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Natural Hybridization Between the Teiid Lizards *Cnemidophorus tesselatus* (Parthenogenetic) and *C. tigris marmoratus* (Bisexual): Assessment of Evolutionary Alternatives

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ABSTRACT

Annual hybridization is taking place between representatives of the parthenogenetic lizard *Cnemidophorus tesselatus* ($2n = 46, 47$) and males of the bisexual species *C. tigris marmoratus* ($2n = 46$) in desert grassland habitats at Arroyo del Macho, Chaves County, New Mexico. This raises the question of whether a new triploid parthenogenetic species may be originating as a consequence of this activity. Hybrids were collected in each of four years (1996–1999), and 20 of 21 hybrids collected (12 males and 8 females) were available for study. Although a triploid parthenogenetic species (*Cnemidophorus exsanguis*, $3n = 69$) and a diploid bisexual species (*C. inornatus*, $2n = 46$) were also found at the hybridization site, the genealogy of the hybrids was determined unequivocally with karyotypic and electrophoretic evidence (34 loci tested). The specimens examined electrophoretically included an adult female and one of her laboratory-reared daughters, which demonstrated for the first time clonal inheritance in *C. tesselatus* pattern class E.

The population of *C. tesselatus* at Arroyo del Macho is characterized by two karyotypic cytotypes. The ancestral one ($2n = 46$) occurs at about half the frequency of the derived cytotype ($2n = 47$), which apparently was produced by centric fission of the ancestral X-chromosome from *C. tigris*. In contrast, the occurrence of the two cytotypes was reversed and strongly asymmetrical in the hybrids; only one of nine hybrids possessed the fissioned X-chromosome. This individual was significantly different in 12 meristic characters from the sample of hybrids with intact X-chromosomes. Predictably, principal components scores for this individual fell outside the 95% confidence ellipse of scores of the other eight hybrids that

were karyotyped. The skewed ratio and multiple phenotypic differences suggest that hybrids inheriting a fissioned X-chromosome might be at a selective disadvantage compared to hybrids with intact X-chromosomes.

All 20 hybrids closely resemble *C. tesselatus* in most color pattern features. However, these hybrids, like *C. tigris marmoratus,* lack lateral stripes. Because the population of *C. tesselatus* at Arroyo del Macho has lateral stripes (or their remnants), hybrids can be readily distinguished from *C. tesselatus* by this color pattern feature. Compared to the two parental species, hybrids had a significantly lower mean number of scales around midbody, but hybrids resembled either *C. tesselatus* or *C. tigris marmoratus* in other univariate meristic characters. This mosaic pattern of resemblance was simplified to a three-dimensional depiction of variation using principal components analysis. Each of two principal components expressed the resemblance of hybrids to one of the two parental species. A third component reflected the difference between hybrids and both parental species. A canonical variate analysis of meristic characters demonstrated the multivariate distinctiveness of each group—hybrids, *C. tesselatus,* and *C. tigris marmoratus.* However, based on Mahalanobis D^2 distances, the closest morphological resemblance among hybrids and parental species was between hybrids and the maternal species, *C. tesselatus.*

Nine additional museum specimens, suspected of being *C. tesselatus* × *C. tigris marmoratus* hybrids, were identified, as such, by a canonical variate analysis using our samples of *C. tesselatus, C. tigris marmoratus,* and hybrids from Arroyo del Macho as a priori groups. These nine individuals document hybridizations between *C. tesselatus* and *C. tigris marmoratus* at two additional localities in Chaves County, New Mexico, two localities in Sierra County, New Mexico, and a cluster of sites near Presidio, Presidio County, Texas. Previously, several of these hybrids had been misidentified as male *C. tesselatus.*

The reproductive systems of female and male hybrids were compared histologically to those of *C. tesselatus* and *C. tigris marmoratus,* respectively. Sexually mature and reproductive adults of *C. tesselatus* usually have oocytes in the ovary, complete and well-organized ovarian follicle walls, inconspicuous connective tissue and fewer vacuoles in the well-vascularized ovary, the distal oviduct with a thin mucosa, well-developed alveolar glands restricted to the middle oviduct, a proximal oviduct with a thick mucosa and well-developed folds, and small mesonephric tubules. Female hybrids have a poorly defined follicular epithelium with little vascularization in small ovaries, empty or fluid-filled follicles without oocytes, few or no cilia in the middle oviduct, and numerous abnormally large mesonephric tubules. There is no evidence that *Cnemidophorus tesselatus* × *C. tigris marmoratus* females can produce viable and fertile eggs. Although hybrid males are capable of producing sperm that appear normal and were present in the epididymides, the allotriploid chromosome complement reduces the chance that sperm would carry genetically balanced sets of information.

