimeter of parietal and interparietal scales (PSC; Appendix 1) 15; back of head covered with small granules, smaller than middorsal ones on body; supraoculars 4, 1st and 4th smallest, 2nd largest, separated from superciliaries by 1 or 2 rows of granules (LSG, 13-12; Appendix 1), except 1st supraocular broadly contacts first 2 superciliaries. Last supraocular separated from frontoparietals by row of small scales (circumorbital semicircles, 3-5); postnasal 1 on each side; loreal 1 on each side, large, somewhat rectangular; preocular 1 on each side, with distinct ridge (keel). A row of 3 suboculars, the 2 anterior ones with a suborbital ridge continuing anteriorly onto the preocular, the 2nd subocular longest. Postoculars irregular and varied in size and shape; superciliaries 6-6,

the 3rd (left) or 2nd and 3rd (right) longest, the anterior ones elongate, posterior ones basically quadrangular. A few somewhat enlarged supratemporals; somewhat enlarged scales anterior to ear opening; central region of temple with small, roundish granules. Ear opening large, surrounded by small scales forming a smooth edge; external auditory meatus short, tympanum clearly visible; large supralabials 6-6, followed by small ones; suture between 5th and 6th below center of eye; the 3rd, 4th, and 5th longest. Lower eyelid with semitransparent disc of 5 enlarged palpebrals; pupil shape basically round to somewhat oval horizontally, with small irregularity on lower edge.

Mental trapezoid with convex anterior edge; postmental basically pentagonal; 7 pairs of chinshields (= sublabials of some authors) curving posteriorly and dorsally to the lower labials, only those of anterior pair in contact at midline; chinshields, from 2nd–7th pair separated in part or completely from infralabials by interlabial scales (7-7); 7 enlarged anterior infralabials, followed by small scales; 3rd infralabial (left side) or 4th (right side) largest.

Gulars small, flat, rounded, juxtaposed to slightly imbricate, somewhat larger on the anterior part of the throat, smaller posteriorly, from level of ear openings (GUL, 17; Appendix 1). Mesoptychial scales (on anterior edge of distinct gular fold) abruptly enlarged, slightly imbricate, smooth, about 13 enlarged ones across throat. Scales on nape and side of neck similar to dorsal and lateral body scales but smaller.

Dorsal and lateral scales irregularly granular, in indistinct transverse and oblique rows; ventrals large, usually somewhat rhomboidal, usually wider than long, imbricate, smooth, mostly in 8 longitudinal and 28 transverse rows (axilla to groin), the anterior rows on chest interrupted by a small triangular area of smaller scales. Number of dorsal granules around midbody 63; preanal area with 4 clearly enlarged, smooth, irregularly shaped, juxtaposed or slightly imbricate scales plus smaller ones.

Femoral pores 18-19, usually each pore surrounded by three small scales (medial one largest); midventrally, 2 scales separate the femoral pore series of each side.

Scales on dorsal and lateral aspects of tail basically rectangular, obliquely keeled, slightly mucronate, somewhat imbricate, in transverse rows, keels forming longitudinal ridges (TBS, 22; Appendix 1); scales under tail wider, smooth, more imbricate; scales on regenerated part of tail small, irregular, keeled, in transverse rows; tail round in cross section.

Scales on upper and anterior surfaces of upper arm, on upper and anterior surfaces of forearm, on anterior and lower surfaces of thighs, and on lower surfaces of lower legs large, smooth, imbricate. Scales on lower and posterior surfaces of upper arm, on posterior and ventral surfaces of forearm, on posterior and upper surfaces of thighs, and on upper, anterior, and posterior surfaces of lower legs small, granular, juxtaposed (but postantebrachials on forearms enlarged, angular, and irregular in shape). Lamellae on ventral surface of 4th finger 16-15. Lamellae on ventral surface of 4th toe 33-32, usually in single row on fingers but often paired on toes; fingers and toes somewhat laterally compressed; palms and soles with small, irregular, juxtaposed, flat scales (some tubercular); upper surfaces of hands and feet with large, imbricate, smooth, flat scales.

Color and Pattern of Holotype in Life. On January 11, 2011, with an adult SVL of 77 mm and age of about 29 months (Fig. 1, upper), coloration was as follows: dorsum reddish-brown with small pale cream to tan light dots and somewhat larger spots; 6 extremely subtle gray to tan stripes as seen from above, more evident if one looks over

the lizard from low in the rear, as in aiming a rifle; paravertebral light stripes wavy or zigzag, other light stripes basically straight; top of head brown; arms reddish-brown with few dark brown to black markings; legs similar to arms but with more black markings; very few pale tan dots dorsally on thighs, not as evident as in most *A. exsanguis*; some light tan spots present dorsally on rump, not as evident as in most *A. exsanguis*; dorsal surface of tail mostly reddish-brown on base, becoming greenish-brown distally.

Chin, throat (up to lower edge of ear opening), and chest pale blue; tan beneath arms and on chest; very pale blue to gray on abdomen, underside of legs, and underside of base of tail; light tan with gray smudges on underside of medial and distal tail.

Color and Pattern of Holotype in Preservative (70% Ethanol). The following changes occurred after preservation. The light dots and spots on the body are pale gray or cream. Only four largely faded gray light stripes are barely evident, more visible posteriorly than anteriorly. No light dots or spots visible on thighs. Dorsal surface of tail reddish-brown, becoming brown posteriorly, then reddish-brown distally.

Ontogenetic Development of Color Pattern in Life. The following notes on three typical individuals of *A. neavesi* of different ages were taken on January 11, 2011. The youngest, MCZ R-192243 (= SIMR 10400; Fig. 2, upper), was 16 days old at a SVL of 36 mm. Dorsum brown (not reddish-brown as in adults) with 6 bold, conspicuous light stripes (yellow, but the lateral stripe tends toward cream); paravertebral light stripes wavy or zigzag, others basically straight; dorsum essentially unspotted (except on limbs), with few hints of tiny pale dots in the dorsolateral and lateral dark fields; top of head light brown; dorsal tail at base similar to posterior body but becoming conspicuous bluish-green; arms dorsally dark brown with yellow spots; legs similar to arms but also with short yellow stripes; ventral surfaces very light tan, but underside of tail is bluish-green.

