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# Hybridization Between the Endangered Unisexual Gray-Checkered Whiptail Lizard (*Aspidoscelis dixoni*) and the Bisexual Western Whiptail Lizard (*Aspidoscelis tigris*) in Southwestern New Mexico

CHARLES J. COLE,[1] CHARLES W. PAINTER,[2] HERBERT C. DESSAUER,[3] AND HARRY L. TAYLOR[4]

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[1] Division of Vertebrate Zoology (Herpetology), American Museum of Natural History (cole@amnh.org).
[2] Herpetologist, Endangered Species Program, New Mexico Department of Game and Fish, Santa Fe, NM 87504 (cpainter@state.nm.us).
[3] Division of Vertebrate Zoology (Herpetology), American Museum of Natural History; Department of Biochemistry and Molecular Biology, Louisiana State University Health Science Center, New Orleans, LA 70112.
[4] Division of Vertebrate Zoology (Herpetology), American Museum of Natural History, Department of Biology, Regis University, Denver CO 80221 (htaylor@regis.edu).

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## ABSTRACT

Hybridization between the unisexual *Aspidoscelis dixoni* and the bisexual *Aspidoscelis tigris punctilinealis* in southwestern New Mexico is documented by observations and analyses of external morphology (coloration, size, scalation), chromosomes (karyotypes), nuclear gene products (allozymes), and mitochondrial DNA. The locality (Hidalgo County, Antelope Pass of the Peloncillo Mountains, centered at 10.5 km west of Animas), consisting of only a few square kilometers, is the only place where this particular unisexual clone of *A. dixoni* exists. Because of its extreme rarity in recent years, *A. dixoni* has been listed as an Endangered Species in New Mexico, and the status of its populations has received intense study. Today, the cause(s) of endangerment remains unknown, although we hypothesize that interspecific competition may be the problem.

*Aspidoscelis dixoni* is a diploid unisexual species that normally reproduces by parthenogenetic cloning, as demonstrated here with genetic data from laboratory-reared lizards. However, fertilization of its eggs in Antelope Pass is possible if mating occurs with a male of the syntopic bisexual species *A. tigris punctilinealis*. The resulting hybrids closely resemble their maternal parent morphologically, but they are triploid and the females observed to date have been sterile.

*Aspidoscelis t. punctilinealis* is a recent invader of southwestern New Mexico. It is the dominant species of whiptail lizard today in the low-elevation, semiarid habitat of creosote desertscrub in Antelope Pass. The present rarity of *A. dixoni* in Antelope Pass, in contrast to its abundance a few decades ago, may result from negative interactions with this dominant species, including asymmetrical destabilizing hybridization.

Only a few other populations of *A. dixoni* are known to exist, each in a limited area in southwestern Texas, so there is a hiatus of nearly 500 km between the small and restricted populations in New Mexico and Texas. Comparative genetic data presented here indicate that although these populations are similar, the population in New Mexico represents a unique clone. It has three alleles at 3 nuclear gene loci (among 31 examined) that distinguish it from the Texan populations, and it lacks a microchromosome that occurs in Texan populations. In addition, in this paper we present new comparative genetic data confirming that the origin of *A. dixoni* itself was from a hybrid between an *A. tigris marmorata* ♀ × *A. gularis septemvittata* ♂, consistent with earlier studies.

## INTRODUCTION

The gray-checkered whiptail lizard, *Aspidoscelis dixoni* (Scudday, 1973), occurs in extremely limited areas in New Mexico and Texas, with population centers widely separated from each other (fig. 1). As its distribution in New Mexico is confined to a few square kilometers in Antelope Pass in the Peloncillo Mountains, centered about 10.5 km west of Animas, Hidalgo County (Degenhardt et al., 1996), and individuals were rarely seen in recent years, the New Mexico Department of Game and Fish (NMDGF) has listed it as an Endangered Species.

When first recognized as a distinctive entity or clone, *A. dixoni* was allocated to pattern class F of *Cnemidophorus tesselatus* (see Zweifel, 1965), known only from New Mexico. Later, Scudday (1973) named it *Cnemidophorus dixoni* (= *Aspidoscelis dixoni* following the generic allocation of Reeder et al., 2002), and he recognized two pattern classes, A and B, both occurring mostly in Texas. Most recently, Walker et al. (1994) and Cordes and Walker (2006) recognized the New

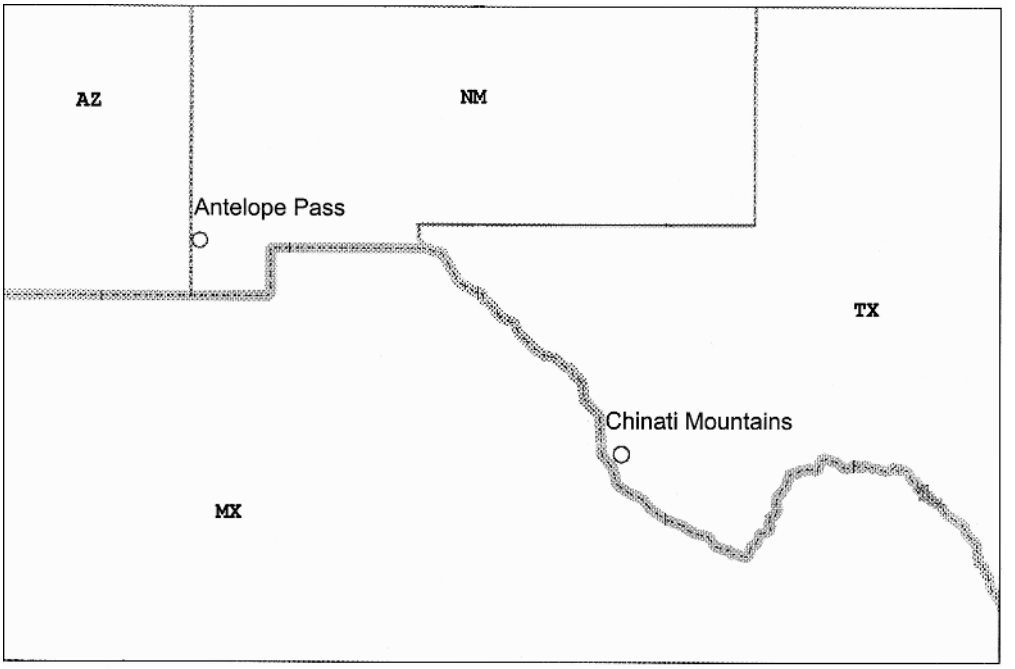


Fig. 1. The global distributional range of *Aspidoscelis dixoni*. This species occurs in two areas that are separated by approximately 500 km from which no specimens are known. The two areas are Antelope Pass, Peloncillo Mountains, Hidalgo County, New Mexico, and Chinati Mountains, Presidio County, Texas. Initialisms are as follows: AZ, Arizona; MX, Mexico; NM, New Mexico; and TX, Texas. Modified from Topo USA 2.0, DeLorme, Yarmouth, Maine (1999).

Mexican population as a distinctive form, which they referred to as *A. dixoni* pattern class C, or *A. dixoni* C, which, with its extremely limited global distribution consisting of only a few square kilometers in southwestern New Mexico, is one of the most endangered lizards in the world.

Owing to the extreme rarity of *A. dixoni* C in New Mexico recently, one of us (C.W.P.) and the NMDGF conducted extensive ecological and capture-mark-release-recapture studies largely to determine the status of this lizard, which had been thought by some herpetologists in the 1980s to be extinct (James R. Dixon and colleagues were unable to find any in several attempts, personal commun.). This intense field program (Sias and Painter, 2002) continued for seven years (1987–1993) and included a series of pit-fall traps in grids (without drift fences) and arrays (with drift fences) that extended across approximately 9 km of Antelope Pass. A total of 552 pit-fall traps in 31 grids and arrays were inspected every other day throughout the lizard activity season. During these 447,650 trap days, a total of 1,281 different individuals of the conspicuously abundant bisexual Arizona desert whiptail, *Aspidoscelis tigris punctilinealis* (see Taylor and Walker, 1996, for the use of this subspecific name) were captured in the study area. Although *A. dixoni* C previously was known to be reasonably abundant in the area (they were readily observed on any warm sunny day during lizard activity season in the sandy lower elevations of Antelope Pass in the 1960s; Zweifel, personal commun. and personal observations, C.J.C.), only 220 individuals of *A. dixoni* were found in the seven recent years of trapping (Sias and Painter, 2002). In addition, three of the lizards captured appeared morphologically similar to *A. dixoni* but with subtle aspects of color and pattern that suggested to the keen-eyed field assistants (Barney Tomberlin and Anthony Snell) that they might be hybrids between *A. dixoni* and *A. t. punctilinealis*, the only bisexual species of whiptail lizard found in Antelope Pass (addi-

tional unisexual species found there were *A. exsanguis* [extremely rare in Antelope Pass but abundant elsewhere], *A. sonorae* [extremely rare in Antelope Pass but abundant elsewhere], and *A. uniparens* [abundant in Antelope Pass and elsewhere]).

While we were studying these possible hybrids, several projects were underway that were relevant to this research: (1) C.J.C. was keeping two living female presumptive hybrids in the *Aspidoscelis* colony at the AMNH to determine whether they would lay eggs, and also some captive *A. dixoni* were reproducing there; (2) Dessauer et al. (2000) were studying extensive hybridization and gene flow in *A. t. punctilinealis* × *A. tigris marmorata* in passes immediately to the north of Antelope Pass, Peloncillo Mountains, Hidalgo County, New Mexico; (3) Taylor et al. (2001) were analyzing frequent production of sterile female hybrids between *A. tesselata* × *A. tigris marmorata* near Roswell, Chaves County, New Mexico; (4) Reeder et al. (2002) were determining that North American species of *Aspidoscelis* are not congeneric with South American species of *Cnemidophorus*; and (5) James E. Cordes (Louisiana State University at Eunice) kindly sent us some living individuals of *Aspidoscelis dixoni* of pattern classes A and B from Texas.

The specimens from James E. Cordes allowed us to conduct comparative genetic analyses (karyotypes, allozymes, mtDNA) of *A. dixoni* from throughout its known range. These data can now be understood in the context of the important comparative histocompatibility data recently published by Cordes and Walker (2006).

With all of the relevant studies listed above completed, we can now address the following questions in this report: (1) Were the presumptive hybrids of *A. dixoni* C × *A. t. punctilinealis* from New Mexico actually such hybrids? (2) If so, were the female hybrids sterile or could they clone themselves? (3) Do nonhybrid individuals of *A. dixoni* clone themselves in captivity? (4) Do the new data confirm that *A. dixoni* itself is of hybrid origin, and if so, are its ancestral species probably the same taxa that were proposed in earlier reports? (5) What is the genetic relationship between the New Mexican and Texan populations of *A. dixoni* and between both of these and *A. tesselata* (see Walker et al., 1997, for use of this specific name)? (6) Is it possible that recent competition and destabilizing hybridization with *A. t. punctilinealis* are the causes of endangerment of *A. dixoni* C in New Mexico, the only place where this clone exists?

## MATERIALS AND METHODS

### Fieldwork

Pit-fall traps, some with and some without drift fences to guide lizards into the traps, were used to capture animals alive (Sias and Painter, 2002; fig. 2 here; traps were modified from Campbell and Christman, 1982, and Jones, 1986). Trapping stations in Antelope Pass, Peloncillo Mountains, centered approximately 10.5 km west of Animas, Hidalgo County, New Mexico, were established for varying periods of time at 31 different local sites within habitat thought to have been available to *A. dixoni*. Traps were open continuously between April 1 and October 15 each year from 1987 to 1993, and each trap was checked every other day during this period, for a grand total of 447,650 trap days. Lizards that were captured were taken to the laboratory for recording the following information: (1) weight (to the nearest 0.5 g with spring scales in 1987–1988, then to the nearest 0.05 g with an electronic balance in 1989–1993); (2) snout-vent length (to the nearest millimeter with a rule); and (3) sex and reproductive condition, when possible. Most individuals were uniquely toe-clipped and released at the capture site within 48 hr, although some were preserved for later study; presumptive hybrids and a few nonhybrids of *A. dixoni* C were maintained in captivity to determine whether they would reproduce and for examination of coloration, karyotypes, allozymes, mitochondrial DNA, and scalation (see below). See appendix 1 for a list of the specimens examined.

### Captive Maintenance

Lizards were maintained in captivity by methods that have been described elsewhere (Cole and Townsend, 1977; Townsend, 1979; Townsend and Cole, 1985). Nevertheless,

Fig. 2. Cherry array with drift fence and pitfall traps, where one of the hybrids was found, Antelope Pass, Peloncillo Mountains, Hidalgo County, New Mexico. Photo by C.J.C., June 8, 1990.

some problems remain to be solved to maximize success in captive maintenance (Porter et al., 1994).

### Karyotype Analysis

We used previously published methods (sodium citrate cell suspension, methanol and glacial acetic acid fixation, flame drying) for preparing and studying standard, giemsa-stained chromosomes (Cole, 1979). For *A. dixoni* C from New Mexico, we examined 21 cells at mitotic metaphase from bone marrow of three females. For *A. dixoni* A from Texas, we examined 26 cells at mitotic metaphase from bone marrow of three females captured in the field by James E. Cordes and two laboratory-reared offspring from two additional adults. For *A. tigris*, we have examined dozens of specimens from widespread localities representing many subspecies, including an individual from Antelope Pass, all of which are karyotypically identical to each other (e.g., Lowe et al., 1970b; Dessauer et al., 2000; Taylor et al., 2001). We also examined 12 cells at mitotic metaphase from bone marrow of one female hybrid of *A. dixoni* C × *A. t. punctilinealis* from New Mexico. Individuals karyotyped are specified in Specimens Examined (see appendix 1).