Although the annual production of hybrids could affect the long-term success of this local population of *C. tesselatus,* two lines of evidence indicate that hybridization is unlikely to result in its extirpation. First, the population of *C. tigris marmoratus* at Arroyo del Macho is tightly associated with a microhabitat dominated by creosote bush. Because creosote bush is distributed there in small, widely scattered patches, the density of *C. tigris marmoratus* is relatively low, and many individuals of *C. tesselatus* escape insemination. This was evident from an absence of sperm in the reproductive tracts of 11 individuals of *C. tesselatus* collected during the peak reproductive season (May and June) of three different years. Second, reproductively mature individuals of *C. tesselatus* are significantly larger than comparable females of *C. tigris marmoratus.* This translates into larger clutches, with the mean clutch size of *C. tesselatus* being twice as large as that of *C. tigris marmoratus.* The disparity in mean clutch size in conjunction with habitat constraints on *C. tigris marmoratus* probably explains why *C. tesselatus* outnumbers both *C. tigris marmoratus* and hybrids by a ratio of approximately 2:1 at the hybridization site.

Although hybridization between *C. tesselatus* and *C. tigris marmoratus* appears to be an annual event at Arroyo del Macho, there is no evidence that a new triploid parthenogenetic species is resulting from this hybridization activity—all female hybrids examined were sterile. Nevertheless, the hybridization taking place at Arroyo del Macho is a remarkable natural experiment in progress, with either evolutionary alternative—speciation vs. destabilizing hybridization—adding to an understanding of the dynamics between parthenogenetic and bisexual species in sympatric associations.

INTRODUCTION

Interspecific hybridization is the only pathway to the origin of parthenogenesis in *Cnemidophorus,* and it is an important speciation mechanism in this genus (see reviews by Dessauer and Cole, 1989; Moritz et al., 1989; Parker et al., 1989; Sites et al., 1990). With few exceptions, parthenogenetic species of *Cnemidophorus* appear to be ecologically resilient and able to coexist successfully with bisexual congeners where their ranges overlap (Case, 1990; Taylor et al., 2000). These assemblages of parthenogenetic and bisexual species represent natural experiments for testing hypotheses concerning the relative reproductive success of individuals with radically different reproductive modes in a variety of taxonomic combinations and ecological contexts (Medica, 1967; Christiansen, 1971; Schall, 1978, 1993; Cuellar, 1979, 1993; Mitchell, 1979; Paulissen et al., 1992; Price et al., 1993).

The success or failure of sympatric populations of parthenogenetic and bisexual species is influenced by a number of interactions. Destabilizing hybridization is one possibility, where genetic contributions from male congeners disrupt (in hybrids) the adaptive combination of genetic information inherited from the parthenogenetic parent (Lynch, 1984). These hybridizations represent one of the theoretical constraints affecting the establishment of a parthenogenetic species from a viable, reproductively competent, F_1 hybrid (Wright and Lowe, 1968; Maslin, 1971; Cole, 1975; Cuellar, 1977; Vrijenhoek, 1989). However, the diversity of triploid parthenogenetic species of *Cnemidophorus* (Maslin and Secoy, 1986; Vrijenhoek et al., 1989; Cole and Dessauer, 1993; Wright, 1993) is incontrovertible evidence that hybridization between diploid parthenogens and males of bisexual species occasionally generates new adaptive combinations of genetic information. Each triploid species of *Cnemidophorus* reemphasizes the importance of hybridization as a speciation mechanism in this genus by having one hybridization event (the one responsible for its origin) superimposed on another (the hybridization responsible for initiating parthenogenesis in the lineage to which its maternal parent belongs).