The next youngest, SIMR 9575 (Fig. 2, lower), was noted at about 9 months old at a SVL of 62 mm and with colors and pattern intermediate between the youngest and oldest individuals (as described for the holotype). Dorsal ground color becoming reddish-brown; light stripes becoming less conspicuous, especially anteriorly, becoming gray and significantly faded; lateral light stripe tannish-cream; top of head darker brown; spots on arms and legs now tan; distal tail now brownish-green; ventral surfaces as on younger individuals, without blue.

The next oldest lizard, MCZ R-192218 (= SIMR 8092) is a sister of the holotype (in the same clutch) and was about 29 months old when noted, with a SVL of 68 mm, about 9 mm shorter than the holotype at the time. She was similar to the holotype except as follows: dorsum and sides with considerably more light spots (mostly tan); tail becoming brownish-green posterior to base; chest, abdomen, and ventral surfaces of legs and tail light gray to pale blue.

The ontogenetic changes can be summarized as follows: dorsal ground color changes from brown to reddish-brown; dorsal light stripes change from being conspicuous and yellow or cream to become tan or gray and extremely faded; upon hatching, dorsal body is essentially unspotted, but later develops light spots, which in largest adults become less conspicuous; top of head changes from light brown to brown; tail changes from conspicuous bluish-green to greenish-brown; arms and legs change from dark brown to reddish-brown and tend to lose the light spots and stripes; ventral surfaces change from very light tan to gray, pale blue, or darker blue.

Karyotype. Lutes *et al.* (2011) described and illustrated the karyotype. It consists of the three haploid sets of chromosomes of *A. exsanguis* (as they were cloned) plus a haploid set of *A. inornata*. The karyotype of *A. exsanguis* involved is slightly modified from the ideal theoretical triploid condition. The modifications are identical to those found in the maternal triploid *A. exsanguis* that were collected in Alamogordo, New Mexico, in 2003–2005, and their laboratory offspring, as follows: (A) one of the largest macrochromosomes (a metacentric with a subterminal secondary constriction on one arm) had apparently undergone centric fission into two smaller telocentric chromosomes; and (B) two microchromosomes were apparently missing. These modifications were not found in the original field-caught tetraploid hybrid or in the *A. exsanguis* collected with it (Neaves, 1971), which indicates that two or more karyotypic clones of *A. exsanguis* have been present in Alamogordo since 1967. This is not particularly unusual for parthenogenetic whiptails (Lowe *et al.*, 1970b; Cole, 1979).

Reproduction. Individuals reproduce by parthenogenetic cloning (Lutes *et al.*, 2010, 2011).

Etymology. The specific epithet, a noun in the genitive singular case, honors Dr. William B. Neaves, who was awarded a Ph.D. at Harvard University. Dr. Neaves' graduate studies on unisexual whiptail lizards (Neaves and Gerald, 1968, 1969; Neaves, 1969, 1971) provided important early insights into the molecular genetics, origins, and speciation of parthenogens through hybridization, as well as the origin of a tetraploid hybrid lizard of *A. exsanguis* × *A. inornata* that he discovered in the field in Alamogordo, Otero County, New Mexico, which was the inspiration for the present laboratory hybridization project.

Comments. The specimen selected as the holotype is an F_2 generation lizard that produced cloned offspring. The F_1 lizards were true hybrids with two parents of different species, but individuals of subsequent generations were not hybrids; they had only one parent from which they were cloned. It is a paradox of convention for unisexual lizards of hybrid origin that the animals of the F_2 generation and beyond are considered to represent a named species, but the F_1 female hybrids of which they are a clone are not considered to be members of a species.

Specimens of *A. neavesi* examined for the present report are members of three lineages derived from two clutches of F_1 hybrid eggs of *A. exsanguis* × *A. inornata* that were produced at the SIMR from three F_1 females that cloned themselves (Lutes *et al.*, 2011). These cloned lineages ultimately were a product of one female's ovaries and one male's testes. We treat the lineages as one species (following Cole, 1990), rather than three, although some authors have suggested that theoretically each lineage that stems from a hybrid zygote should be treated as a separate species (Frost and Wright, 1988; Frost and Hillis, 1990). There were genetic differences in microsatellite loci detected among the F_1 hybrids owing to fertilizations by different spermatozoa from *A. inornata* and a derived mutant allele of MS 14 that was found in an F_3 female (SIMR 9706; Lutes *et al.*, 2011). This kind of genetic variation is found within gonochoristic species as well. Despite such genetic differences, individuals of the different lineages are very similar to each other in morphology (see below). Nevertheless, reproduction varied widely within the three lineages discussed in this paper. For example, at the time of this writing the lineage of AMNH R-176077–176148 (= SIMR 4921 lineage) had produced about 250 offspring, whereas the lineage of MCZ R-192211 (= SIMR 5983 lineage) had produced only about 54.

TABLE 1. DESCRIPTIVE STATISTICS FOR 10 MORPHOLOGICAL CHARACTERS IN A POOLED SAMPLE OF THE F_2, F_3, AND F_4 GENERATIONS OF THREE LINEAGES OF *ASPIDOSCELIS NEAVESI*.

Character[a]	Mean ± 1 SE (range)	Sample Size (*N*)
SPV	4.6 ± 0.06 (3–6)	113
GAB	63.6 ± 0.18 (58–69)	125
FP	38.0 ± 0.14 (32–41)	124
SDL-T	31.8 ± 0.10 (29–35)	123
SDL-F	15.0 ± 0.07 (13–17)	122
GUL	16.8 ± 0.13 (13–20)	113
COS	9.1 ± 0.10 (6–12)	121
TBS	18.6 ± 0.18 (15–24)	125
PSC	14.7 ± 0.12 (11–18)	118
LSG	25.9 ± 0.20 (21–35)	116

[a]See Appendix 1 for detailed descriptions of characters.

Normally, new species are named not because they are newly formed but because they became recently known to science. In this case, *A. neavesi* really is a new species; its date of origin was August 12, 2008, when the first F_2 individual hatched.

INTRASPECIFIC COMPARISONS

Scalation. Basic quantitative morphological data for 125 specimens of *A. neavesi* are provided by descriptive univariate statistics for 10 meristic scalation characters (Appendix 1; Table 1). The following additional characters were examined also.

Condition of enlarged ventral preanal scales: in Type I there are 3 enlarged scales, 2 bordering the vent and 1 anterior to these. In Type II there are 2 enlarged scales, 1 bordering the vent and 1 anterior to it. In Type III the pattern is different from both Type I and II. Of 124 specimens examined, 40% had Type I, 8% had Type II, and 52% had Type III.

Number of rows of enlarged ventral plates across belly at midbody: there were 8 in all 124 specimens examined.