### Allozymes and DNA

Genotypes at 31 nuclear gene loci, based on phenotypes of tissue protein activities on starch gels following electrophoresis, were determined for five wild-caught and three laboratory-reared specimens (all females) of *A. dixoni* C and two specimens of presumptive hybrids of *A. dixoni* C × *A. t. punctilinealis*. Five nonhybrid *A. t. punctilinealis* from Antelope Pass were examined also, and their genetic data were consistent with data from those *punctilinealis* that were among the 607 specimens of *A. tigris* analyzed from additional sites nearby (Dessauer et al., 2000). Three

specimens of *A. dixoni* pattern class A and two of B from Texas were also compared with these specimens, and all lizards analyzed are listed in the Specimens Examined (see appendix 1).

Methods of collecting and storing tissues followed Dessauer et al. (1996a). Methods of preparing homogenates, conducting electrophoresis, 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). Dessauer et al. (1996b) used allele-specific oligonucleotide probes to determine haplotypes of mitochondrial DNA (mtDNA) present in certain individuals in order to confirm maternity of hybrids and of clonal species of hybrid origin.

### Morphology

Photographs and notes on colors and pattern were recorded prior to preservation of the lizards. Characters of size and scalation were noted as discussed by Cole et al. (1988) and Dessauer et al. (2000). Descriptive univariate statistics of the eight useful characters are provided later, in table 3.

Seven meristic characters for each individual were used in a principal components analysis (PCA) to generate scores extracted from the correlation matrix. This depicted the pattern of morphological variation without a priori identification of specimens to group. We then used our samples of *A. dixoni* C and *A. t. punctilinealis* as a priori groups and a step-wise selection of meristic characters (*F* probabilities of 0.15 for character entry and 0.20 for character removal; Costanza and Afifi, 1979) in a discriminant analysis (DA) to identify which parental taxon the hybrids (entered in the model as unclassified) most closely resembled. Finally, a canonical variate analysis (CVA) was run, using the same step-wise, character selection procedure and samples of *A. dixoni* C, *A. t. punctilinealis*, and the hybrids as three a priori groups for maximally discriminating among the groups. We used SPSS 14.0 (from SPSS, Inc., Chicago, Illinois) and SYSTAT 11.0 (from SYSTAT Software, Inc., Richmond, California) for statistical analyses.

## RESULTS AND DISCUSSION

### Coloration and Identification of the Hybrids

Dorsally and laterally, *A. dixoni* C has a ground color of small dark brown or black squares and rectangles separated by light stripes and cross bars in a checkered pattern (Degenhardt et al., 1996). Anteriorly, the light stripes and bars are grayish tan, but gradually they become a brighter tan or cream to yellow posteriorly. An orange-brown wash may be present on the posterior body (fig. 3). Ventrally, the throat is light tan with a slight touch of orange and without dark spots. The chest is paler than the throat, with a few small black dots. There is a distinct tan color posterolaterally on the abdomen, but the belly is cream or white without black. The color beneath the arms, legs, and tail is pale tan.

Dorsally and laterally, *A. t. punctilinealis* has a brown to black ground color with grayish tan light spots and usually four similarly colored stripes. The dorsum is not a checkered pattern (fig. 3). Laterally, individuals of this taxon are black with cream spots (light gray anteriorly), which may tend to coalesce into vertical rows, but the sides are not barred as typically they are in the subspecies *A. t. marmorata*. Ventrally, the throat and chest are black, which extends well onto the anterior abdomen (fig. 4). The light parts of the abdomen, especially posterolaterally, are white, not tan. The color beneath the arms is black, beneath the legs, light gray with darker gray smudges, and beneath the tail, gray.

→

Fig. 3. Dorsolateral views of three whiptail lizards (*Aspidoscelis*). **Upper**, diploid unisexual *A. dixoni* C from Antelope Pass (AMNH R-148360, body length 96 mm). **Middle**, triploid female hybrid of *A. dixoni* C × *A. tigris punctilinealis* from Antelope Pass (AMNH R-148141, body length 93 mm). **Lower**, diploid bisexual *A. t. punctilinealis* male from Antelope Pass (AMNH R-148113, body length 90 mm).

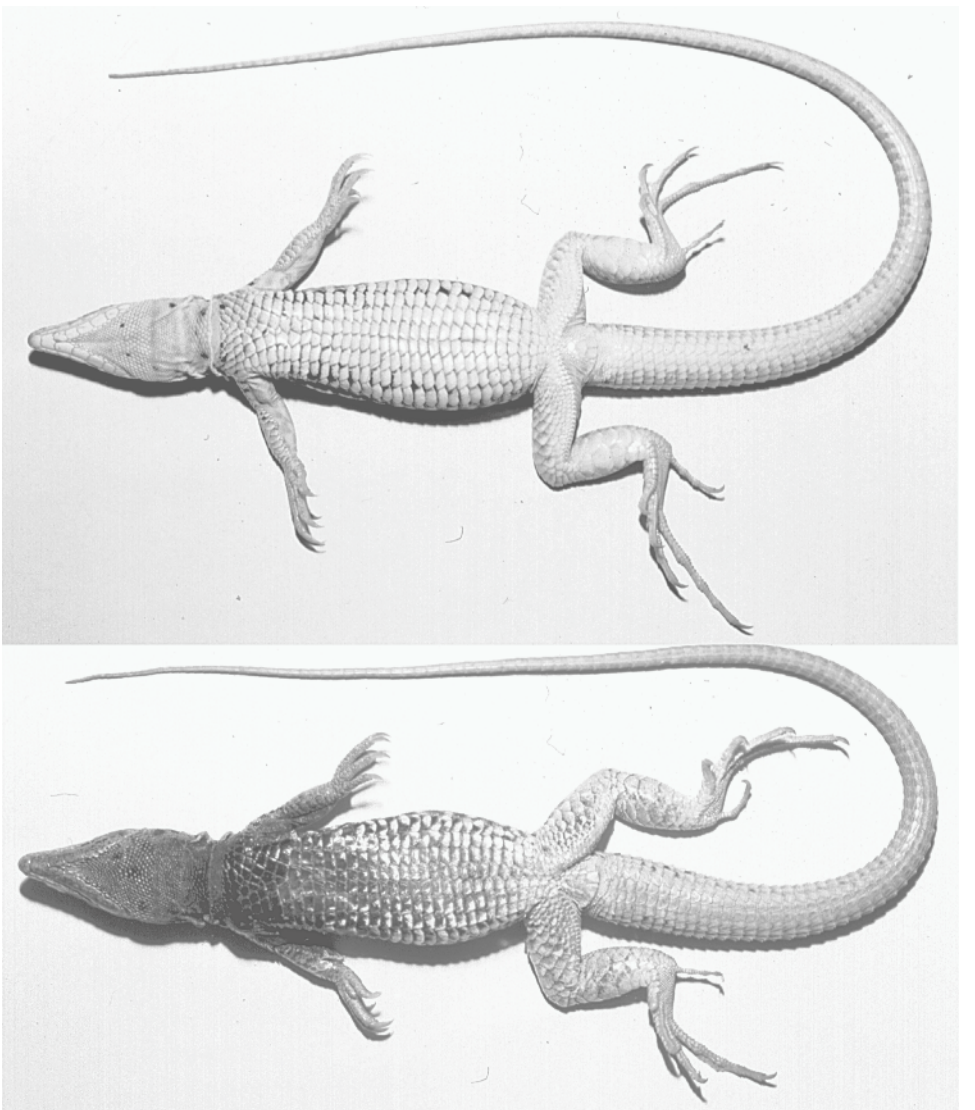

Fig. 4. Ventral views (black-and-white) of two of the same lizards shown in figure 3. **Upper**, hybrid (AMNH R-148141); **lower**, *A. t. punctilinealis* (AMNH R-148113). In *A. dixoni* C (no photograph of ventral view) there is no black on the abdomen or throat, although there may be a few small black dots on the chest.

Color notes were taken from one female presumed (and later verified) hybrid of *A. dixoni* C × *A. t. punctilinealis* (figs. 3, 4). Dorsally she had six light stripes and spots (grayish tan anteriorly, deeper tan posteriorly) on the black ground color. Laterally, some of the light bars were broken into spots. Ventrally, the throat was pale orange with a few round black dots. The chest was light orange with a few black spots. The abdomen was cream with a trace of tan posterolaterally and a few black spots laterally. The color beneath the arms was light orangish tan with light gray mottling; beneath the legs and tail was light tan.

Clearly, the hybrids are most similar to *A. dixoni* C, but they have some coloration characters that are intermediate between the

TABLE 1
**Colors and Patterns for Distinguishing Among *Aspidoscelis dixoni* C, *A. t. punctilinealis*, and Their Hybrids**

| Character | *A. dixoni* C | Hybrids | *A. t. punctilinealis* |
|---|---|---|---|
| Dorsal pattern | checkered | 6 pale stripes | usually 4 pale stripes |
| Lateral pattern | checkered | light bars/spots | pale spots, some coalescing |
| Posterior dorsal body | usually with orange-brown wash | no wash | no wash |
| Throat color | light tan, touch of orange | pale orange | black |
| Black spots on throat? | usually not | a few black dots | throat all black |
| Chest color | as throat but paler | light orange | black |
| Black spots on chest? | a few black dots | a few black spots | chest all black |
| Abdomen pale color | cream with tan posterolaterally | cream, trace of tan posterolaterally | white, including posterolaterally |
| Abdomen with black? | no | a few spots | many spots |
| Beneath arms | pale tan | light orangish tan, light gray mottling | black |
| Beneath legs | pale tan | pale tan | light gray with darker gray smudges |
| Beneath tail | pale tan | pale tan | gray |

two parental types (figs. 3, 4; table 1). In particular, their sides are less regularly checkered than in *A. dixoni*, their pale orange throat has round black dots, their chest has larger black spots, and their abdomen has only a trace of tan posterolaterally plus a few black spots.

## Karyotypes

Individuals of *Aspidoscelis dixoni* A from Texas have a karyotype with a diploid number of 47 chromosomes consisting of two rather different haploid genomes that reflect its own origin by hybridization. As with other clones in the *A. tesselata* complex, the hybrid origin of the unisexual *A. dixoni* was apparently of *A. t. marmorata* ♀ × *A. gularis septemvittata* ♂ (Neaves, 1969; Parker and Selander, 1976; Densmore et al., 1989; Dessauer and Cole, 1989; Dessauer et al., 1996b). The karyotype of *A. dixoni* A is modified from the ancestral $F_1$ hybrid condition in one respect (see below), although, as seen in most clones of *A. tesselata* (see Dessauer and Cole, 1989: 57; Taylor et al., 2001: 24), it is close to the original hybrid state.

The haploid set of chromosomes originally inherited from the maternal *A. t. marmorata* ($n = 23$) includes three large set I biarmed macrochromosomes + eight smaller set II biarmed intermediate-sized macrochromosomes + 12 set III microchromosomes (Lowe et al., 1970b; Taylor et al., 2001). The second largest chromosome in set I of the ancestral *A. tigris* has a dotlike satellite on the end of one arm, set off by the nucleolar organizer region (Ward and Cole, 1986), which is often difficult to see, and the third largest chromosome is the sex chromosome (Cole et al., 1969; Bull, 1978). In both *A. dixoni* A and C this haploid complement has been modified in that the X chromosome has apparently undergone centric fission and is now represented by two smaller set II telocentric macrochromosomes, as found in some clones of *A. tesselata* (see Taylor et al., 2001). Consequently, the pattern of macro- and microchromosomes in the modified ancestral *tigris* genome is 2 + 10 + 12 instead of the expected 3 + 8 + 12.

In *A. dixoni* also, the haploid set of chromosomes originally inherited from the paternal *A. g. septemvittata* ($n = 23$) includes one large set I metacentric macrochromosome (which has a subterminal secondary constriction [nucleolar organizer; Ward and Cole, 1986] on one arm followed by an elongate satellite that is at least twice the size of the satellite in *A. t. marmorata*) +12 smaller set II intermediate-sized telocentric or subtelocentric macrochromosomes + 10 set III microchromosomes (fig. 5). However, in *A. dixoni* C from New Mexico, one of the microchromosomes (possibly one from either parent) is apparently missing (for illustrative purposes, shown here as missing from the