Cnemidophorus neotesselatus (Walker et al., 1997a) is a well-documented example of the evolution of triploidy in this genus. The origin of *C. neotesselatus* has been traced to a hybridization event between a parthenogenetic female *C. tesselatus* and a male of *C. sexlineatus* (Neaves, 1969; Neaves and Gerald, 1969; Parker and Selander, 1976; Dessauer and Cole, 1989). The maternal species, *C. tesselatus,* originated from hybridization between two bisexual species, *C. tigris marmoratus* and *C. gularis septemvittatus* (Neaves, 1969; Parker and Selander, 1976; Dessauer and Cole, 1989; Dessauer et al., 1996b). Although a haploid genome from *C. sexlineatus* was added to the allodiploid genome of *C. tesselatus,* the new allotriploid combination of genetic information was stable, and neither fertility nor development was negated. In fact, the *sexlineatus* genome may have provided ecological opportunities to *C. neotesselatus* that were not exploited by *C. tesselatus* (Parker, 1979a), as evidenced by disjunct populations of *C. neotesselatus* located west of the principal range of *C. tesselatus* (Walker et al., 1997a, 1997b; Taylor et al., 1999a).

Hybrids derived from crosses between parthenogenetic females and bisexual males are relatively rare in museum collections, and the majority of these are males, suggesting that an equivalent number of female hybrids has been overlooked. Most female hybrids are difficult to detect because of a tendency for the hybrid color pattern to resemble most closely the color pattern of the parthenogenetic parent (Lowe et al., 1970a; Walker et al., 1994; Hardy and Cole, 1998). In addition, hybridization tends to be documented by relatively few hybrids, making it easy to assume that the hybridization is not affecting the parental populations negatively.

The list below provides examples of hybridization between parthenogenetic and bisexual species of *Cnemidophorus.* The species name of the parthenogenetic parent is listed first: *C. exsanguis* × *C. inornatus* (Neaves, 1971); *C. laredoensis* × *C. gularis* (Walker et al., 1989a, 1989b, 1991a); *C. maslini* × *C. deppei* (Fritts, 1969; for current taxonomy see Moritz et al., 1992; Taylor and Cooley, 1995a, 1995b; Hernandez-Gallegos et al., 1998; Manríquez-Morán et al., 2000);

C. neomexicanus × *C. inornatus* (Axtell, 1966; Taylor and Medica, 1966; Wright and Lowe, 1967a; Christiansen and Ladman, 1968; Cole et al., 1988; for current taxonomy see Taylor and Walker, 1996b; Walker, 1997; Smith et al., 1997; International Commission on Zoological Nomenclature, 1999); *C. neomexicanus* × *C. tigris* (Dessauer et al., 2000); *C. neotesselatus* × *C. sexlineatus* (Taylor et al., 1989; Walker et al., 1990, 1994; for current taxonomy see Walker et al., 1997a); *C. sonorae* × *C. tigris punctilinealis* (Lowe et al., 1970a; for current taxonomy see Taylor and Walker, 1996a); *C. tesselatus* × *C. gularis* (Walker et al., 2000); *C. tesselatus* × *C. sexlineatus* (Walker et al., 1994); *C. tesselatus* × *C. tigris marmoratus* (Taylor et al., 1989); and *C. velox* × *C. tigris septentrionalis* (Taylor et al., 1989).

In the only field study specifically addressing the effects of hybridization on parthenogenetic and bisexual parental species, hybridization between parthenogenetic females of *C. laredoensis* and males of *C. gularis* did not affect adversely either species at several localities (Paulissen et al., 1992). Therefore, hybridization between parthenogenetic and bisexual species can (1) serve as a speciation mechanism leading to new polyploid taxa, (2) be inconsequential to the well-being of either hybridizing population, or (3) be detrimental to the perpetuation of one or both parental species because of the frequency with which it occurs.