Number of ventral scales separating the series of femoral pores: of 123 specimens examined, 87% had 2 scales, 12% had 3 scales, and 1% (1 individual) had 4 scales.

Condition of postantebrachial scales on the forearm: all of the 124 specimens examined had enlarged scales.

Condition of mesoptychial scales across the throat: all 124 specimens examined had abruptly enlarged scales, but 3 specimens had some irregular small scales in the middle of the series.

Statistical Comparisons of Two Lineages. Here we address the question of whether there are differences between the two best represented lineages, that of MCZ R-192209 (= SIMR 4919 lineage) and AMNH R-176077–176148 (= SIMR 4921 lineage). These two lineages differed significantly in 2 of the 10 meristic characters (SPV and FP; Table 2), but we included all 10 characters in a PCA of the two lineages. There were no multivariate outliers among the 88 specimens with complete data in these two lineages. We used the 10 principal components generated by the PCA as potential candidate variables for a CVA, of which PC1 and PC7 were selected by the stepwise selection criteria (see Materials and Methods) for inclusion in the CVA model (Table 3).

The two lineages differed significantly in CV1 (Table 2). However, this statistical difference was counterbalanced by evidence that possible biological significance is open to question. For example, the Wilks' lambda value (0.840) from the CVA indicated that only about 16% of the total variation was explained by meristic differences between the two lineages. Additionally, the small eigenvalue of 0.190 for CV1 indicated that the discriminant function was weak (Table 3), and only 69.3% of the specimens were classified correctly to their respective lineages by the CVA model (Table 4).

It was not surprising that the difference in CV1 was statistically significant because these two lineages differed statistically in

TABLE 2. COMPARISON OF MEANS OF MORPHOLOGICAL CHARACTERS OF TWO LINEAGES OF *ASPIDOSCELIS NEAVESI*.

Character[a]	SIMR Lineage[b]		Is the Difference Significant?		
	4921 ($N = 56$)	4919 ($N = 32$)	Y/N	t_{86}	P
SPV	4.4 ± 0.07 (3–5)	4.8 ± 0.12 (3–6)	Yes	−2.468	0.02
FP	38.0 ± 0.22 (32–40)	38.8 ± 0.18 (36–41)	Yes	−2.479	0.02
GAB	64.0 ± 0.24 (58–68)	63.4 ± 0.41 (59–69)	No	1.225	0.22
SDL-T	31.9 ± 0.15 (29–35)	32.1 ± 0.19 (30–35)	No	−0.940	0.35
SDL-F	15.1 ± 0.11 (13–17)	15.2 ± 0.12 (13–16)	No	−0.845	0.40
GUL	16.7 ± 0.16 (13–20)	17.0 ± 0.23 (15–19)	No	−1.090	0.28
COS	9.0 ± 0.14 (6–11)	9.4 ± 0.21 (8–12)	No	−1.498	0.14
TBS	18.6 ± 0.26 (15–23)	19.2 ± 0.35 (16–24)	No	−1.384	0.17
PSC	14.7 ± 0.18 (11–17)	14.7 ± 0.25 (12–18)	No	−0.073	0.94
LSG	25.9 ± 0.26 (22–30)	26.4 ± 0.37 (23–33)	No	−1.191	0.24
CV1	−0.326 ± 0.121 (−2.677 to 1.761)	0.570 ± 0.204 (−2.323 to 2.406)	Yes	−4.040	<0.001

[a]Mean ± SE and range limits are shown for 10 univariate characters described in Appendix 1 and CV1. Only specimens with complete data for all 10 univariate characters are included. All variances were homogeneous (Levene's Test probabilities: 0.05–0.93).

[b]Lineages are described in Appendix 2. SIMR 4921 lineage is AMNH R-176077–176148, and the lineage of SIMR 4919 is the lineage of MCZ R-192209.

two univariate characters (see above; Table 2). But is there a possibility that evolutionary divergence of the lineages is occurring at a low level of resolution? There appears to be a trend in a progressive separation of the two lineages across generations, as seen in a decrease in mean CV1 from the F_2 to the F_4 generations in the AMNH lineage and an increase in mean CV1 from the F_3 to the F_4 generations in the MCZ lineage. Meristic divergence, as measured by CV1, reached a level of separation at the F_3 generation of the AMNH lineage and F_4 generation of the MCZ lineage that became a statistically significant difference in CV1 between the two lineages (Table 5). There were too few F_2 specimens with complete data in the MCZ lineage to include in this lineage-generation combination of comparisons. The possibility that morphological divergence is taking place between lineages in this newly formed parthenogenetic species is worth monitoring, and this hypothesis can be tested by including more individuals and future generations in the analysis.

INTERSPECIFIC COMPARISONS

Coloration. Here we compare colors and patterns of *A. neavesi* with its ancestral species, *A. inornata* and *A. exsanguis*. Lutes *et al.* (2011) illustrated adults of the three species in color (their fig. 1A:9911). *Aspidoscelis inornata* is so different from the others that it is easily identified before capture in the field. Its dorsum is dark brown with 6–7 conspicuous yellow stripes, no spots, a bright blue tail, blue on the ventral surfaces (darker in males than females), and only minor changes in color tones during ontogenetic development. The strong difference between *A. inornata* and the other two species is interesting because *A. inornata* is one of the three ancestral species of *A. exsanguis* (having provided at least 33% of the overall genome of *A. exsanguis*; Dessauer and Cole, 1989; Reeder *et al.*, 2002); consequently, it provided at least 50% of the overall genome of *A. neavesi*. The strong similarities between *A. exsanguis* and *A. neavesi* reflects matriclinous inheritance (i.e., having a greater

TABLE 3. LOADINGS: CORRELATIONS BETWEEN CHARACTERS AND EITHER PRINCIPAL COMPONENTS (PC) OR A CANONICAL VARIATE (CV1) FROM MULTIVARIATE ANALYSES OF TWO LINEAGES OF *ASPIDOSCELIS NEAVESI*.