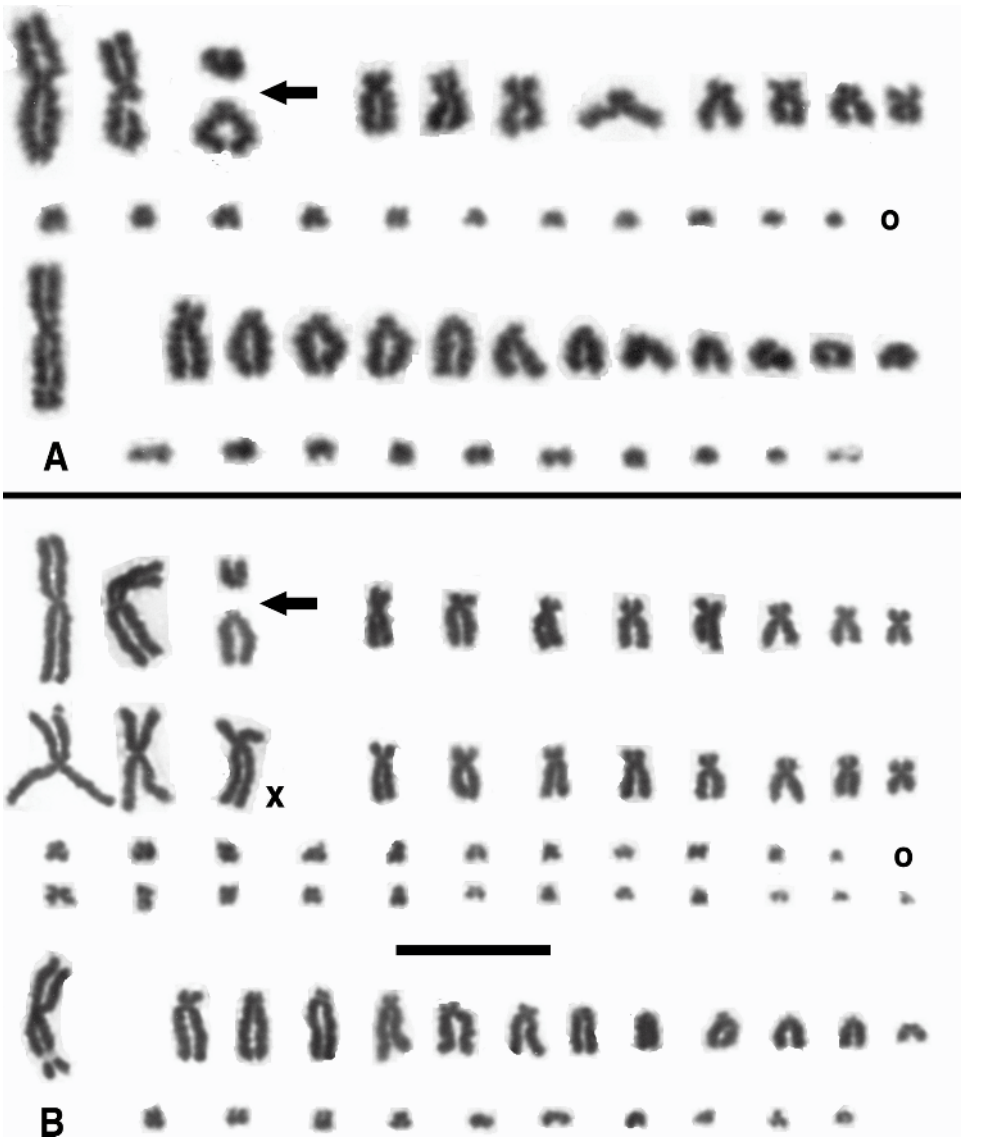


Fig. 5. Karyotypes of two whiptail lizards (*Aspidoscelis*) from Antelope Pass, Peloncillo Mountains, Hidalgo County, New Mexico. **A**, Diploid unisexual *A. dixoni* C (AMNH R-148360, fig. 3), with $2n = 46$ chromosomes. This taxon is a clone with its ultimate ancestor having been an $F_1$ hybrid between *A. tigris marmorata* (haploid complement of chromosomes in the upper two rows) × *A. gularis septemvittata* (haploid complement in the lower two rows of **A**). Note that the X chromosome that was ultimately inherited from *marmorata* (third largest, upper row) was apparently fissioned at the centromere (arrow) forming two additional set II-sized macrochromosomes (here arranged to suggest the ancestral unfissioned state in *marmorata*). Note also that the missing microchromosome of *A. dixoni* C is illustrated as missing from the *marmorata* genome (o at the right end of the second row), although we do not know from which genome it was actually lost. **B**, Triploid hybrid female of *A. dixoni* C × *A. t. punctilinealis* (AMNH R-148141, fig. 3), with $3n = 69$ chromosomes arranged in six rows. Rows 2 and 4 represent the haploid complement most recently inherited from *A. t. punctilinealis*, including its intact X chromosome (third largest). Rows 1, 3, 5, and 6 represent the two haploid complements inherited from *A. dixoni* C, including the fissioned X (arrow) chromosome and missing microchromosome (o), as in **A**, above. Bar represents 10 μm.

*marmorata* complement; fig. 5). In contrast, the full ancestral complement of 22 microchromosomes is present in *A. dixoni* A from Texas. In addition, the two laboratory-hatched offspring of *A. dixoni* A had the same karyotype, comprised of unpaired sets of chromosomes, consistent with clonal inheritance in the laboratory.

In summary, the modified diploid karyotype of *A. dixoni* C consists of 46 chromosomes, with 3 large biarmed macrochromosomes + 22 smaller macrochromosomes (2 being the fissioned X chromosome) + 21 microchromosomes that were originally inherited as two morphologically different haploid genomes (fig. 5). Considering the fissioned ancestral X chromosome from *A. t. marmorata* and the absence of one of the ancestral microchromosomes, the three specimens of *A. dixoni* C examined had a unique karyotype, and this remained constant in samples collected over the years spanning 1966–1987.

The karyotype of *A. tigris punctilinealis* is identical to that of *A. t. marmorata*, with $n = 23$ chromosomes (Cole et al., 1969; Lowe et al., 1970b; Dessauer et al., 2000). Consequently, a hybrid between *A. dixoni* C and *A. t. punctilinealis* would be predicted to have $3n = 69$ chromosomes, including the cloned diploid set from the maternal *A. dixoni* C and the additional haploid set from the paternal *A. t. punctilinealis*. The suspected hybrid female karyotyped was, in fact, a triploid with precisely the combination of chromosomes expected from an offspring of *A. dixoni* C × *A. t. punctilinealis* (fig. 5), having 6 large set I biarmed macrochromosomes + 30 smaller set II macrochromosomes (2 being the fissioned X chromosome from *dixoni*) + 33 set III microchromosomes.

## Reproduction in the Laboratory

The two hybrid individuals of *A. dixoni* C × *A. t. punctilinealis* maintained alive in captivity were of adult size when preserved (93 and 104 mm in snout-vent length). One of the hybrids lived in good health in captivity for nearly 23 months and the other for more than 29 months, but neither ever appeared gravid nor did they lay any eggs during this period. In contrast, one *A. dixoni* C from New Mexico that was maintained alive in captivity laid a clutch of six eggs over a captive period of 8.5 months, although none hatched. Another individual of *A. dixoni* C from New Mexico laid six clutches of eggs ($N = 5$–6 per clutch for a total of 33 eggs) over a period of 26 months, of which three eggs hatched. In addition, individuals of *A. dixoni* from Texas also reproduced in captivity.

Four $P_1$ generation (field-captured) lizards of *A. dixoni* A from Texas produced 22 clutches ($N = 2$–6 per clutch for a total of 69 eggs, of which 23 hatched) over a period of up to 46 months per lizard; six $F_1$ lizards produced 28 clutches ($N = 1$–5 per clutch for a total of 96 eggs, of which 28 hatched) over a period of up to 24 months per lizard; and five $F_2$ lizards produced 7 clutches ($N = 1$–4 per clutch for a total of 18 eggs, of which none hatched) over a period of up to 10 months per lizard.

We conclude that the triploid hybrid females were not capable of parthenogenetic cloning, although their mothers were. This is consistent with the report of Taylor et al. (2001) concerning hybridization between *A. tesselata* ♀ × *A. t. marmorata* ♂ from the vicinity of Roswell, Chaves County, New Mexico, for which a larger number of sterile triploid female hybrids was analyzed. As in that study, triploid hybrids between *A. dixoni* C and *A. t. punctilinealis* theoretically could be either male or female, depending on whether fertilization was by a spermatozoan bearing the X chromosome or the Y chromosome. The hybrid sex ratio should be 50:50, and it was (2 females:1 male).

Finally, although the Texan individuals of *A. dixoni* produced more offspring than the New Mexican lizards in captivity, one must not read too much into this comparison. Reproduction in captive *Aspidoscelis* is negatively affected by aspects of the captive environment in complex ways that are not fully understood (Townsend and Cole, 1985; Porter et al., 1994).

## Biochemical Genetics

Protein electrophoresis of allele products representing 31 nuclear gene loci plus ASO dot-blot tests identifying the haplotype of mtDNA (table 2) provide extensive genetic

TABLE 2
**Genotypes or Alleles[a] at 32 Gene Loci[b] in Samples of *Aspidoscelis*[c]**

| Locus | MAR | SEP | DIX C[d] | HYB | PUN | TES | DIX AB | BUR |
|---|---|---|---|---|---|---|---|---|
| *Oxidoreductases* | | | | | | | | |
| ADH | a | b | ab | aab | a | ab | ab | a |
| IDDH | b,a | a | aa | aab | b | aa | aa | a |
| LDH1 | a | b | ab | aab | a | ab | ab | b |
| sMDH | a | b[e] | a**c**[e] | aac | a | ab | ab | b,c |
| sMDHP | a | b | ab | abb | b | ab | ab | c |
| sSOD | b | a | ab | abb | b | ab | ab | a |
| *Transferases* | | | | | | | | |
| sAAT | a | b | ab | aab | a | ab | ab | a |
| mAAT | b | a | ab | abb | b | ab | ab | a |
| *Hydrolases* | | | | | | | | |
| EST2 | b | c | bc | bcc | c | bc | bc | c |
| PEPA | b,c | c,b,d | cd | bcd | b | cd | **d**d | c,b |
| PEPB | b | b | bb | abb | a | bb | bb | a,b |
| PEPD | c | c | cc | acc | a | cc | cc | d,c |
| ADA | a | c,b | ac | aac | a | ac | ac | b,a |
| *Lyase* | | | | | | | | |
| sACOH | c | b | bc | bcc | c | bc | bc | c,d,b |
| *Isomerases* | | | | | | | | |
| MPI | a | b | a**a** | aaa | a | ab | ab | b,c |
| GPI | c,a | c | ac | acc | d,c | ac | ac | c |
| PGM2 | a | b | ab | aab | a | ab | ab | b |
| PGM3 | a | c,b | ab | aab | a | ab | ab | c,d |
| *Blood proteins* | | | | | | | | |
| TF | b | b | bb | bbc | c | bb | bb[f] | b |
| ALB | a | b | ab | aab | a | ab | ab[f] | c |
| *mtDNA* | | | | | | | | |
| ASO | M | — | M | M | P | M[g] | M[f] | — |

[a] Genotypes are presented for the hybrids of *A. dixoni* × *A. tigris punctilinealis* (HYB) and for unisexual taxa (DIX C, TES, and DIX A and B), as these did not vary from individual to individual. For bisexual taxa (MAR, SEP, PUN, and BUR) only the most common alleles are listed, in order from the highest frequency to the lowest. If no a-allele is shown (e.g., EST2) we have found it in species of *Aspidoscelis* that are not included here.

[b] Alleles are designated in alphabetical order according to decreasing anodal migration of their allozymes. For multilocus enzymes, loci are numbered in order of decreasing anodal migration of their isozymes. Methods follow Harris and Hopkinson (1976), Murphy et al. (1996), and Dessauer et al. (2000; EST2 in particular); s, cytosolic enzyme; m, mitochondrial enzyme. Abbreviations for loci are as follows: ADH, alcohol dehydrogenase; IDDH, L-iditol dehydrogenase; LDH, L-lactate dehydrogenase; MDH, malate dehydrogenase; MDHP, malate enzyme; SOD, superoxide dismutase; AAT, aspartate aminotransferase; EST, esterase; PEP, peptidase; ADA, adenosine deaminase; ACOH, aconitase hydratase; MPI, mannose-6-phosphate isomerase; GPI, glucose-6-phosphate isomerase; PGM, phosphoglucomutase; TF, transferrin; ALB, albumin; and ASO, allele-specific oligonucleotide of mitochondrial DNA (mtDNA; M, *A. tigris marmorata*; P, *A. tigris punctilinealis*). Boldface alleles in *A. dixoni* at sMDH, PEPA, and MPI are distinctive in one way or another (see text). The following 11 loci were invariant (i.e., all individuals possessed one and the same allele in the homozygous state): G3PDH (glycerol-3-phosphate dehydrogenase, except for some variation found within *A. burti*); LDH2; mMDH; s and m IDH (isocitrate dehydrogenase); mSOD; CK1 (creatine kinase); AK (adenylate kinase); ESTD (except for some variation found within *A. burti*); PEPE (except for some variation found within *A. burti*); and HB (hemoglobin, except that this was not tested for *A. dixoni* class B from Texas).

[c] Abbreviations for taxa and individuals are as follows: MAR, *A. tigris marmorata*; SEP, *A. gularis septemvittata*; DIX C, *A. dixoni* from New Mexico; HYB, hybrids of *A. dixoni* from New Mexico × *A. tigris punctilinealis* (PUN); TES, *A.*

TABLE 2
*(Continued)*

*tesselata* pattern class E-C from Macho Draw, NM (see Taylor et al., 2001, 2003); DIX AB, *A. dixoni* from Texas (class A and class B individuals were analyzed and they were identical at all loci, but individuals of class B could not be tested for TF, ALB, and ASO); BUR, *A. burti burti* and *A. b. stictogramma*.

[d] The data for DIX C include several field-captured individuals plus three offspring produced by one of them in the laboratory (see Specimens Examined, appendix 1). As all individuals were identical, with the heterozygosities at all loci maintained from mother to offspring, this constitutes evidence of clonal inheritance in *A. dixoni*.

[e] At sMDH, we have found the c-allele in some individuals of *A. gularis gularis* also, so it would not be surprising to discover that it also occurs in *A. g. septemvittata*.

[f] We did not have the opportunity to record data for four loci in individuals of *A. dixoni* class B from Texas: TF, ALB, ASO, and HB, the last of which was invariant in all of the other lizards.

[g] We did not test ASO for individuals of TES E-C from Macho Draw, but all other specimens of TES (all pattern classes) tested have had M (Dessauer et al., 1996b), so this is a reasonable assumption for these also.

data for comparing specimens of *A. dixoni* of all three pattern classes, its close relatives, offspring, and hybrids involving *A. dixoni*. Alleles representing heterozygous phenotypes usually are expressed equally, with banding patterns clearly indicating ploidy and the dosage of each allele (Neaves, 1969; Neaves and Gerald, 1969; Dessauer and Cole, 1984). Occasionally, however, phenotypes for parthenogens of hybrid origin suggest that one allele at a polymorphic locus is more active than another at the same locus, as discussed for PEPA by Taylor et al. (2001). Scoring such loci can be clarified by examining control gels using homogenates of different tissues on which samples from both the parthenogen and hybrids are compared side by side.

The biochemical data (table 2) are based on six specimens of *A. dixoni* C from Antelope Pass, New Mexico, and three offspring produced by one of them in the laboratory; five *A. dixoni* (classes A and B) from Texas; six *A. t. punctilinealis* from Antelope Pass; two hybrids of *A. dixoni* C × *A. t. punctilinealis*; 11 *A. tesselata* E-C from Macho Draw, New Mexico (Taylor et al., 2001); 15 *A. g. septemvittata*; 4 *A. b. burti*; 20 *A. burti stictogramma*; and more than 600 additional *A. tigris* from the Animas Valley and vicinity of the Peloncillo Mountains, New Mexico, where *A. t. punctilinealis* and *A. t. marmorata* interbreed (Dessauer et al., 2000). These data provide evidence bearing on the following questions: (1) Is *A. dixoni* C of hybrid origin, consistent with earlier studies, and if so, what are its ancestral species? (2) Does *A. dixoni* reproduce by parthenogenetic cloning? (3) Are the presumptive hybrids between *A. dixoni* C × *A. tigris punctilinealis* from New Mexico actually such hybrids? (4) What is the genetic relationship between the New Mexican and Texan populations of *A. dixoni* and between these and *A. tesselata*?