An ideal situation for studying hybridization between parthenogenetic and bisexual species would be one in which hybridization was occurring frequently during each reproductive season. This would maximize the chance of observing an unlikely event (speciation) and provide an opportunity to determine if the hybridization were having an effect on either parental population. We recently discovered an example of natural hybridization that fulfills these conditions at Arroyo del Macho, north of Roswell, Chaves County, New Mexico. The *Cnemidophorus* assemblage at this locality includes two bisexual species (*C. inornatus* and *C. tigris marmoratus*), a triploid parthenogenetic species (*C. exsanguis*), and a diploid parthenogenetic species (*C. tesselatus* of pattern class E [Zweifel, 1965]).

As is generally true for *Cnemidophorus* hybrids, the first hybrids in this study were discovered incidental to a different project. Discovery of three color pattern classes of *Cnemidophorus tesselatus* in sympatry at Sumner Lake State Park, De Baca County, New Mexico (Taylor et al., 1997), provided the impetus for subsequent attempts to clarify the reproductive characteristics, distribution, and pattern class identity of other populations of this species in the Pecos River Drainage in neighboring Guadalupe and Chaves counties. A locality record in Zweifel (1965) led to a visit of the Arroyo del Macho site in 1996 to determine if this population of *C. tesselatus* were extant, and three individuals were collected from the western perimeter of the hybridization site. In 1997, the sample was augmented by 23 additional individuals collected closer to the center of the study area. At that time, the 26 specimens were thought to be representatives of *C. tesselatus.*

In the laboratory, internal examinations for the presence of eggs revealed that 5 of the 26 specimens had testes and vasa deferentia, suggesting that they might be hybrids. Equally interesting, and in sharp contrast to the other 17 females in the sample, 4 females had obviously abnormal (poorly developed) ovaries and oviducts, indicating that these individuals might be hybrids as well. Additional morphological evidence of their hybrid status came from a canonical variate analysis of eight meristic scalation characters (identified in Materials and Methods). Females with functional reproductive tracts and the unusual males were used as a priori groups of *C. tesselatus* and putative hybrids, respectively, and the four atypical females were included as unknowns for classification to group. The four atypical females were unequivocally assigned to the group of putative male hybrids (all Ps > 0.91) rather than to *C. tesselatus.* In addition, an external color pattern marker indicated that the nine suspected hybrids shared the same parental species—all had the fundamental color pattern of *C. tesselatus* but lacked the pale lateral stripe characterizing *C. tesselatus* at this locality.

Therefore, the Roswell area had a UFO (unidentified fecund organism) presumably

fertilizing eggs produced by *C. tesselatus*. The paternal parents of the hybrids would have to be males of either *C. inornatus* or *C. tigris marmoratus* because these are the only bisexual species of *Cnemidophorus* at the site. If the unusual lizards were hybrids they would be triploids, having inherited the full diploid complement of cloned maternal chromosomes from *C. tesselatus* plus a new haploid set of paternal chromosomes. The extraordinary ratio of 9 putative hybrids to 17 *C. tesselatus* prompted additional sampling of the *Cnemidophorus* assemblage at the Arroyo del Macho site in 1998 for karyotypic, electrophoretic, and histological analyses. Karyotypes would confirm the ploidy of the suspected hybrids and the definitive identification of the paternal species as well, because *C. inornatus* and *C. tigris marmoratus* have diagnostically distinctive haploid complements of chromosomes (Lowe et al., 1970b). Protein electrophoresis would provide similar information, augmenting the extensive databases for these taxa provided by Cole et al. (1988), Dessauer and Cole (1989), and Dessauer et al. (2000), and it would provide a more detailed assessment of genetic variation among the hybrids and their parental species. A histological component would address the reproductive functionality of male hybrids and confirm sterility of female hybrids based on what appeared to be anatomical deficiencies. Discovery of two karyotypic cytotypes in the 1998 samples of *C. tesselatus* and hybrids, and an apparent discrepancy in the ratios of the two cytotypes in the two groups, led to additional sampling of *C. tesselatus* and hybrids in 1999.