	Character[a]	PC1	PC7
Principal components analysis	SPV	0.651	0.086
	FP	0.651	−0.133
	GAB	0.640	−0.533
	COS	0.388	0.346
	SDL-T	0.429	0.254
	PSC	0.266	−0.216
	GUL	0.363	0.329
	LSG	0.185	0.201
	SDL-F	0.119	0.169
	TBS	0.202	0.047
Eigenvalues		1.884	0.719
Proportion of variation (%)		18.8	7.2
Canonical variate analysis	Character	CV1	
	PC7[b]	0.756	
	PC1[b]	0.588	
	PC6	0.072	
	PC9	0.046	
	PC8	0.046	
	PC2	−0.038	
	PC3	−0.036	
	PC10	−0.032	
	PC5	−0.024	
	PC4	0.009	
Eigenvalue		0.190	
Proportion of intergroup variation (%)		100	

[a]Univariate characters are described in Appendix 1 and the lineages of AMNH R-176077–176148 (= SIMR 4921 lineage) and MCZ R-192209 (= SIMR 4919 lineage) in Appendix 2.

[b]Of the 10 principal components, only PC1 and PC7 were selected as having discrimination value for the CVA.

TABLE 4. CLASSIFICATION RESULTS OF A CANONICAL VARIATE ANALYSIS OF TWO SIMR LINEAGES OF *ASPIDOSCELIS NEAVESI*.

	N	Lineage 4921, No. (%)	Lineage 4919, No. (%)
Original grouped specimens[a]			
Lineage 4921	56	40 (71.4)	16 (28.6)
Lineage 4919	32	10 (31.3)	22 (68.8)
Jackknifed classification			
Lineage 4921	56	40 (71.4)	16 (28.6)
Lineage 4919	32	11 (34.4)	21 (65.6)

[a]A priori groups are in the first column, and the row for each a priori group shows the number of individuals assigned to each group by the CVA model (Table 3). Overall classification success was 70.5% for the original grouped specimens and 69.3% for the jackknifed classification. Lineages are described in Appendix 2. The SIMR 4921 lineage is AMNH R-176077–176148 and the SIMR 4919 lineage is of MCZ R-192209.

resemblance to the maternal ancestral species) following the most recent episode of the three hybridization events in the ancestry of *A. neavesi* (see below).

Detailed notes on color and pattern comparing *A. neavesi* and *A. exsanguis* were made on preserved specimens. Young individuals of *A. exsanguis* (based on MSB 95084 and MSB 95086 [= SIMR 9982 and 9989, respectively], with SVLs of 53 and 59 mm, respectively) differ from similar-sized *A. neavesi* only in having the emerging light spots become more random in distribution, including appearing on the light stripes. Large adults of *A. exsanguis* (e.g., as MCZ R-192216 [= SIMR 7190], at 78 mm SVL) differ from adults of *A. neavesi* by having the following characteristics: dorsal light spots larger and more conspicuous, especially across rump; dorsal thighs with large, abundant, conspicuous light spots (these disappear in large *A. neavesi*); medial region of tail dorsally greenish-brown (brown in large *A. neavesi*); more cream, less blue on ventral surfaces than *A. neavesi* (compare Figs. 1 upper and lower).

Comparisons in preservative between *A. neavesi* and two male tetraploid hybrids of *A. exsanguis* × *A. inornata* collected in the field follow. Natural hybrid MCZ R-101991 with a SVL of 69 mm is similar in all respects to *A. neavesi* of similar size and noted in life (e.g., SIMR 9575 at 62 mm SVL), except that on the natural hybrid, the paravertebral light stripes converge posterior to the neck and

TABLE 5. DESCRIPTIVE STATISTICS AND TESTS OF SIGNIFICANCE AMONG SIMR LINEAGE-GENERATION COMBINATIONS FOR CANONICAL VARIATE 1 (CV1) IN *ASPIDOSCELIS NEAVESI*.

	Descriptive Statistics				
Generation[a]	4921-F_2	4921-F_3	4921-F_4	4919-F_3	4919-F_4
N	4	30	23	16	14
Mean ± SE (range)	−0.54 ± 0.772 (−2.41 to 1.36)	−0.36 ± 0.181 (−2.68 to 1.76)	−0.20 ± 0.14 (−1.70 to 0.85)	0.52 ± 0.36 (−2.32 to 2.41)	0.62 ± 0.23 (−0.71 to 1.88)
Lineage[b]	Probabilities (*P*) That CV1 Means Are the Same				
4921-F_2	1.0				
4921-F_3	0.999	1.0			
4921-F_4	0.989	0.956	1.0		
4919-F_3	0.734	0.230	0.373	1.0	
4919-F_4	0.639	0.016	0.035	0.999	1.0

[a]Sample size, mean ± SE and range of variation of CV1 are shown.
[b]Lineages are described in Appendix 2. The SIMR 4921 lineage is AMNH R-176077–176148, and the SIMR 4919 lineage is of MCZ R-192209.

continue immediately adjacent to each other as a gray vertebral area, then separate again at the hip. In life, this natural hybrid also had brilliant blue ventral surfaces (Neaves, 1971). Natural hybrid UCM 29196, also with a SVL of 69 mm, was similar except that the paravertebral stripes were like those of *A. neavesi*, including some zigzag, contrary to Taylor *et al.* (1967). Three large male hybrids of *A. exsanguis* × *A. inornata* formed at SIMR (MCZ R-192210 [= SIMR 5134]; MCZ R-192238 [= SIMR 9682]; and MCZ R-192239 [= SIMR 9683]) were similar to large *A. neavesi*, but two had larger and more conspicuous dorsal spots, and they had considerable blue ventrally.

In summary, male hybrids usually are very similar to female hybrids, to *A. neavesi*, and to *A. exsanguis*, especially when active individuals are observed in the field. If collected, the male hybrids stand out in particular because of the deeper blue ventral surfaces and because of their hemipenes (absent in *A. exsanguis*). Alternatively, female hybrids or specimens of *A. neavesi*, if collected, would be more easily misidentified as *A. exsanguis*. Nevertheless, by comparing specimens carefully with Figure 1, the color notes, the karyotype, and the morphological data presented here, one can consistently distinguish between *A. neavesi* and *A. exsanguis*.

Scalation and Multivariate Statistics. As with the intraspecific comparisons (see above), a PCA was used to develop uncorrelated characters (principal components) from the raw meristic data, to be used in a follow-up CVA (Tables 6, 7). There were no multivariate outliers among the 110 specimens with complete data in these three taxa.