Is *A. dixoni* C of Hybrid Origin, and, if so, What Are Its Ancestral Species?: For the 31 protein loci analyzed (table 2), all of the specimens of *A. dixoni* C from New Mexico had alleles in the heterozygous state at 15 loci, or 48%. This very high level of heterozygosity, compared with about 5% in nonhybrids of bisexual taxa of *Aspidoscelis* (Parker and Selander, 1976; Dessauer and Cole, 1989), clearly indicates that *A. dixoni* had a hybrid origin. In addition, the data (table 2) are consistent with the ancestry of *A. dixoni* C being the result of hybridization between an *A. t. marmorata* ♀ × *A. g. septemvittata* ♂, similar to that of the diploid parthenogen, *Aspidoscelis tesselata* (Neaves, 1969; Parker and Selander, 1976; Dessauer and Cole, 1989; Densmore et al., 1989; see below).

There have been few genetic data directly comparing *A. tesselata* and *A. dixoni* C published to date, so conclusions concerning their close relationship have been based mostly on morphology (Zweifel, 1965; Scudday, 1973), until very recently. The few data published on allozymes, for about five loci, are consistent with concluding that there is a close relationship between these taxa (Neaves, 1969). In addition, mtDNA of *A. dixoni* (Densmore et al., 1989; Dessauer et al., 1996b) clearly implicates *A. t. marmorata* as its maternal parent. Most important of all, a recent report on histocompatibility among all color pattern classes of *A. dixoni* and between

these and *A. tesselata* most strongly suggests that *dixoni* and *tesselata* share as a common ancestor one $F_1$ hybrid female that cloned herself (Cordes and Walker, 2006) and from which the pattern classes and other genetically variant clones differentiated owing to postformational divergence. The allozyme data presented here (table 2) are consistent with these data, indicating that the origin of *A. dixoni* was from a hybrid between *A. t. marmorata* ♀ × *A. g. septemvittata* ♂.

We compared proteins representing 31 nuclear gene loci for *A. dixoni* C from New Mexico, *A. t. marmorata*, and *A. g. septemvittata* (table 2). For 29 of these loci, *A. dixoni* C is consistent with having had a hybrid origin with one gamete from *A. t. marmorata* and one from *A. g. septemvittata*, which is consistent also with its high heterozygosity. Of the 35 individual alleles identified in New Mexican *dixoni*, 33 (94%) were found in *marmorata* and *septemvittata*. The two exceptional alleles were the c-allele at sMDH and the second a-allele at MPI (fig. 6C,D). Interestingly, at both the sMDH and MPI loci, the Texan *dixoni* (pattern classes A and B) differed from the New Mexican ones and the Texan samples had the expected hybrid genotype (*A. t. marmorata* × *A. g. septemvittata*) at both of these loci (table 2). Consequently, the allozymes of the Texan *dixoni* A and B more closely represent a perfect $F_1$ hybrid, with but one exceptional allele. That is the second d-allele at PEPA in the Texan *dixoni* (fig. 6B). Considering that the histocompatibility data strongly indicate that the New Mexican and Texan *dixoni* share one individual ancestral hybrid, and they share this with *A. tesselata* as well (Cordes and Walker, 2006), we conclude that these allelic differences reflected in allozymes are a result of postformational events, similar to those discussed by Taylor et al. (2003) for various clones of *A. tesselata*. For New Mexican *dixoni* C, the second a-allele at MPI could have stemmed from biased gene conversion (Hillis et al., 1991). The unexpected c-allele at sMDH in New Mexican *dixoni* C could have resulted from a postformational point mutation, and it is interesting that we have seen evidence for this same allele in some individuals of *A. g. gularis*, a close relative of *A. g. septemvittata*.

Early in this investigation, H.C.D. discovered the unexpected c-allele at sMDH in New Mexican *A. dixoni* and realized that the New Mexican and Texan specimens differed at this locus. Initially, we wondered whether these populations could have had separate hybrid origins, and if so, which species might be a candidate for the paternal ancestor for New Mexican *A. dixoni* C. Consequently, we added *Aspidoscelis burti stictogramma* to the comparisons because it is another large, spotted species of the *sexlineata*-group (Lowe et al., 1970b; Reeder et al., 2002) that occurs in southwestern New Mexico within 65 km (to the south) of Antelope Pass. Could *A. dixoni* C have originated through past hybridization between *A. t. marmorata* ♀ × *A. b. stictogramma* ♂? This comparison ruled out *A. b. stictogramma* as a likely ancestor of New Mexican *A. dixoni*, because eight loci were mismatched (table 2; ADH, sMDHP, sAAT, PEPA, ADA, MPI, PGM3, and ALB), although the c-allele occurs at sMDH in *burti*. Therefore, consistent with the histocompatibility data (Cordes and Walker, 2006), the allozyme data indicate that the ancestor of *A. dixoni* (all three pattern classes) ultimately was a hybrid between *A. tigris marmorata* ♀ × *A. gularis septemvittata* ♂.

The haplotype of mitochondrial DNA in two of the specimens of *A. dixoni* C from New Mexico, the triploid hybrid of *A. dixoni* C × *A. t. punctilinealis* and two of the *A. dixoni* A from Texas was determined by Dessauer et al. (1996b). All of these specimens had the mtDNA of *A. t. marmorata* (table 2), as expected, owing to the maternal inheritance of mtDNA, because ultimately *A. t. marmorata* was the maternal ancestor of the clonal *A. dixoni* in its hybrid origin (*A. t. marmorata* ♀× *A. g. septemvittata* ♂), and the mtDNA of *A. dixoni* was inherited by the recent hybrids with *punctilinealis*.

Does *A. dixoni* Reproduce by Parthenogenetic Cloning?: Table 2 includes protein data from a field-captured adult *A. dixoni* C from New Mexico (AMNH R-148360, see Specimens Examined, appendix 1) and three offspring that she produced in the *Aspidoscelis* colony at the American Museum of Natural History (AMNH R-148364–66). The mother and all of her daughters (all from the same

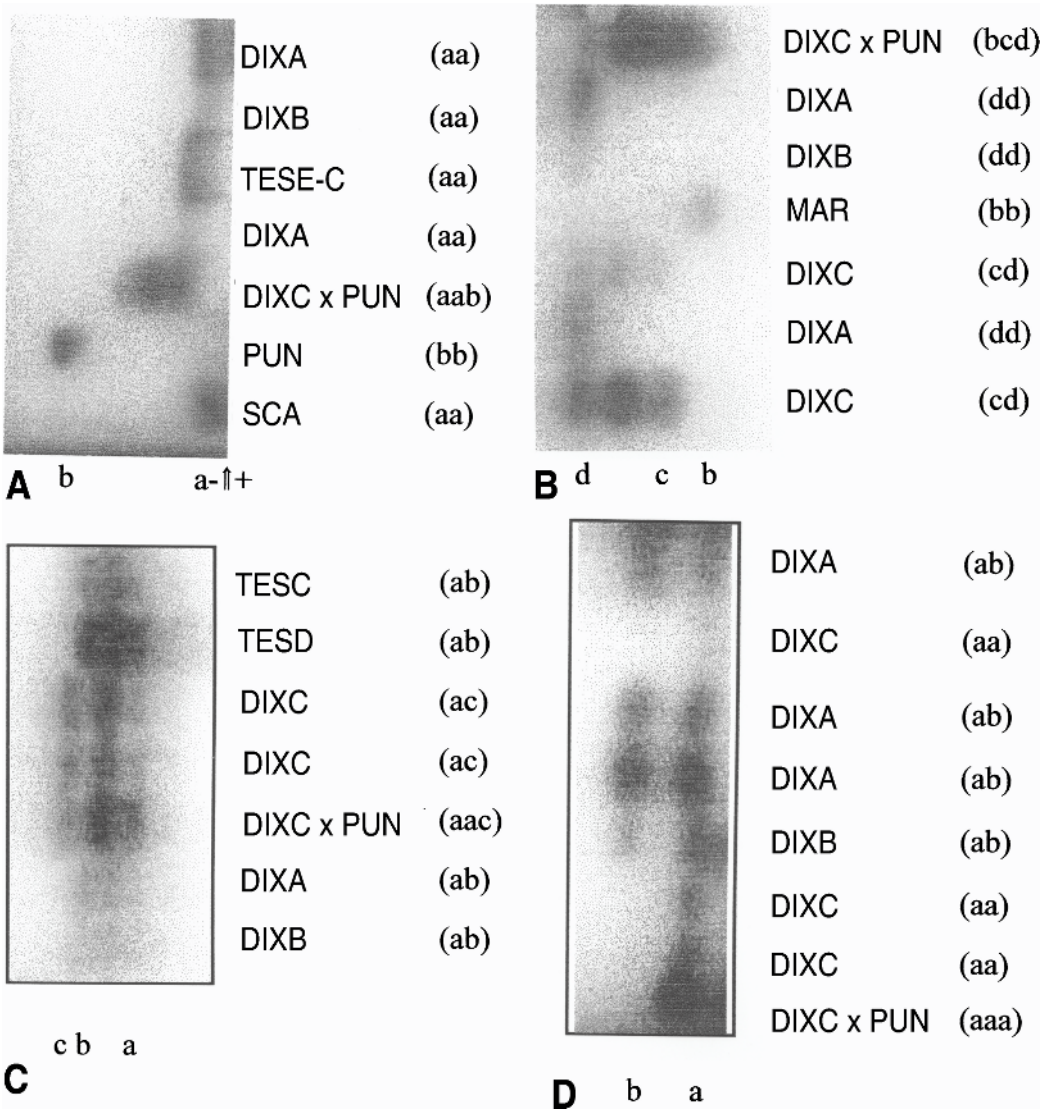


Fig. 6. Electrophoretic phenotypes representing products of four gene loci of *Aspidoscelis*. **A**, IDDH, a tetrameric enzyme, from liver homogenates of seven lizards. Note that the b-allele from PUN is very faint but present in the hybrid. **B**, PEPA, a dimeric enzyme, from muscle homogenates of seven lizards. Note that New Mexican *dixoni* differ from Texan *dixoni* and that the hybrid is triallelic. **C**, sMDH, a dimeric enzyme, from muscle homogenates of seven lizards. Note that New Mexican *dixoni* differ from Texan *dixoni* and the hybrid shows two doses of the a-allele. **D**, MPI, a monomeric enzyme, from muscle homogenates of eight lizards. Note that New Mexican *dixoni* differ from Texan *dixoni*. Letters below gels identify allozymes based on alleles present (table 2). Lanes for individual lizards are labeled beside the gel (with genotype) as follows: DIXA, *A. dixoni* A; DIXB, *A. dixoni* B; DIXC, *A. dixoni* C; DIXC × PUN, hybrid of *A. dixoni* C × *A. t. punctilinealis*; MAR, *A. t. marmorata*; SCA, *A. g. scalaris*, which at these loci are often the same as *A. g. septemvittata*; TESC, *A. tesselata* C-E; TESD, *A. tesselata* D; TESE-C, *A. tesselata* E-C; PUN, *A. t. punctilinealis*. Anode is to the right, and arrow in **A** indicates position of sample application.

clutch of eggs) were identical in their genotypes at all loci, with the heterozygosity at the 15 heterozygous loci being preserved in all of the offspring. In addition, these individuals had the same genotype at all loci as the other five field-collected specimens of *A. dixoni* C from New Mexico. Clearly, this demonstrates that *A. dixoni* C normally reproduces by parthenogenetic cloning.

ARE THE PRESUMPTIVE HYBRIDS BETWEEN *A. DIXONI* C × *A. TIGRIS PUNCTILINEALIS* ACTUALLY SUCH HYBRIDS?: Two of the lizards identified as hybrids in the field were tested biochemically (AMNH R-148141 and 148142). Both specimens were triploids and their genotypes were identical to each other (table 2). At each locus, these triploids had a combination of alleles that matched perfectly predictions expected for lizards that inherited the cloned, diploid genome from the maternal *A. dixoni* C (including her mtDNA) plus a haploid genome consisting of the alleles found commonly in the local *A. t. punctilinealis* males. For most loci, this conclusion was based on recording the relative band densities (reflecting gene dosage) on gels representing heterozygous loci for the diploids (both parental forms) and triploids, and the pattern was clear. At PEPA, the hybrids were most clearly triallelic (fig. 6B), bearing the c- and d-alleles from *dixoni* C and the b-allele from *punctilinealis*. The triploid hybrids also had the unusual c-allele at sMDH (fig. 6C), which occurred in all *dixoni* C from New Mexico, as opposed to those (pattern classes A and B) from Texas. In addition, for the following four loci, *dixoni* C is a homozygote, but *punctilinealis* commonly bears a different allele, and at each of these loci the hybrids were heterozygotes with the predicted genomes: IDDH (fig. 6A), PEPB, PEPD, and TF (table 2). Consequently, the level of heterozygosity in the triploid hybrids (61%) was significantly higher than in their maternal parent (48%).