The availability of a large number of contemporary hybrids from Arroyo del Macho provides an exceptional opportunity to examine the effects of hybridization on a specific pair of unisexual and bisexual species. Goals of this study were to (1) verify the hybrid status of the atypical specimens; (2) identify the parental species; (3) determine, histologically, if fertile female hybrids were being produced, including the possibility of the origin of a new triploid parthenogenetic species; (4) provide diagnostic features of the color pattern of these hybrids and a method of identifying hybrids of similar genealogy from other localities; (5) determine univariate and multivariate patterns of morphological variation among hybrids and the parental species; (6) determine the pattern of genetic variation in the Arroyo del Macho populations of parental species and hybrids; (7) determine if intragroup morphological variation in *C. tesselatus* and hybrids is associated with cytotypic differences; and (8) describe the relationship between habitat and hybridization activity and determine the possible effects of hybridization on the interacting populations.

MATERIALS AND METHODS

Morphological Analysis

Samples of *C. tesselatus* ($N = 41$), *C. tigris marmoratus* ($N = 4$), and hybrids ($N = 20$) were collected at the Arroyo del Macho locality during four consecutive years (1996–1999). Museum numbers and specific collecting data are provided in the appendix. We collected an additional sample of 27 individuals of *C. tigris marmoratus* from a large population located approximately 18 km southeast of the Arroyo del Macho locality to improve statistical comparisons and minimize the possibility of over-collecting representatives of the small Arroyo del Macho population.

Eight meristic scalation characters were used to assess variation within and among the parental populations and their hybrids. These included SDL-T4 (the number of subdigital lamellae in the anterior row of the fourth toe of one foot) and SDL-F4 (the number of subdigital lamellae on the fourth finger of one hand). Left or right counts were chosen randomly for individuals with undamaged digits. In addition, we determined FP (the sum of femoral pores on both thighs) and GAB (the number of granular dorsal scales in a single row around the midbody). There are eight longitudinal rows of enlarged scales covering the ventral body surface. The third ventral row on either side of the midsagittal line terminates anteriorly in the axillary region. The 15th ventral scale posterior to this terminus established the point for beginning the GAB count. We also determined GS (the number of granular gular scales bordering the medial edges of the eight anterior sublabials [four on each side, or their sub-

divisions] and the posterior mental) and LSG (the sum of lateral supraocular granules on both sides of the head). These granular scales are located between the supraoculars and superciliary scales (see Burt, 1931: fig. 3), and the count includes all scales anterior to the suture line between the third and fourth supraoculars. We also scored individuals for COS (the bilateral total of circumorbital scales) as standardized by Wright and Lowe (1967a) and PSC (the sum of all scales, including occipitals, contacting the outer perimeter of parietal and interparietal scales; see Burt, 1931: fig. 2). Snout–vent length (SVL), measured to the nearest 1 mm from tip of snout to posterior edge of the preanal scales, was used to ascertain size at reproductive maturity, to determine whether the lateral stripes in hybrids were absent at hatching or lost during color pattern ontogeny, and to estimate the number of generations represented in our samples of *C. tesselatus* and hybrids.

Four meristic characters based on color pattern features were included in comparisons between two karyotypic cytotypes found in our samples of *C. tesselatus* and hybrids. A dissection microscope was used to score individuals for these characters (at 7×). One character is PSVF (pale segments in the vertebral field), a linear count of the number of pale elements (vertebral stripe fragments and other spots) occupying the midsagittal region of the vertebral dark field, a field between the two pale paravertebral stripes. The count was made from the posterior edge of the interparietal head scale to the first row of keeled scales at the base of the tail. Use of this character would be misleading for a number of other populations of *C. tesselatus* lacking pale elements in the midsagittal region of the vertebral field. Three other characters are based on the total number of interruptions of the paired longitudinal pale stripes by black pigment: IDLS (interruptions of dorsolateral stripes); IPVS (interruptions of paravertebral stripes); and, for *C. tesselatus* only, ILS (interruptions of lateral stripes). All stripe interruptions were counted irrespective of width (i.e., single rows or multiple rows of dark scales).

We used SPSS 10.0, SYSTAT 9.0, and BMDP/Dynamic 7.0 software (all from SPSS, Chicago, Illinois) for statistical analyses. The linear and quadratic discriminant analysis procedure (5M) for computing Mahalanobis D^2 distances among centroids of CV scores can be found in BMDP Statistical Software (1992); all other procedures and tests are described in Wilkinson et al. (1996) and SPSS (1999).