Because a PCA reflects all of the intragroup and intergroup variation in one pooled sample (i.e., without classification of individuals into a priori groups), we wanted to see if the PCA would objectively cluster the specimens into three separate groups representing *A. exsanguis, A. inornata*, and *A. neavesi*. The three species were clearly separated by the first two principal components (PC1 and PC2), which accounted for approximately 44% of the total meristic variation (Table 7; Fig. 3). However, *A. neavesi* did not differ significantly from *A. exsanguis* for PC1, and *A. neavesi* did not differ significantly from *A. inornata* for PC2 (Table 6). Scores for PC1 and PC2 placed

TABLE 6. COMPARISON OF MEANS AMONG MORPHOLOGICAL CHARACTERS OF *ASPIDOSCELIS NEAVESI* AND ITS PROGENITOR SPECIES, *A. EXSANGUIS* AND *A. INORNATA*.

Character[a]	Group[b]		
	A. exsanguis ($N = 10$)	*A. neavesi* ($N = 90$)	*A. inornata* ($N = 10$)
FP	19.5 ± 0.17 (19–20) A	19.2 ± 0.12 (13–21) A	15.0 ± 0.26 (14–16) B
SPV	4.4 ± 0.16 (4–5) A	4.6 ± 0.06 (3–6) A	8.3 ± 0.45 (5–10) B
PSC	15.1 ± 0.31 (14–17) A	14.7 ± 0.14 (11–18) A	18.7 ± 1.37 (14–25) B
SDL-T	29.8 ± 0.25 (28–31) A	32.0 ± 0.12 (29–35) B	28.5 ± 0.64 (25–31) C
LSG	31.0 ± 0.68 (28–34) A	26.1 ± 0.21 (22–33) B	24.3 ± 2.57 (17–39) B
GAB	69.7 ± 0.45 (68–71) A	63.7 ± 0.21 (58–69) B	60.9 ± 1.75 (52–70) C
TBS	21.7 ± 0.56 (19–25) A	18.7 ± 0.21 (15–24) B	19.2 ± 0.74 (15–22) B
COS	9.4 ± 0.34 (7–11) A	9.1 ± 0.12 (6–12) A	9.0 ± 0.42 (7–11) A
SDL-F	13.6 ± 0.16 (13–14) A	15.1 ± 0.08 (13–17) B	14.4 ± 0.34 (12–16) A
GUL	17.2 ± 0.29 (15–18) A	16.8 ± 0.14 (13–20) A	16.2 ± 0.53 (13–19) A
PC1	0.33 ± 0.068 (0.05 to 0.67) A	0.27 ± 0.048 (−1.10 to 1.26) A	−2.73 ± 0.326 (−4.10 to −0.86) B
PC2	2.11 ± 0.116 (1.43 to 2.64) A	−0.26 ± 0.062 (−1.99 to 1.85) B	0.04 ± 0.504 (−2.14 to 2.73) B
CV1	0.06 ± 0.233 (−1.09 to 0.84) A	0.86 ± 0.101 (−2.38 to 2.73) B	−7.83 ± 0.475 (−10.41 to −5.59) C
CV2	−4.35 ± 0.222 (−5.30 to −3.28) A	0.44 ± 0.093 (−2.05 to 3.27) B	0.40 ± 0.607 (−3.40 to 3.51) B

[a]Mean ± SE and range limits are shown for PC1, PC2, CV1, CV2, and 10 univariate characters described in Appendix 1. Tukey multiple comparison tests were used; groups sharing the same capital letter are not significantly different at $\alpha = 0.05$.

[b]Samples comprise specimens with complete data for all 10 univariate characters.

TABLE 7. LOADINGS: CORRELATIONS BETWEEN CHARACTERS AND EITHER PRINCIPAL COMPONENTS (PC) OR CANONICAL VARIATES (CV) FROM MULTIVARIATE ANALYSES OF *ASPIDOSCELIS NEAVESI* AND ITS PROGENITOR SPECIES, *A. EXSANGUIS* AND *A. INORNATA*.

	Character[b]	PC1	PC2	PC3	PC4	PC5	PC6
Principal components analysis[a]	FP	0.782	0.076	0.171	−0.099	0.075	0.309
	SPV	−0.737	0.082	−0.195	0.349	0.020	−0.154
	PSC	−0.726	0.263	0.116	0.191	0.093	0.280
	SDL-T	0.700	−0.245	0.033	0.183	0.154	0.109
	LSG	0.249	0.707	0.302	0.033	0.069	−0.462
	GAB	0.458	0.670	−0.270	0.095	−0.041	−0.202
	TBS	0.022	0.591	−0.404	0.125	0.490	0.361
	COS	−0.060	0.401	0.686	0.293	−0.340	0.279
	SDL-F	0.207	−0.447	0.245	0.634	0.434	−0.185
	GUL	0.358	−0.044	−0.472	0.494	−0.568	0.072
Eigenvalue		2.620	1.801	1.164	0.960	0.911	0.712
Proportion of variation (%)		26.2	18.0	11.6	9.6	9.1	7.1
Canonical variate analysis	Character	CV1	CV2				
	PC1	0.672	−0.134				
	PC6	0.060	0.043				
	PC2	−0.046	−0.686				
	PC3	0.099	0.176				
	PC4	−0.049	0.165				
	PC7	−0.022	0.152				
	PC9	−0.016	−0.102				
	PC5	0.027	0.098				
	PC10	−0.056	0.065				
Eigenvalue		6.359	1.948				
Proportion of intergroup variation (%)		76.5	23.5				

[a]Loadings are not shown for PC7, PC9, and PC10 that summarized, respectively, only 6.2%, 3.9%, and 3.3% of the meristic variation; PC8 was not included in the CVA model.

[b]Univariate characters are described in Appendix 1.

the laboratory hybrid male (MCZ R-192239 [= SIMR 9683]) well inside the cluster of *A. neavesi*. In contrast, the field-caught presumptive hybrid male (UCM 19196) was outside the clusters of the three species but closest to the cluster of *A. exsanguis* (Fig. 3).

Next we used our samples of *A. exsanguis, A. inornata,* and *A. neavesi* as a priori (preidentified) groups for a CVA, and the hybrid males (MCZ R-192239 and UCM 29196) were included as unclassified individuals for assignment to the most similar a priori group. Our objectives were (1) to determine how well the three species could be discriminated with meristic characters, (2) to quantify the morphological resemblance of *A. neavesi* to each of its progenitor species, and (3) to determine the species alliance in multivariate space of the tetraploid hybrid males of *A. exsanguis* × *A. inornata*.