The specimens under discussion were collected in Antelope Pass of the Peloncillo Mountains, Hidalgo County, New Mexico. A few kilometers to the north there are additional passes (Granite Gap and Steins Pass) where there is extensive interbreeding between two bisexual taxa, *A. t. punctilinealis* and *A. t. marmorata*, apparently as a result of recent secondary contact (Dessauer et al., 2000), and a new hybrid zone involving these taxa could develop one day in Antelope Pass as well, as a specimen of *A. t. marmorata* (AMNH R-148140) was recently found west of Animas. One might question, therefore, whether there is evidence of recently contributed *marmorata* alleles in the hybrids. The four most informative loci by which the local *A. t. marmorata* and *A. t. punctilinealis* differ are sMDHP, EST2, PEPB, and TF; in addition, PEPD shows significant allelic frequency differences. At each of these loci, the older, original ancestral *marmorata* allele was found in a single dose as expected in the local *A. dixoni* C, and the *punctilinealis* allele was found in the recently formed triploid hybrids. All of the genetic data were consistent with concluding that the triploids were a consequence of a cloned, diploid ovum from an *A. dixoni* C having been fertilized by a haploid spermatozoan from an *A. t. punctilinealis*.

WHAT IS THE GENETIC RELATIONSHIP BETWEEN THE NEW MEXICAN AND TEXAN POPULATIONS OF *A. DIXONI* AND BETWEEN BOTH OF THESE AND *A. TESSELATA*?: Data in table 2 provide the first comparison of allele products from nuclear genes for samples of *A. dixoni* C from New Mexico and pattern classes A and B from Texas. In addition, these are compared with individuals of *A. tesselata* pattern class E-C from Macho Draw, New Mexico (see Taylor et al., 2003, for the E-C pattern class designation).

There were no allozyme differences found between individuals of *A. dixoni* of classes A and B from Texas. For the genes tested, they were identical (table 2). In addition, the Texan *A. dixoni* were nearly identical to *A. tesselata* class E-C, differing by only one allele, the second d-allele at PEPA (fig. 6B). For PEPA, *tesselata* has a cd genotype, which is consistent with inheritance of the c-allele from *A. t. marmorata* and the d-allele from *A. g. septemvittata*. As the cd combination is also found in *A. dixoni* C from New Mexico (fig. 6B), we hypothesize that it occurred in the parthenogenetic ancestor of Texan *dixoni* and that their c-allele experienced biased gene conversion to d (Hillis et al., 1991), particularly as all of these classes are histocompatible (Cordes and Walker, 2006).

A single allele each at sMDH and MPI also differ between the New Mexican and Texan samples of *A. dixoni* (fig. 6C,D), with Texan *dixoni* A and B having the original ancestral hybrid condition. Histocompatibility and the data in table 2 are consistent with hypothesizing that the c-allele at sMDH in New Mexican specimens resulted from point mutation(s) and the second a-allele at MPI in New Mexican specimens resulted from biased gene conversion. As mentioned above, all available data considered together indicate that all of the population samples of *A. dixoni* and *A. tesselata* share a common ancestor that was a parthenogen of hybrid origin from a single hybrid zygote (Cordes and Walker, 2006). This is consistent also with the clonal diversity found within *A. tesselata*. For example, Taylor et al. (2003) discussed evidence of a common ancestor for three allozyme clones involving sACOH, MPI, and GPI of *A. tesselata* of pattern class C-E from Conchas Lake State Park, New Mexico. As with some of the clones of *A. tesselata* also, the clones of *A. dixoni* have extremely limited geographic distributions. For example, the clone of *A. dixoni* C in New Mexico is unique (allozymes and karyotype) and today is known to occur only in an area of a few square kilometers in Antelope Pass. Whether the color pattern class of the original $F_1$ hybrid of *A. t. marmorata* × *A. g. septemvittata* mostly resembled *A. dixoni* or *A. tesselata* E or neither remains unknown.

### Size, Scalation, and Multivariate Analyses

Morphological data were recorded for 16 *Aspidoscelis dixoni* C, 15 *A. t. punctilinealis*, and 3 of their hybrids, all from Antelope Pass, Hidalgo County, New Mexico. As the first 2 hybrids found were females, only females of the bisexual *A. t. punctilinealis* were examined, to avoid possible influence of a Y chromosome. The third hybrid was a male, but none of this should matter, as Dessauer et al. (2000) showed that none of these characters is sexually dimorphic in the local *A. tigris*.

Univariate statistics for each character and results of statistical tests, including those on principal component scores and canonical variates, are presented in table 3. Variances were homogeneous across samples for all characters except GS (number of gular scales), so we used analysis of variance (ANOVA) and Tukey HSD post hoc tests to determine whether significant differences in these characters occurred among the three groups. We used a Kruskal–Wallis test (chi-square = 2.354, 2 *df*; $P = 0.31$) to verify the nonsignificant differences in GS indicated by the ANOVA ($F_{2,\ 27} = 1.361$; $P = 0.27$). These tests indicated that the hybrids differed significantly ($P < 0.05$) from their maternal parent (*A. dixoni* C) in the GAB, IFS, and PC2 characters, but they were similar to their maternal parent in the ILS, FP, SDL-T4, and PC1 characters. Each group (*A. dixoni* C, *A. t. punctilinealis*, and hybrids) was distinctive in both the CV1 and CV2 characters (table 3).

Three multivariate analyses (PCA, DA, and CVA) were used to determine the patterns of morphological variation between and among individuals and groups. Snout-vent length was excluded from these analyses because our samples did not include the same proportions of various age or size classes.

The first multivariate procedure, a PCA of meristic characters (factoring a correlation matrix), generated scores summarizing the pattern of variation among individuals independent of group membership. An ordination of principal component scores (fig. 7) depicted the two parental taxa as being separated from each other on PC1, with hybrids resembling their maternal parent, *A dixoni*, on this axis. Factor loadings indicated that SDL-T4, ILS, FP, and COS were more important in summarizing variation on PC1 than PC 2, whereas GAB, IFS, and GS contributed principally to PC2 (table 4).

For the remaining multivariate procedures, we used step-wise selection of meristic characters ($F$ probabilities of 0.15 for character entry and 0.20 for character removal) to isolate those meristic characters that contributed significantly to the discrimination between and among groups. The model for the DA used functions determined from meristic characters of the parental taxa, with hybrids included as unclassified in the analysis. Three of seven characters were selected as having value in discriminating the two a priori

TABLE 3

**Descriptive Statistics of Morphological Characters Among Parthenogenetic *Aspidoscelis dixoni*, Bisexual *A. tigris*, and Their Hybrids**

Mean ± SE are subtended by sample size and range limits. * = *A. tigris* is significantly different from the other two groups for this character; ** = *A. dixoni* and hybrids are significantly different from one another for this character; *** = hybrids are significantly different from the other groups for this character; **** = each group is significantly different from the other groups.

| | Group | | |
|---|---|---|---|
| Character[a] | *A. dixoni* | Hybrids | *A. tigris* |
| ILS* | 20.4 ± 0.69 | 19.3 ± 1.67 | 12.7 ± 0.46 |
| | 15 (17–27) | 3 (16–21) | 14 (10–15) |
| FP* | 42.0 ± 0.42 | 44.0 ± 0.58 | 37.1 ± 0.66 |
| | 19 (39–47) | 3 (43–45) | 14 (33–42) |
| SDL-T4* | 39.6 ± 0.29 | 40.3 ± 0.67 | 31.4 ± 0.36 |
| | 19 (37–42) | 3 (39–41) | 14 (29–33) |
| PC1* | 0.91 ± 0.112 | 0.46 ± 0.034 | −0.94 ± 0.135 |
| | 13 (0.41 to 1.69) | 3 (0.41 to 0.52) | 14 (−1.82 to −0.28) |
| GAB** | 96.2 ± 1.30 | 84.0 ± 1.16 | 91.5 ± 1.43 |
| | 19 (91–115) | 3 (82–86) | 14 (85–101) |
| IFS** | 5.6 ± 0.16 | 4.3 ± 0.33 | 5.1 ± 0.16 |
| | 19 (5–7) | 3 (4–5) | 14 (4–6) |
| COS | 16.2 ± 0.47 | 17.7 ± 0.67 | 15.3 ± 0.82 |
| | 19 (13–21) | 3 (17–19) | 14 (11–22) |
| GS | 21.5 ± 0.50 | 22.0 ± 2.52 | 23.1 ± 0.68 |
| | 13 (19–25) | 3 (19–27) | 14 (19–27) |
| SVL | 91.6 ± 1.70 | 92.3 ± 6.94 | 84.0 ± 1.53 |
| | 17 (81–104) | 3 (80–104) | 14 (72–92) |
| PC2*** | −0.15 ± 0.212 | 1.76 ± 0.639 | −0.24 ± 0.225 |
| | 13 (−2.04 to 0.81) | 3 (0.48 to 2.41) | 14 (−1.55 to 1.10) |
| CV1**** | 4.26 ± 0.262 | 6.06 ± 0.253 | −5.25 ± 0.296 |
| | 13 (2.92 to 5.49) | 3 (5.79 to 6.57) | 14 (−7.12 to −3.16) |
| CV2**** | 0.92 ± 0.302 | −3.36 ± 0.299 | −0.14 ± 0.259 |
| | 13 (−1.00 ± 2.80) | 3 (−3.79 to −2.78) | 14 (−1.71 to 1.40) |

[a]The characters are as follows: ILS, number of interlabial scales (left side); FP, total number of femoral pores; SDL-T4, number of subdigital lamellae on 4th toe of left foot; GAB, number of granules (scales) around midbody; IFS, number of scales between femoral pore rows; COS, number of circumorbital scales; GS, number of gular scales; PC1 and PC2, scores on the first two principal components; CV1 and CV2, scores on the first two canonical axes; SVL, length of body from snout to vent of adults (one *dixoni* of SVL = 59 mm not included).

groups (ILS, FP, and SDL-T4, with function coefficients of 0.227, 0.162, and 0.785, respectively). All individuals of *A. dixoni* C and *A. t. punctilinealis* were classified to the correct a priori group, *tigris* having a mean CV1 of −4.394 (range, −6.366 to −2.447) and *dixoni* having a mean CV1 of 4.732 (range, 2.576 to 7.443). Hybrids resembled their mother, with each of the three hybrids being classified with a probability of 1.0 to the maternal *A. dixoni* C.

Our CVA incorporated the three hybrids as a third a priori group and a CVA model using five of seven characters (SDL-T4, FP, ILS, GAB, and GS) for distinguishing among the three groups (table 5). Eigenvalues of 27.119 and 1.672 summarized 94.2% and 5.8% of the univariate variation on axes CV1 and CV2, respectively. Each group was meristically distinctive (fig. 8), and each individual was classified correctly to its a priori group. As also shown by the PCA, the first axis (CV1) separated *A. dixoni* C and the hybrids from *A. t. punctilinealis*, but, unlike the PCA, the CVA separated the hybrids from both parents on CV2. This CVA reiterated the meristic resemblance of the hybrids to *A. dixoni* C, their maternal progenitor

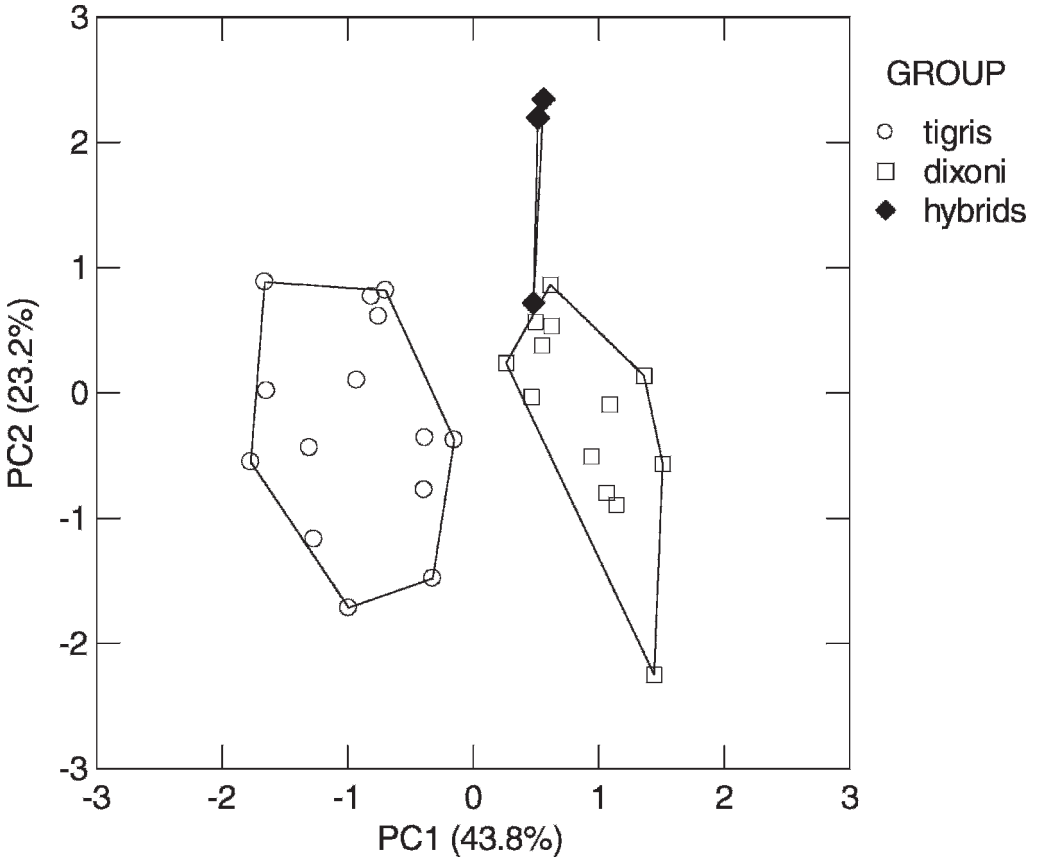


Fig. 7. Pattern of morphological variation expressed by the distribution of scores on the first two principal components extracted from a correlation matrix of seven meristic characters of 30 specimens of *Aspidoscelis* from Antelope Pass, Hidalgo County, New Mexico. The specimens include representatives of two species (*A. dixoni* C and *A. t. punctilinealis*) and hybrids between them. Note that the three hybrids cluster most closely to their maternal parent.