An absence of significant sexual dimorphism, determined by Student's *t*-tests, allowed us to pool males and females into single samples of *C. tigris marmoratus* and hybrids. Appropriate test results from either pooled or separate sample variances were selected depending on homogeneity or heterogeneity of sample variances (determined using Levene's test), respectively. We used one-way analyses of variance (ANOVA) to determine univariate differences among *C. tesselatus, C. tigris marmoratus,* and hybrids. Significant ANOVAs were followed by Tukey HSD multiple comparison tests to identify specific pairs of significantly different samples. An alternative multiple comparison test, Tamhane's T2, was substituted for Tukey's HSD for characters with heterogeneous sample variances.

We used a principal components analysis (PCA) to depict the pattern of meristic variation among hybrids and parental species. The PCA was based on the correlation matrix, and factors with eigenvalues greater than 1.0 were selected for interpretation.

Quadratic canonical variate analyses (CVAs) were used, in instances of unequal covariance matrices (as revealed by Box's *M* tests), to determine the multivariate distinctiveness of particular groups. The first CVA provided a contrast of within- and between-sample variation among *C. tesselatus, C. tigris marmoratus,* and hybrids. We used color pattern characteristics to assign individuals to a priori groups and jackknifing to classify unassigned individuals into those groups. We included in each analysis only individuals with complete data for eight meristic characters: 38 of 40 representatives of *C. tesselatus,* 26 of 31 representatives of *C. tigris marmoratus,* and 20 of 20 hybrids. We used a second quadratic canonical variate analysis to substantiate that nine male specimens, resembling *C. tesselatus* in color pattern but collected from other localities (see Speci-

mens Examined, appendix), could be identified as *C. tesselatus* × *C. tigris marmoratus* hybrids. Some of these specimens had been damaged during collection, and the number of lateral supraocular granules, number of gular scales, number of scales contacting the outer perimeters of parietal and interparietal scales, and the number of circumorbital scales could not be determined for certain individuals. The number of subdigital lamellae for the fourth finger was not being used as a character when the nine suspected hybrids were examined, so this character also was excluded from the analysis. Therefore, this CVA model was based on number of femoral pores, number of subdigital lamellae on the fourth toe, and number of granules around the midbody. Our samples of *C. tesselatus*, *C. tigris marmoratus*, and hybrids were used as a priori groups, and the nine unusual males were included in the model as unassigned for classification to group. We also ran a linear canonical variate analysis (removing three meristic characters to achieve equality of covariance matrices) to obtain Mahalanobis distances (D^2) for comparing the pattern of meristic similarity among the Arroyo del Macho hybrids and their parental species.

Karyotypic Analysis

We used previously published methods for preparing and studying standard, giemsa-stained chromosomes (Cole, 1979). For *C. tesselatus*, we examined 54 cells at mitotic metaphase from bone marrow of 10 females (see Specimens Examined, appendix). For hybrids, we examined 55 cells at mitotic metaphase from bone marrow of four females and five males, plus testicular material from one of these males.

Electrophoretic Analysis

Genotypes at 34 gene loci, based on phenotypes of tissue proteins, were determined for 11 specimens (all females) of *Cnemidophorus tesselatus*, 4 (3 males, 1 female) of *C. tigris marmoratus*, and 10 (6 males, 4 females) of suspected hybrids, all from the hybridization site near Roswell (see Specimens Examined, appendix). Methods of collecting, preparing, and storing tissue samples followed Dessauer et al. (1996a). Methods of preparing tissue homogenates, conducting electrophoresis, localizing specific proteins, and scoring gel phenotypes in the context of gene products followed Harris and Hopkinson (1976), Murphy et al. (1996), and, particularly for lizards of the genus *Cnemidophorus*, Dessauer et al. (2000).