Our samples of 10 *A. exsanguis* and 10 *A. inornata* are from the same locality as the female hybrid (MCZ 101991) that was collected in 1967 in Alamogordo, New Mexico, and reported by Neaves (1971). Of 129 individuals in the four lineages of *A. neavesi*, 39 were excluded from statistical analyses because they could not be scored for

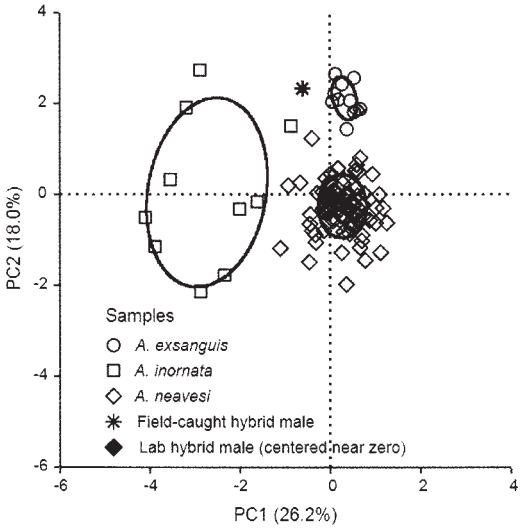

Figure 3. Scatterplot of principal component scores of 90 specimens of *A. neavesi* and 10 each of its progenitor species, *A. exsanguis* and *A. inornata*. Sample centroids are centered on the 50% confidence ellipses of group scores, and axis percentages are percentages of variance explained by PC1 and PC2 (Table 7). The field-caught hybrid male (UCM 29196) is clearly visible, but the laboratory hybrid male (MCZ R-192239 [= SIMR 9683]) is buried within the cluster of *A. neavesi*.

one or more meristic characters, mostly owing to damage incurred when specimens were dissected. For the 10 univariate meristic characters, *A. neavesi* resembled *A. exsanguis* in FP, SPV, and PSC; resembled *A. inornata* in LSG and TBS; and was significantly different from both progenitor species in SDL-T, GAB, and SDL-F (Table 6).

We included all 10 univariate characters in a PCA, and 9 of the 10 principal components were selected for inclusion in the CVA model (Table 7). Wilks' lambda (0.046) from the CVA indicated that about 95% of the total variation was explained by meristic differences among the three species. Large eigenvalues (Table 7) and an overall classification success of 99.1% (Table 8) were evidence of a strong discrimination. There was only one misclassification (Table 8)—specimen AMNH R-176081 (= SIMR 10310) from the F_2 generation of the AMNH lineage of *A. neavesi* was misclassified to the *A. exsanguis* group based on a marginal probability of 0.51. This assignment was automatically made by the CVA model because the probability for assigning this specimen to the *A. neavesi* group (0.49) was smaller. The assignment of the unclassified laboratory hybrid male (MCZ R-192239 [= SIMR 9683]) to the *A. neavesi* group was robust ($P = 0.999$). In contrast, the unclassified field-caught hybrid male (UCM 29196) was assigned to the *A. exsanguis* group with a high level of probability ($P = 0.989$).

The spatial relationships of the three species in the CVA are shown in Figure 4. The positions of the three clusters on the

Table 8. Classification results of a canonical variate analysis of *Aspidoscelis neavesi* and its progenitor species, *A. exsanguis* and *A. inornata*.

Original Grouped Specimens[a]	*N*	No. *A. neavesi* (%)	No. *A. exsanguis* (%)	No. *A. inornata* (%)
A. neavesi	90	89 (98.9)	1 (1.1)	0
A. exsanguis	10	0	10 (100)	0
A. inornata	10	0	0	10 (100)
Laboratory hybrid male[b]	1	1	—	—
Field-caught hybrid male[c]	1	—	1	—

[a]A priori groups are in the first column, and the row for each a priori group shows the number of individuals assigned to each group by the CVA model (Table 7). Overall classification success was 99.1% for the three a priori groups. A jackknifed classification gave the same results.

[b]This individual (MCZ R-192239 [= SIMR 9683]) was included in the CVA as unclassified.

[c]This individual (UCM 29196) was included in the CVA as unclassified.

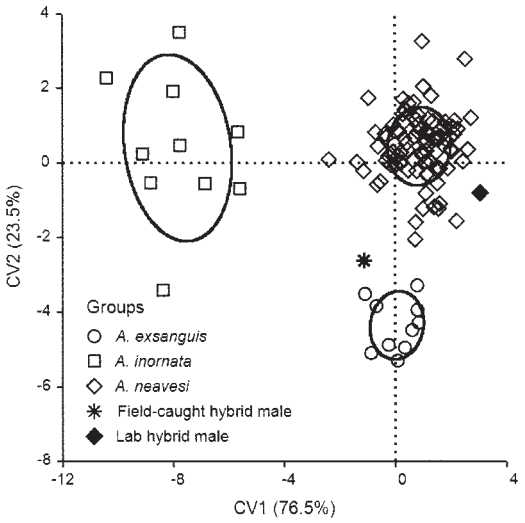

Figure 4. Scatterplot of canonical variate scores of 90 specimens of *A. neavesi* and 10 each of its progenitor species, *A. exsanguis* and *A. inornata*. The CVA model is shown in Table 7. Sample centroids are centered on the 50% confidence ellipses of group scores, and axis percentages are contributions of canonical variates CV1 and CV2 to the discrimination (Table 7). The laboratory hybrid male is MCZ R-192239 (= SIMR 9683), and the field-caught hybrid male is UCM 29196.

CV1 axis (which expresses the greater proportion of variation) indicate that *A. neavesi* is more similar to *A. exsanguis* than *A. inornata* in multivariate space. This was confirmed by pairwise Mahalanobis D^2 distances (smaller distances = greater resemblance) of 23.6 between *A. neavesi* and *A. exsanguis*, 75.6 between *A. neavesi* and *A. inornata*, and 84.9 between *A. exsanguis* and *A. inornata*. Therefore, *A. neavesi* is matriclinous in resembling the maternal ancestral species. All three species differed significantly in mean CV1 (Table 6), but *A. neavesi* clearly resembled *A. inornata* on the CV2 axis, as substantiated by the absence of a significant difference in CV2 between these two groups (Table 6). The hybrid males are peripheral to the respective groups to which they were assigned (Fig. 4), but their classification probabilities near unity leave little doubt that these assignments based on meristic characters are robust.

ACKNOWLEDGMENTS

We thank Rick Kupronis and his team of dedicated reptile technicians at SIMR, David Jewell, Alex Muensch, Christina Piraquive, and Kristy Winter, for excellent husbandry and herpetocultural skills. We also thank Jillian Kupronis and Aracely Lutes for contributions to genotyping analysis. The following individuals at museum repositories provided efficient curating of the specimens: Margaret Arnold, David Dickey, Darrel Frost, David Kizirian, and Christopher Raxworthy (AMNH); Jonathan Losos, Joe Martinez, and José Rosado (MCZ); and Tom Giermakowski and Howard Snell (MSB).