TABLE 4
**Factor Loadings for Two Principal Components Derived from Meristic Variation Among *Aspidoscelis dixoni*, *A. tigris*, and Their Hybrids**

| Character[a] | PC 1 | PC 2 |
|---|---|---|
| SDL-T4 | 0.880 | 0.261 |
| ILS | 0.854 | 0.108 |
| FP | 0.829 | 0.200 |
| COS | 0.483 | 0.146 |
| GAB | 0.422 | −0.833 |
| IFS | 0.579 | −0.696 |
| GS | −0.358 | −0.553 |
| Eigenvalue | 3.065 | 1.626 |
| Total explained variation | 43.8% | 23.2% |

[a]Characters are defined in table 3.

TABLE 5
**Discriminant Functions Used in a CVA to Distinguish *Aspidoscelis dixoni*, *A. tigris*, and Their Hybrids**

| Character[a] | CV 1 | CV 2 |
|---|---|---|
| ILS | 0.492 | 0.358 |
| GS | 0.343 | −0.587 |
| GAB | −0.339 | 1.210 |
| FP | 0.523 | −0.711 |
| SDL-T4 | 0.985 | 0.015 |
| Eigenvalue | 27.119 | 1.672 |
| Total Explained Variation | 94.2% | 5.8% |

[a]Characters are defined in table 3.

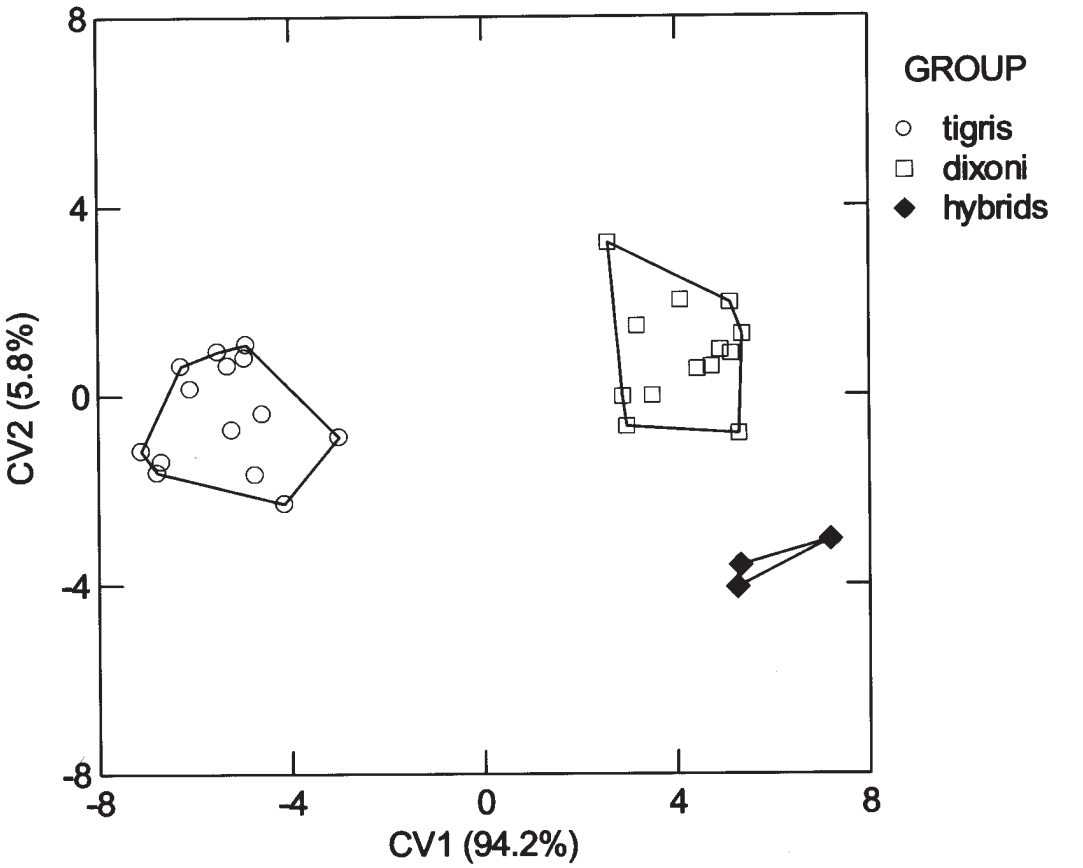


Fig. 8. Pattern of morphological distinctiveness expressed by the distribution of canonical variate scores derived from a canonical variate analysis of five meristic characters of 30 specimens of *Aspidoscelis* from Antelope Pass, Hidalgo County, New Mexico. The specimens include representatives of two species (*A. dixoni* C and *A. t. punctilinealis*) and hybrids between them. Note that the three hybrids cluster most closely to their maternal parent.

(Mahalanobis $D^2$ between centroids = 21.6) rather than to *A. t. punctilinealis*, their paternal progenitor ($D^2$ between centroids = 138.4).

The lizard photographs (fig. 3) and statistical analyses (figs. 7, 8) show that the hybrids of *A. dixoni* C ♀ × *A. t. punctilinealis* ♂ most closely resemble their clonal maternal parent, while differing significantly from their paternal parent. This pattern of hybrids morphologically resembling their clonal parent has been found in nearly all of the studies that have included karyotypic and/or biochemical genetic evidence of hybridization between clonal all-female species of *Aspidoscelis* and males of bisexual congeners, as follows: *A. sonorae* ♀× *A. t. punctilinealis* ♂ (Lowe et al., 1970a); *A. sonorae* ♀× *A. t. marmorata* ♂ (Cole, 1979; Hardy and Cole, 1998); *A. laredoensis* ♀ × *A. g. gularis* ♂ (Walker et al., 1989, 1991); *A. neotesselata* ♀ × *A. sexlineata viridis* ♂ (Walker et al., 1990); *A. tesselata* ♀ × *A. s. viridis* ♂ (Walker et al., 1994); *A. neomexicana* ♀ × *A. tigris* ♂ (Dessauer et al., 2000); *A. tesselata* ♀ × *A. t. marmorata* ♂ (Taylor et al., 2001); and *A. neomexicana* ♀ × *A. s. viridis* ♂ (Manning et al., 2005). In each of these instances of hybridization, the maternal parent's genomes were cloned, whether consisting of two or three haploid sets of chromosomes, whereas the paternal parent contributed one haploid set of chromosomes in the spermatozoan.

Fig. 9. Antelope Pass, Peloncillo Mountains, Hidalgo County, New Mexico. Photo by C.W.P., April 1, 1987, taken from 13 km west of Animas at 31°56′ 9″ N, 108°56′37″ W in WGS 84.

## Distribution of the Parental Species and Hybrids in Antelope Pass and Suggestions for Future Monitoring

For a period of seven years (1987–1993) C.W.P. maintained a survey of the lizards of Antelope Pass, Peloncillo Mountains, Hidalgo County, New Mexico (centered at approximately 10.5 km west of Animas), for the New Mexico Department of Game and Fish (Sias and Painter, 2002). The study focused on *A. dixoni* C, which is listed as an Endangered Species by the NMDGF. For this survey, grids and arrays of pit-fall traps were established at 31 sites in low-elevation habitats where *A. dixoni* C was most likely to be found, based on an earlier study by Zweifel (1965). The stimulus for this study was that the population of *A. dixoni* C was thought by some herpetologists to be extinct in the late 1980s or early 1990s (J. R. Dixon, personal commun.), although during the 1960s they were so abundant that one could go there in the appropriate season and see several individuals on any day with good weather (Zweifel, personal commun., and personal observations, C.J.C.).

Antelope Pass (figs. 2, 9), at 1300–1500 m elevation, consists of rocky hillsides with slopes and bajadas covered with gravel. Bottomlands have sandy soil with gravel, and the arroyo bottoms are quite sandy. Overall, the habitat is Chihuahuan Desert desertscrub. Literally in and beside the main arroyo in the bottomland there are a few trees (desert hackberry, *Celtis pallida*, desert willow, *Chilopsis linearis*, juniper, *Juniperus*) and conspicuous shrubs, listed from most to least abundant (estimated), being mesquite (*Prosopis glandulosa*), Apache plume (*Fallugia paradoxa*), fourwing saltbush (*Atriplex canescens*), littleleaf sumac (*Rhus microphylla*), and some allthorn (*Koeberlinea spinosa*). The broad expanse of lowland up and away from the arroyo includes, listed from most to least

TABLE 6
**Number of Individuals Found at Different Trap Sites Where *A. dixoni* and/or *A. t. punctilinealis* Were Found in Antelope Pass**

| Site | No. *A. dixoni* | No. *A. tigris* | Coordinates in WGS 84 | Symbol, figs. 10, 11 |
|---|---|---|---|---|
| Barbara | 4 | 9 | 32°00′19″N, 108°54′47″W | dot |
| Beacon | 0 | 5 | 31°56′14″N, 108°57′31″W | square |
| Baja | 11 | 1 | 31°57′45″N, 108°55′19″W | dot |
| Budweiser | 6 | 17 | 31°56′33″N, 108°56′01″W | dot |
| Cherry | 55 | 1 | 31°56′49″N, 108°55′20″W | dot |
| Debbie | 0 | 50 | 31°56′46″N, 108°59′23″W | square |
| East | 29 | 765 | 31°56′21″N, 108°56′09″W | dot |
| Heather | 0 | 51 | 31°56′47″N, 108°59′54″W | square |
| Hill | 55 | 1 | 31°57′05″N, 108°53′59″W | dot |
| Jennifer | 2 | 0 | 31°57′43″N, 108°54′18″W | triangle |
| Kate | 8 | 1 | 31°56′59″N, 108°54′25″W | dot |
| Kristin | 4 | 1 | 31°55′36″N, 108°53′18″W | dot |
| Lynn | 1 | 4 | 32°00′49″N, 108°54′51″W | dot |
| Maria | 3 | 0 | 31°57′16″N, 108°55′23″W | triangle |
| Michelle | 2 | 0 | 31°59′05″N, 108°55′46″W | triangle |
| Marcy | 1 | 0 | 31°56′28″N, 108°53′16″W | triangle |
| Norte | 0 | 7 | 31°56′53″N, 108°55′46″W | square |
| Railroad | 0 | 32 | 31°56′08″N, 108°56′24″W | square |
| Sandy | 3 | 0 | 31°58′36″N, 108°54′09″W | triangle |
| Susan | 5 | 0 | 31°56′52″N, 108°53′45″W | triangle |
| Tiffany | 7 | 0 | 31°57′21″N, 108°55′11″W | triangle |
| Valencia | 6 | 0 | 31°54′50″N, 108°54′31″W | triangle |
| West | 18 | 339 | 31°56′20″N, 108°56′54″W | dot |

abundant, creosotebush (*Larrea tridentata*), *Prosopis glandulosa*, cholla, yucca (*Yucca elata*), prickly pear (*Opuntia*), *Koeberlinea spinosa*, and a few barrel cacti (*Ferocactus*). "Dwarf desert-holly (*Perezia nana*), snakeweed (*Gutierrezia sarothrae*), and zinnia (*Zinnia acerosa*) are common perennial herbs. Grass cover is sparse with muhly grass (*Muhlenbergia* sp.) and tobosa grass (*Hilaria mutica*) most abundant" (Sias and Painter, 2002: 1). Plants on the hillsides are more widely scattered than in the lowlands, consisting mostly of *Larrea*, and here juniper trees are more frequent. Also present, mostly higher up on the rockier soils, are agaves (*Agave*), small cacti (including *Mammillaria*), and scattered ocotillos (*Fouquieria splendens*). Running from south to north are a few narrow, short, rocky-sloped, tree-supporting side canyons that do not have sandy lowlands with dense stands of *Larrea* and *Prosopis*. These side canyons, with rocky soils, appear to be unfavorable microhabitat for *A. t. punctilinealis*, and one wonders whether they could constitute small refuges for *A. dixoni*.

During the survey period 1,281 different individuals of *A. t. punctilinealis* were found, not counting the many recaptures. Only 220 individuals of *A. dixoni* C were found during this period, as were 3 hybrids of *A. dixoni* × *A. t. punctilinealis*. Clearly, the two species use the same habitat frequently, as 1,104 of the *A. t. punctilinealis* were found at only two of the sites (referred to as East and West), and 47 of the *A. dixoni* were found at the same two sites. However, two other sites (Debbie and Heather, the westernmost sites) produced either 50 or 51 *A. t. punctilinealis*, respectively, but no *A. dixoni* (table 6).