We are also grateful to Jonathan Losos and José Rosado of the MCZ and to Mariko Kageyama and Christy McCain of the UCM for lending us specimens that were needed for this research. Additionally, Charles Painter, New Mexico Department of Game and Fish, helped with permit matters, and Carol Townsend (AMNH) assisted in many ways.

Most of all, we are deeply indebted to William B. Neaves for spearheading the research on parthenogenetic lizards at the SIMR and for guidance, encouragement, and assistance in our work. This work was funded by the Stowers Institute for Medical Research and the Howard Hughes Medical Institute.

Appendix 1

Abbreviations for morphological characters used are as follows: COS, number of circumorbital semicircle scales (total of both sides of head; following Wright and Lowe, 1967); GAB, number of dorsal granules (scales) around midbody, following Wright and Lowe (1967); FP, number of femoral pores (for intraspecific comparisons we used

the total of both legs summed, but for interspecific comparisons we increased the sample size by using the right leg only, but the left if the right was not clear); GUL, number of gular scales, following Cole *et al.* (1988); LSG, number of lateral supraocular granules (total of both sides of head, whether in 1 or 2 rows) between the supraoculars and superciliaries, counting forward from an imaginary line extended from the suture between the 3rd and 4th supraoculars toward the superciliaries, following Walker *et al.* (1966); PSC, total number of scales in contact with outer perimeter of parietal and interparietal scales, following Cole *et al.* (2010); SDL-F, number of subdigital lamellae on the right 4th finger but using the left if the right is missing, following Taylor *et al.* (2001); SDL-T, number of subdigital lamellae on the right 4th toe but using the left if the right is missing, following Cole *et al.* (1988); SPV, number of granules (scales) between the paravertebral light stripes, following Wright and Lowe (1967); SVL, snout-vent (body) length in millimeters; TBS, number of enlarged dorsal scales around dorsal aspect of base of tail; the count is made while holding the hind legs at the hip perpendicular to the body and counting on an imaginary line along the posterior edges of the legs, but not including lateral granules on the tail.

Appendix 2

Specimens Examined

Aspidoscelis exsanguis: MCZ R-100419; MSB 95084 (= SIMR 9982); MSB 95086 (= SIMR 9989); MCZ R-192263 (SIMR 11575); MCZ R-192287–192288 (SIMR 13218–13219); and MCZ R-192289–192292 (SIMR 13273–13276). In addition, one specimen illustrated (Fig. 1, lower, SIMR 13209) has not reached a permanent repository, as it is still alive. All specimens were either caught in the city park, Alamogordo, Otero County, New Mexico, or reared at SIMR from stock obtained at that locality.

Aspidoscelis inornata: MCZ R-100425; MCZ R-192214 (SIMR 6636); MCZ R-192215 (SIMR 6755); MCZ R-192221 (SIMR 8453); MCZ R-192222 (SIMR 8678); MCZ R-192223 (SIMR 8680); MCZ R-192228 (SIMR 8860); MCZ R-192230 (SIMR 9098); MCZ R-192232 (SIMR 9427); and MCZ R-192264 (SIMR 11678). All specimens were either caught in the city park, Alamogordo, Otero County, New Mexico, or reared at SIMR from stock obtained at that locality.

Aspidoscelis neavesi: All of these specimens are paratypes except for SIMR 9575 and SIMR 9706 (see below). For economy of space, we do not list the correlated SIMR catalog numbers for these specimens, which are included on an individual basis in the catalogs at the AMNH and MCZ. The two lineages of which specimens were compared by PCA and CVA stem from F_1 hybrid females SIMR 4921 and MCZ R-192209, although those used in the PCA and CVA are only the ones with all data available for the complete suite of 10 characters, and the F_1 hybrid females were not included. Specimens from the AMNH R-176077–176148 series (= SIMR 4921 lineage) are the following: F_2 generation, AMNH R-176077–176085; F_3 generation, AMNH R-176086–176120; and F_4 generation, AMNH R-176121–176148. Specimens from the MCZ R-192209 lineage are the following: F_2 generation, MCZ R-192218–192219, MCZ R-192224–192227, MCZ R-192229, MCZ R-192236–192237, MCZ R-192243, and MCZ R-192256; F_3 generation, MCZ R-192233–192234, MCZ R-192241–192242, MCZ R-192244–192246, MCZ R-192248–192255, MCZ R-192258–192260, MCZ R-192262, MCZ R-192265, MCZ R-192267–192268, MCZ R-192276, and MCZ R-192279; F_4 generation, MCZ R-192261, MCZ R-192266, MCZ R-192269–

192275, MCZ R-192277–192278, and MCZ R-192280–192286. Individuals from another lineage, the MCZ R-192211 (= SIMR 5983) lineage, excluding the F_1 female are MCZ R-192229, MCZ R-192236–192237, MCZ R-192254, and MCZ R-192256. In addition, two specimens noted for a few characters have not reached a permanent repository: SIMR 9575 (an F_2 individual of the MCZ R-192209 lineage, used for color notes, is still alive) and SIMR 9706 (an F_3 individual of the MCZ R-192209 lineage, noted for a microsatellite mutation [Lutes *et al*., 2011] is still alive). The last two individuals are not paratypes. The individual ancestors of these lineages, *A. exsanguis* (SIMR 71) and *A. inornata* (SIMR 69), were both reared at SIMR from stock obtained in the city park, Alamogordo, Otero County, New Mexico.

F_1 laboratory hybrids of *A. exsanguis* × *A. inornata*: MCZ R-192209–192211 and MCZ R-192238–192239. These individuals were reared at SIMR from parental stock that was caught in the city park, Alamogordo, Otero County, New Mexico.

F_1 hybrids of *A. exsanguis* × *A. inornata* that were found in nature: MCZ R-101991, a female, from the city park, Alamogordo, Otero County, New Mexico, and (presumed hybrid) UCM 29196, a male, from 2 mi W, 1 mi S Mesilla, Doña Ana County, New Mexico.

LITERATURE CITED

Cole, C. J. 1979. Chromosome inheritance in parthenogenetic lizards and evolution of allopolyploidy in reptiles. *Journal of Heredity* **70:** 95–102.

Cole, C. J. 1990. When is an individual not a species? *Herpetologica* **46:** 104–108.

Cole, C. J., C. H. Lowe, and J. W. Wright. 1969. Sex chromosomes in teiid whiptail lizards (genus *Cnemidophorus*). *American Museum Novitates* **2395:** 1–14.

Cole, C. J., H. C. Dessauer, and G. F. Barrowclough. 1988. Hybrid origin of a unisexual species of whiptail lizard, *Cnemidophorus neomexicanus*, in western North America: new evidence and a review. *American Museum Novitates* **2905:** 1–38.

Cole, C. J., L. M. Hardy, H. C. Dessauer, H. L. Taylor, and C. R. Townsend. 2010. Laboratory hybridization among North American whiptail lizards, including *Aspidoscelis inornata arizonae* × *A. tigris marmorata* (Squamata: Teiidae), ancestors of unisexual clones in nature. *American Museum Novitates* **3698:** 1–43.

Dawley, R. M., and J. P. Bogart (eds.). 1989. Evolution and ecology of unisexual vertebrates. *New York State Museum Bulletin* **466:** 1–302.

Dessauer, H. C., and C. J. Cole. 1989. Diversity between and within nominal forms of unisexual teiid lizards, pp. 49–71. *In* Dawley, R. M., and J. P. Bogart, eds. *Evolution and Ecology of Unisexual Vertebrates.* New York, New York State Museum Bulletin 466.

Fritts, T. H. 1969. The systematics of the parthenogenetic lizards of the *Cnemidophorus cozumela* complex. *Copeia* **1969:** 519–535.

Frost, D. R., and D. M. Hillis. 1990. Species in concept and practices: herpetological applications. *Herpetologica* **46:** 87–104.

Frost, D. R., and J. W. Wright. 1988. The taxonomy of uniparental species, with special reference to parthenogenetic *Cnemidophorus* (Squamata: Teiidae). *Systematic Zoology* **37:** 200–209.

ICZN. 1999. *International Code of Zoological Nomenclature*. Fourth Edition London, The International Trust for Zoological Nomenclature.

James, E. 1823. *Account of an Expedition from Pittsburgh to the Rocky Mountains, Performed in the Years 1819 and '20*. Volume 2. Philadelphia, H. C. Carey and I. Lea.

Jombart, T., S. Devillard, and F. Balloux. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* **11:** 94.

Lowe, C. H., J. W. Wright, C. J. Cole, and R. L. Bezy. 1970a. Natural hybridization between the teiid lizards *Cnemidophorus sonorae* (parthenogenetic) and *Cnemidophorus tigris* (bisexual). *Systematic Zoology* **19:** 114–127.

Lowe, C. H., J. W. Wright, C. J. Cole, and R. L. Bezy. 1970b. Chromosomes and evolution of the species groups of *Cnemidophorus* (Reptilia: Teiidae). *Systematic Zoology* **19:** 128–141.

Lutes, A., W. B. Neaves, D. P. Baumann, W. Wiegraebe, and P. Baumann. 2010. Sister chromosome pairing maintains heterozygosity in parthenogenetic lizards. *Nature* **464:** 283–286.

Lutes, A., D. P. Baumann, W. B. Neaves, and P. Baumann. 2011. Laboratory synthesis of an inde-

pendently reproducing vertebrate species. *Proceedings of the National Academy of Sciences* **108:** 9910–9915.

Neaves, W. B. 1969. Adenosine deaminase phenotypes among sexual and parthenogenetic lizards in the genus *Cnemidophorus* (Teiidae). *Journal of Experimental Zoology* **171:** 175–183.

Neaves, W. B. 1971. Tetraploidy in a hybrid lizard of the genus *Cnemidophorus* (Teiidae). *Breviora* **381:** 1–25.

Neaves, W. B., and P. Baumann. 2011. Unisexual reproduction among vertebrates. *Trends in Genetics* **27:** 81–88.

Neaves, W. B., and P. S. Gerald. 1968. Lactate dehydrogenase isozymes in parthenogenetic teiid lizards (*Cnemidophorus*). *Science* **160:** 1004–1005.

Neaves, W. B., and P. S. Gerald. 1969. Gene dosage at the lactate dehydrogenase b locus in triploid and diploid teiid lizards. *Science* **164:** 557–559.

Neff, N. A., and L. F. Marcus. 1980. *A Survey of Multivariate Methods for Systematics.* New York, American Museum of Natural History.

Reeder, T. W., C. J. Cole, and H. C. Dessauer. 2002. Phylogenetic relationships of whiptail lizards of the genus *Cnemidophorus* (Squamata: Teiidae): a test of monophyly, reevaluation of karyotypic evolution, and review of hybrid origins. *American Museum Novitates* **3365:** 1–61.

Smith, H. M. 1946. *Handbook of Lizards.* Ithaca, New York, Comstock Publishing Co.

Tabachnick, B. G., and L. S. Fidell. 2013. *Using Multivariate Statistics*. 6th ed. Upper Saddle River, New Jersey, Pearson Education Inc.

Taylor, H. L., J. M. Walker, and P. A. Medica. 1967. Males of three normally parthenogenetic species of teiid lizards (genus *Cnemidophorus*). *Copeia* **1967:** 737–743.

Taylor, H. L., C. J. Cole, L. M. Hardy, H. C. Dessauer, C. R. Townsend, J. M. Walker, and J. E. Cordes. 2001. Natural hybridization between the teiid lizards *Cnemidophorus tesselatus* (parthenogenetic) and *C. tigris marmoratus* (bisexual): assessment of evolutionary alternatives. *American Museum Novitates* **3345:** 1–64.

Taylor, H. L., J. M. Walker, J. E. Cordes, and G. J. Manning. 2005. Application of the evolutionary species concept to parthenogenetic entities: comparison of postformational divergence in two clones of *Aspidoscelis tesselata* and between *Aspidoscelis cozumela* and *Aspidoscelis maslini* (Squamata: Teiidae). *Journal of Herpetology* **39:** 266–277.

Walker, J. M., H. L. Taylor, and T. P. Maslin. 1966. Evidence for specific recognition of the San Esteban Whiptail lizard (*Cnemidophorus estebanensis*). *Copeia* **1966:** 498–505.

Wright, J. W., and C. H. Lowe. 1967. Hybridization in nature between parthenogenetic and bisexual species of whiptail lizards (genus *Cnemidophorus*). *American Museum Novitates* **2286:** 1–36.