There were nine sites that produced from one to nine individuals of *A. t. punctilinealis* each. Six of these also produced fewer than 10 *A. dixoni* C each (two produced 0), indicating that marginal habitats that were being avoided were similar for both species. However, the two exceptional sites (Cherry and Hill) produced 55 *A. dixoni* each. Thus, 110 (50%) of

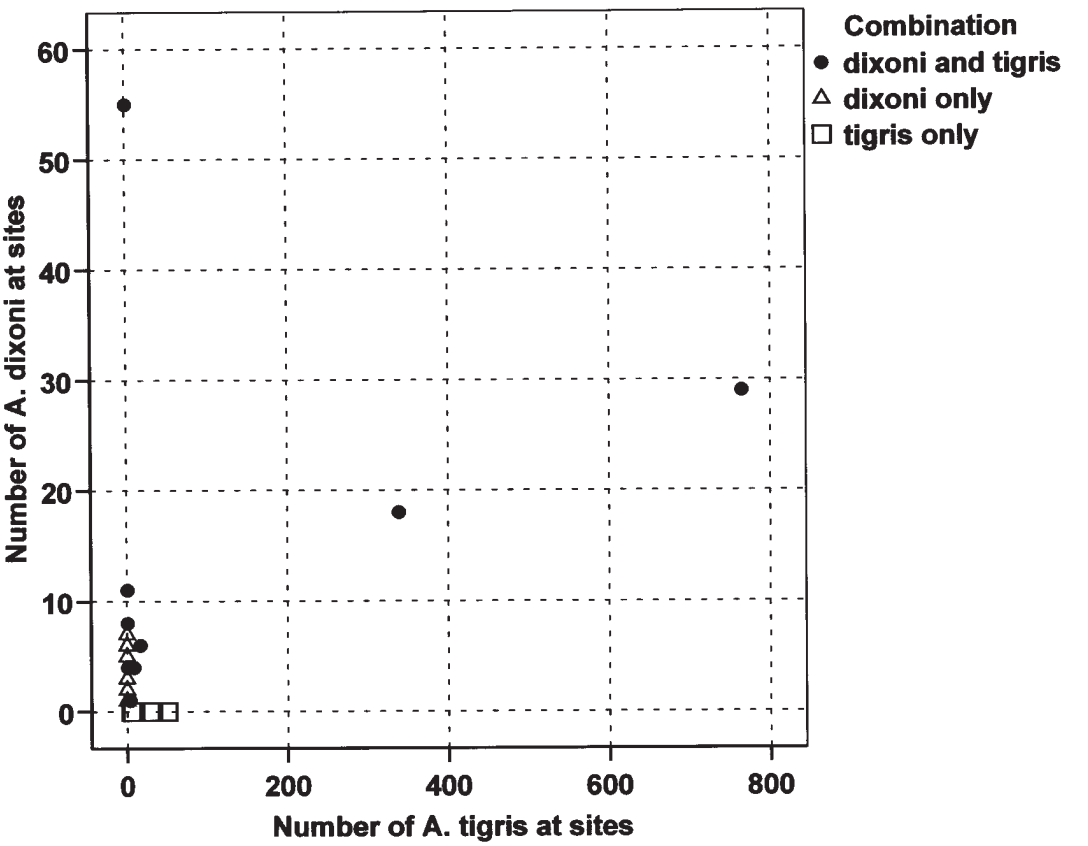


Fig. 10. Relationship between number of *A. dixoni* C and number of *A. tigris punctilinealis* found at each of the trap sites at which either *A. dixoni* or *A. t. punctilinealis* or both were found in Antelope Pass, Hidalgo County, New Mexico. Of 23 sites (table 6), the two with the most examples of *A. dixoni* ($N = 55$ each) had only one individual of *A. t. punctilinealis* each, and the rest of the sites with *dixoni*, except for three, had fewer than 10 *punctilinealis* each.

the *dixoni* found in this seven-year study occurred in local sites that were being used very little by *A. t. punctilinealis*. In addition, there were eight other sites that produced a total of 29 *dixoni* (13% of the overall sample) but no specimens of *A. t. punctilinealis*. Consequently, the majority of the *A. dixoni* C that occurred in Antelope Pass during the survey occurred locally in sites that were little used by *A. t. punctilinealis*.

The broad overlap in habitats used by *A. dixoni* C and *A. t. punctilinealis* in Antelope Pass, and yet the occurrence of *dixoni* at many sites little used by *punctilinealis*, raises the question of whether *dixoni* is being displaced and replaced at optimal sites by *punctilinealis*. Consequently we listed (table 6) and plotted (figs. 10 and 11) the number of individuals found at different trap sites where *A. dixoni* C and/or *A. t. punctilinealis* were found. Note that zero to nine individuals of *A. t. punctilinealis* were found at 15 of the 18 sites (83%) where *A. dixoni* C occurred. Also, zero to eight individuals of *A. dixoni* C were found at 10 of the 15 sites (67%) where *A. t. punctilinealis* occurred.

The three hybrids were found at the following sites: (1) Cherry, on July 22, 1989 (a site where a total of 55 *A. dixoni* and only 1 *A. t. punctilinealis* were found); (2) East, on May 7, 1991 (where a total of 29 *dixoni* and 765 *punctilinealis* were found); and (3) Baja, on July 5, 1993 (where a total of 11 *dixoni* and only 1 *punctilinealis* were found). Interestingly,

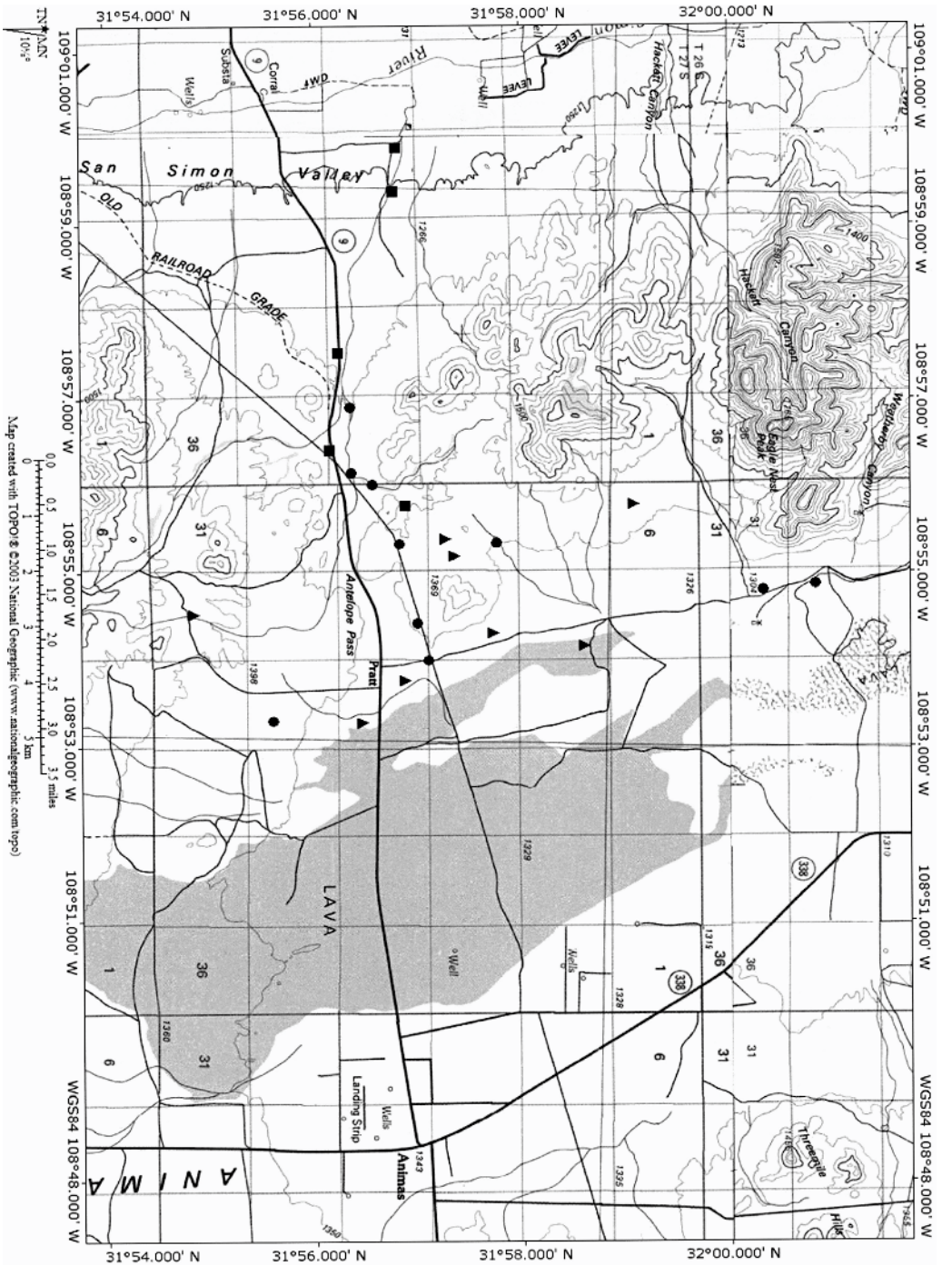


Fig. 11. Topographic map of Antelope Pass, Hidalgo County New Mexico showing the 23 trap sites listed in table 6. Each site can be identified by its coordinates and symbol (table 6). Triangles indicate sites where *A. dixoni* C was found, but not *A. t. punctilinealis*; squares indicate sites where *A. t. punctilinealis* was found, but not *A. dixoni* C; and dots indicate sites where both species were found. This was mapped with TOPO! (2003, National Geographic; www.nationalgeographic.com/topo).

at two of these sites (Cherry, Baja) *A. t. punctilinealis* was far outnumbered by the all-female *A. dixoni*. Consequently, a male *punctilinealis* would far more frequently encounter a female *dixoni* than a conspecific female, which would be conducive to hybridization. The third site (East) had relatively high numbers of both species, but *A. t. punctilinealis* far outnumbered *A. dixoni*, so the chances for hybridization (and significant gamete wastage for *A. dixoni*) would be high, owing to the abundance of males of *punctilinealis*. We hypothesize that the most secure sites for the long-term survival of *A. dixoni* without threat of destabilizing hybridization are those sites lacking *A. t. punctilinealis*. However, if those sites are ecologically marginal for *dixoni* and they have been displaced to those sites by competitive interactions, this would have a negative impact on the long-term survival of *dixoni*.

We do not know when *A. dixoni* arrived in Antelope Pass, assuming it immigrated from elsewhere. The nearest localities today where its ancestral bisexual species occur in sympatry are hundreds of kilometers to the east and southeast in the Chihuahuan Desert. *Aspidoscelis t. punctilinealis*, a Sonoran Desert taxon, recently arrived from the west (via the San Simon Valley, Cochise County, Arizona), probably during the last few hundreds or thousands of years (Dessauer et al., 2000), and the population in Antelope Pass represents nearly the easternmost geographic limit for this taxon. As discussed elsewhere (Dessauer et al., 2000; Taylor et al., 2001), males of *A. tigris* are aggressive breeders during the mating season, and they are less discriminating in mate selection than are most other lizards. Consequently, they occasionally mate with females of other species, including parthenogens. Because females of *A. dixoni* carry an ancestral genome from *A. t. marmorata*, with which *A. t. punctilinealis* freely interbreeds in the area of the Peloncillo Mountains (Dessauer et al., 2000), it is not surprising that *punctilinealis* males mate with *dixoni* also. The known female hybrids were apparently sterile, however, so such matings constitute gamete wastage.

Today, in the Roswell area of New Mexico in Macho Draw, *A. t. marmorata* hybridizes frequently with *A. tesselata* E-C (Taylor et al., 2001), and 50% of the hybrids are sterile females, 50% males. The unisexual *tesselata* remain abundant, however, possibly because they produce twice as many eggs per clutch than does the bisexual *marmorata*, and the *marmorata* are extremely few in number, confined to very limited microhabitat in the area.

In contrast, in Antelope Pass, the bisexual species far outnumbers the parthenogen. We hypothesize that *A. dixoni* C has been experiencing difficulty maintaining itself in Antelope Pass owing to interspecific competitive interactions and asymmetrical destabilizing hybridization with *A. t. punctilinealis*, with which it shares the same preferred microhabitat. This hypothesis could be tested by conducting additional trapping in Antelope Pass. Future trapping could be conducted with immediate on-site release, minimizing handling of the lizards (i.e., without transporting, weighing, measuring, or marking in a laboratory). This would little disturb the lizards and minimize the possibility of introducing disease into the population.

Two of the five important sites to monitor for continuing hybridization would be East and West, where 29 *dixoni* (with 765 *punctilinealis*) and 18 *dixoni* (with 339 *punctilinealis*), respectively, were found (table 6). The other three sites would be Baja, Cherry, and Hill, where only 1 *punctilinealis* was found along with much larger numbers of *dixoni*. At the last three sites, males of *punctilinealis* would have few conspecific females with which to mate. People involved with future monitoring projects must be alert and aware of the similarities of *A. dixoni* C and the hybrids, lest hybrids be overlooked owing to being misidentified as *A. dixoni* C.

Considering that the survey found 220 individuals of *A. dixoni* C and only three hybrids (Sias and Painter, 2002), the impact of destabilizing hybridization to *dixoni* may not appear to be high. Nevertheless, 1,281 individuals of *A. t. punctilinealis* were found in broadly overlapping habitats. The low number of hybrids plus the lack of small ones indicate that they are produced neither consistently nor annually, assuming that no hybrids evaded recognition as such. However, the following

points may be relevant: (1) We do not know the extent of hybrid mortality in eggs and hatchlings that are not observed; (2) *dixoni* is declining in abundance; (3) some *dixoni* occur locally where no *tigris* or very few *tigris* occur; (4) dates of collection and sizes of the three hybrids when found indicate that three different egg clutches were involved; and (5) with the capability of sperm storage in lizards (Hardy and Cole, 1981, reported sperm receptacles in the oviducts of *Aspidoscelis* females, including those of parthenogens), one hybrid mating could have a negative impact on a parthenogenetic female's productivity for two or more seasons. In addition, as male *tigris* mate with multiple females in a breeding season, the gamete wastage is probably insignificant to *A. t. punctilinealis*, so this is asymmetrical destabilizing hybridization, having significant negative impact only on *A. dixoni* C.

There were eight sites in Antelope Pass where *A. dixoni* C were found and no *A. t. punctilinealis* were found (table 6). One might infer that these sites hold the greatest promise for the future survival of *dixoni*, but only 13% of their total number were found at these sites. This could indicate that these sites are of marginal habitat for both species, that *dixoni* was displaced to these suboptimal sites by the dominant *punctilinealis*, and therefore these sites are not very promising for the long-term survival of *dixoni* C. Alternatively, one might infer that those sites where *A. dixoni* outnumbered *A. t. punctilinealis* hold great promise for the future of *dixoni* C. For example, one of the hybrids was found at the Cherry site, where 55 *A. dixoni* and only 1 *punctilinealis* were found. However, at such sites, where males of *punctilinealis* have few conspecific females with which to mate, the frequency of hybridization, and its negative impact on *A. dixoni*, may be greater than at other sites.

Whatever the future fate may be for the unique clone of *A. dixoni* C in Antelope Pass, New Mexico, its long-term survival does not appear to be enhanced by the presence of *A. t. punctilinealis*. Also, considering the small numbers of *A. dixoni* C in scientific collections that were assembled over a period of decades, its absence from the pet trade, and its nonuse as a game species, the decline in this population of *A. dixoni* appears to be a result of natural causes, including desertification, perhaps influenced by effects of human activities for more than a century.

### The Possible Origin of a New Species

The possibility exists that with continued hybridization before (and whether or not) *A. dixoni* C becomes extinct, an unusual triploid female hybrid of *A. dixoni* C × *A. t. punctilinealis* might someday be capable of parthenogenetic cloning, even if the majority of $F_1$ female hybrids are sterile. Should such a clone appear in the future, it would be a unique self-perpetuating entity in ploidy and combination of genomes from ancestral taxa, with a unique history, and this would constitute a new species. Nevertheless, we wonder whether the long-term survival of such a new species, should it arise, would also be seriously threatened by asymmetrical destabilizing hybridization with *A. t. punctilinealis* in Antelope Pass. Ironically, the demise of such a species could be brought on by its progenitor. In any event, personnel involved in future monitoring of sites in Antelope Pass should be aware of this possibility as they discover what may appear to be triploid hybrids.

## SUMMARY AND CONCLUSIONS

1. *Aspidoscelis dixoni* pattern class C is highly endangered, known to exist only in a few square kilometers of southwestern New Mexico, approximately 10.5 km west of Animas, Hidalgo County.

2. This is a diploid unisexual species that normally reproduces by parthenogenetic cloning, and the cause(s) of its recent rarity in New Mexico has been unknown. In this report we present the first genetic comparisons of an adult female *A. dixoni* C and her offspring, which demonstrate clonal inheritance.

3. Additional clones of *A. dixoni* (pattern classes A and B) occur in the Chinati Mountains, Presidio County, Texas. These are disjunct by nearly 500 km from the New Mexican population, and they also occur in very limited areas.

4. In New Mexico, females of *A. dixoni* C occasionally interbreed with males of *A. tigris*

*punctilinealis*, producing triploid hybrids, none of which is known to have reproduced. Considering the initial hybrid origin of *A. dixoni* itself, these hybrids have genomes from the following three taxa: *A. tigris marmorata* ♀; *A. gularis septemvittata* ♂; and *A. tigris punctilinealis* ♂.

5. Comparative genetics (karyotypes and allozymes) and morphological analyses (univariate and multivariate statistics) clearly demonstrate the ancestry of the hybrids between *A. dixoni* C ♀ × *A. t. punctilinealis* ♂ from New Mexico.

6. In addition, we used the same analyses of allozymes to compare individuals representing the three pattern classes of *A. dixoni* from New Mexico and Texas.

7. Individuals of Texan *A. dixoni* A and B were identical to each other in allozymes.

8. However, *A. dixoni* C from New Mexico is unique in lacking one of the microchromosomes found in Texan populations of *A. dixoni* A and in having allelic differences at three allozyme loci (sMDH, PEPA, and MPI). Nevertheless, the New Mexican and Texan populations share possession of a fissioned X chromosome that was originally inherited from *A. t. marmorata*. These populations also share the same individual ancestral $F_1$ female hybrid (*A. t. marmorata* ♀ × *A. g. septemvittata* ♂) with each other and with various pattern classes of *A. tesselata*—groups considered by some taxonomists to be diverse clones of one species (see discussion in Cordes and Walker, 2006). Whether the color pattern class of the original $F_1$ hybrid mostly resembled *A. dixoni* or *A. tesselata* E or neither remains unknown.

9. *Aspidoscelis t. punctilinealis* is a recent invader of Antelope Pass, Hidalgo County, New Mexico, yet it is by far the most dominant species of whiptail lizard there today. Its female hybrids with *A. dixoni* C are apparently sterile and may represent significant gamete wastage for *A. dixoni*, particularly if females produce multiple clutches of inviable or infertile offspring after mating.

10. The present rarity of *A. dixoni* C in Antelope Pass may result from negative interactions with *A. t. punctilinealis*, including competition and asymmetrical destabilizing hybridization (with significant negative impact on *dixoni*, but not on *punctilinealis*).

11. We suggest testing this hypothesis by continuing to monitor for *A. dixoni* and hybrids at any of the following five sites: Baja, Cherry, East, Hill, and West. At these sites, *A. t. punctilinealis* either far outnumbered *A. dixoni* C during the last trapping period or was far outnumbered by it, both conditions which may be conducive to hybridization.

12. Personnel in monitoring projects must be trained to recognize hybrid lizards of both sexes, as they can be very difficult to distinguish from parental taxa.

## ACKNOWLEDGMENTS

This project could not have been completed without the dedicated and professional efforts of Barney Tomberlin and Tony Snell, who worked feverishly on the *A. dixoni* project in Antelope Pass, Hidalgo County, New Mexico. Not only did they first propose the correct identification of the very cryptic hybrid lizards of *A. dixoni* C × *A. t. punctilinealis*, but they untiringly visited the study area no fewer than 1,500 times over the course of seven years, and they individually recorded data on almost 9,000 amphibians and reptiles captured at or near the site. These two people deserve an award!

During the *A. dixoni* project, C.W.P. also received extensive assistance from many colleagues and volunteers who helped maintain and check the pit-fall traps and record original observations. Individuals deserving special mention because of their extraordinary efforts include Tom Moisi, Gerold Merker, and volunteers from the Southwestern Research Station near Portal, Arizona, thanks to its Director at the time, Wade C. Sherbrooke (recently retired; current Director, Dawn S. Wilson). In addition, Dr. Sherbrooke assisted C.J.C. in his field and laboratory work in many ways while he conducted part of this work at the Southwestern Research Station. The late Vincent D. Roth and his gracious wife Barbara provided C.W.P. with laboratory facilities in their home during the early stages of this project.

Mrs. Adeline Hill kindly allowed us to establish trap sites on her ranch and grazing leases for the duration of the *A. dixoni* project, and Lance Tyson (NMDGF) provided coordinates for plotting sites on figure 11. The U.S. Fish and Wildlife Service, Albuquerque Ecological Services Office, provided a significant portion of the funding for this project through various federal aid grants to the New Mexico Department of Game and Fish Endangered Species Program. Bill Merhege and Mark Hakkila (Mimbres District, Bureau of Land Management, Las Cruces, New Mexico) provided considerable funding and logistical support also. In addition, the New Mexico Department of Game and Fish, Share with Wildlife Program, provided funds and other field support, and the Department granted the scientific collecting permits necessary for this work.

Carol R. Townsend and Margaret G. Arnold provided important care for the hybrid lizards while they lived at the *Aspidoscelis* Ranch at the American Museum of Natural History. In addition, Carol R. Townsend assisted in all other aspects of this work and Bartek Jablonski helped to analyze the karyotypes.

We also owe special thanks to James E. Cordes, Louisiana State University at Eunice, and his former major professor, James M. Walker, University of Arkansas, Fayetteville, who sent us living representatives of the two pattern classes of *A. dixoni* from Texas, as well as some tissue samples. Without these specimens, we would not have been able to compare the genetic characters of the disjunct New Mexican and Texan populations. Andrew H. Price (Texas Parks and Wildlife Department) arranged and assisted with collecting important comparative material in Texas, especially of *A. g. septemvittata*. In addition, George L. Bradley and Philip C. Rosen provided C.J.C. access to chromosome data from *A. dixoni* at the University of Arizona, which he had prepared and analyzed in 1966 as a graduate assistant to C. H. Lowe in unpublished collaborative research with Lowe, J. W. Wright, and R. L. Bezy. We also thank Richard G. Zweifel and two anonymous reviewers for helpful comments on the manuscript.

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## APPENDIX 1

### Specimens Examined

Specimens are cited by their individual catalog numbers as follows: AMNH (American Museum of Natural History), CWP (field number of Charles W. Painter), HCD (frozen tissue collection of Herbert C. Dessauer), MSB (Museum of Southwestern Biology, University of New Mexico, Albuquerque), NK (frozen muscle samples from Charles W. Painter), UADZ (University of Arkansas Department of Zoology), and UAZ (University of Arizona). Letters following catalog numbers indicate the kind of data used from the specimens, as follows: a, allozymes; k, karyotypes; m, morphology.

*Aspidoscelis burti burti*: MEXICO: Sonora; Bahia de San Carlos (AMNH R-131433–R-131436, all a).

*Aspidoscelis burti stictogramma*: ARIZONA: Cochise County; Bass Canyon, ca. 0.8 km from Hot Springs Canyon, 50 km (by rd.) WNW Willcox (AMNH R-126767–R-126782, all a). Graham County; Santa Teresa Mountains, 14.5 km (linear) N Klondike (AMNH R-126765, a). Pinal County; vicinity of old CCC Camp, ca. 2.4 km (linear) SW Oracle (AMNH R-126766, a). MEXICO: Sonora; 24.8 km (by Mex. Hwy. 2) ENE Imuris (AMNH R-131436–R-131437, a).

*Aspidoscelis dixoni* A: TEXAS: Presidio County; Mesquite Ranch, San Antonio Canyon, 2.2 km N Campo Nuevo (UADZ 3557, 3560, 3562, and 3613–3615, all a); San Antonio Canyon, 2.3 km N Campo Nuevo (UADZ 3561, a); Indian Springs, 7.5 km NW Campo Nuevo (UADZ 3570, a); head of Pelillos Canyon, 28.5 km NW junction of US Hwy 67 and FM Hwy 170 on FM Hwy 170, then 9.5 km NE on dirt road to Mesquite Ranch, then 14.0 km N to Pelillos Canyon (AMNH R-148426–R-148428, all a, k); two $F_1$ laboratory-reared offspring of two additional different adult females (AMNH R-148369 and R-148411, both k).

*Aspidoscelis dixoni* B: TEXAS: Presidio County; Mesquite Ranch, San Antonio Canyon, 2.2 km N Campo Nuevo (UADZ 3602, a); 28.5 km NW junction of US Hwy 67 and FM Hwy 170 on FM Hwy 170, then 9.5 km NE on dirt road to Mesquite Ranch, then 20.6 km N to Indian Springs (AMNH R-148424 and R-148425, both a).

*Aspidoscelis dixoni* C: NEW MEXICO: Hidalgo County; Antelope Pass, 11 km W Animas (AMNH R-80686–R-80691, all m, AMNH R-84836, m, AMNH R-134864, a, k, m, and UADZ 3552, a); Antelope Pass, 12–13 km W Animas (AMNH 148360, a, k, m, and 3 of her $F_1$ offspring,

AMNH R-148364–R-148366, all a); Peloncillo Mountains, Antelope Pass, 12.9 km (via NM Hwy. 9) W Animas (UAZ 18535, k); Antelope Pass, 13 km W Animas (AMNH R-80681–R-80684, all m, AMNH R-84834, m, AMNH R-86994–R-86995, both m, and NK 3858, 3859, and 3870, all a); Antelope Pass, 13.4 km W Animas (AMNH R-80685, m); Antelope Pass, 16 km W Animas (AMNH R-80680, m); Antelope Pass, site unspecified (CWP 4504, a, m).

*Aspidoscelis gularis scalaris*: MEXICO: Chihuahua; 3.2 km (by Mex. Hwy 45) NW Bachimba (AMNH R-129175, a). Durango; Rio Florido near Canutillo (bridge for Mex. Hwy 45) (AMNH R-129176–R-129179, and an additional lizard frozen whole, all a).

*Aspidoscelis gularis septemvittatus*: TEXAS: Brewster County; 5.6 km (by US Hwy 385) S Marathon (AMNH R-126764, a); 28 km (by US Hwy 385) S Marathon (AMNH R-126906–R-126908, all a); North Rosillos Mountains Preserve, 7.2 km N and 3.2 km W (linear) Rosillos Peak (AMNH R-133254, a); North Rosillos Mountains Preserve, Buttrill Spring, 1.6 km N and 2.7 km W (linear) Rosillos Peak (AMNH R-133255, a). Presidio County: 55.4 km (by TX Hwy 2810) SW Marfa (AMNH R-126903, a); 57.6–60.2 km (by TX Hwy 2810) SW Marfa (AMNH R-126904–R-126905, both a).

*Aspidoscelis tesselata* C-E: NEW MEXICO: San Miguel County; Conchas Lake State Park, South Campground, 49.9 km (by NM Hwy 104) NW Tucumcari (Quay County) (AMNH R-136876, a).

*Aspidoscelis tesselata* D: NEW MEXICO: San Miguel County; Conchas Lake State Park, South Campground, 49.9 km (by NM Hwy 104) NW Tucumcari (Quay County) (AMNH R-136880, a).

*Aspidoscelis tesselata* E-C: NEW MEXICO: Chaves County; N side Arroyo del Macho (33°39′1″N, 104°33′17″W), 22 km N on Hwy. US 285 from junction with Hwy US 70 N of Roswell, then 0.8 km E on Eden Valley Road (AMNH R-146629, R-146631–R-146633, R-146636, R-146638–R-146639, and R-145142–R-145144, all a, k except no k for R-146631).

*Aspidoscelis tigris marmorata*: NEW MEXICO: Hidalgo County; Antelope Pass, 8.9 km W Animas, Hill trap site, T27S R20W SE quarter section 17 (AMNH R-148140, just identified). In addition, allozymes were compared with the hundreds of specimens from nearby localities analyzed by Dessauer et al. (2000). Socorro County; 2.3 km (by road) W San Antonio (AMNH R-131074, a).

*Aspidoscelis tigris punctilinealis*: NEW MEXICO: Hidalgo County; Antelope Pass, 11.3 km W Animas (AMNH R-148114–R-148116, all a); Antelope Pass, 13 km W Animas (AMNH R-148113, a, k). Antelope Pass, along NM Hwy. 9, 12.9–14.5 km W Animas (MSB 2621, 2669, 2671, 2674, 2682, 3039, 3044–3045, 3284–3285, 3293–3294, 3300, and 3311, all m; MSB 3338–3340, all a; and MSB 3341, a, k, m). In addition, allozymes were compared with the hundreds of specimens from nearby localities analyzed by Dessauer et al. (2000).

Hybrids of *A. dixoni* C × *A. t. punctilinealis*: NEW MEXICO: Hidalgo County; Antelope Pass, 10.8 km W Animas, Cherry trap site (table 6), T27S R20W NE quarter section 19 (AMNH R-148141, a, k, m); 11 km W Animas, Baja trap site (table 6), T27S R20W NE quarter section 18 (AMNH R-148142, a, m); 12.2 km W Animas, East trap site (table 6), T27S R21W SE quarter section 24 (AMNH R-148433, m).

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