Scoring electrophoretic phenotypes for unisexual species and for polyploid individuals can be difficult. Alleles determining the phenotype of heterozygotes are usually expressed equally, with banding patterns and densities (activities of reactions) indicating ploidy and dosage of each allele (Neaves and Gerald, 1968, 1969; Dessauer and Cole, 1984). Occasionally, however, phenotypes for individuals of unisexual species suggest that one allele at a polymorphic locus is more active than the other, even in diploid species. For example, at the PEPA locus of the diploid *C. tesselatus* of pattern class E, the d-allele is more highly expressed than the c-allele (see fig. 13). Often, scoring difficult 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 (e.g., Dessauer et al. [2000: 57, fig. 26] for three PGM loci).

The 34 loci analyzed are listed in table 5, in which the body of the table presents genotypes for the loci having alleles in the heterozygous state in *C. tesselatus* and the hybrids. Alleles detected in *C. tigris marmoratus* from the Roswell area are also listed in table 5, including those exhibiting variation, and the 15 invariant loci are footnoted. For each locus, alleles are designated in alphabetical order according to decreasing anodal migration of their allozymes. For multi-locus enzymes, loci are numbered in order of decreasing anodal migration of their isozymes.

Histological Analysis

We determined the reproductive capabilities of hybrids by a histological analysis of gonads and reproductive tracts and comparisons to individuals of *C. tesselatus* and *C. tigris marmoratus* from the hybridization locality. The histological sample consisted of 49 specimens: 3 male *Cnemidophorus tigris*

marmoratus, 26 female *Cnemidophorus tesselatus*, 8 female hybrids (*C. tesselatus* × *C. tigris marmoratus*), and 12 male hybrids (*C. tesselatus* × *C. tigris marmoratus*). After preservation in 10% formalin and storage in 75% ethanol, the following tissues were removed from the left side for histological examination: gonads, accessory reproductive ducts, adrenal gland, and kidney (part). Only a few sections were taken from each male *C. tigris marmoratus* in order to determine reproductive condition. For all other specimens, the tissues were serially sectioned. All tissues were embedded in 56–57°C embedding medium (Paraplast by Lancer) using a tertiary-butyl alcohol process (Weesner, 1960), and they were sectioned at 8 or 10 μm. Every third slide was stained with the Mallory triple connective tissue technique (Pantin method; Presnell and Schreibman, 1997) combined with Harris hematoxylin. The other slides (2 out of each 3) were stained with Harris hematoxylin and eosin (progressive method). Histological and anatomical terminology follows Hardy and Cole (1981).

Reproductive Characteristics of Parental Species

The greater number of representatives of *C. tesselatus* compared to those of *C. tigris marmoratus* at Arroyo del Macho may result from differences in reproductive output as well as availability of preferred microhabitats. Therefore, we estimated clutch size differences between *C. tesselatus* and females of *C. tigris marmoratus* by dissection and scoring individuals for numbers of yolking ovarian follicles larger than 3 mm in diameter or numbers of oviductal eggs and by direct counts of eggs laid by individuals maintained at the AMNH prior to karyotypic and electrophoretic analyses. Six representatives of *C. tesselatus* collected in 1998 were kept alive for various lengths of time in the AMNH. Four of the six laid clutches of eggs, all within 17 days of capture. These clutches, clearly established by the time of capture, were used in our statistical comparisons. Snout–vent lengths of these individuals were measured within 39 days of capture; use of these measurements was justified by the small amount of growth that could have taken place in these reproductively mature individuals in that short period. Only clutches and snout–vent lengths of individuals collected during the same 5-day period in 1998 were used in interspecific comparison.

We estimated the year that each individual *C. tesselatus* and hybrid hatched in order to determine the number of generations of *C. tesselatus* and the number of years of hybridization represented in respective samples. We used snout–vent length in conjunction with month of sampling as an index to age. The age of each individual was determined by estimating the time necessary to attain each snout–vent length (Taylor et al., 1999b)—a combination of the short period available for growth between hatching and hibernation in year one, a second growth period terminating on the date of sampling, and, for older individuals, the approximate number of full annual growth periods in intervening years. We recognize that different hatching dates and variable growth rates introduce uncertainty with respect to the assignment of certain individuals to their appropriate cohort (Walker et al., 1991b), but this degree of precision is not necessary for estimating the number of generations represented in each yearly sample.

RESULTS AND DISCUSSION

Study Site, Habitat, and Microhabitats

The hybridization site is located on the northwest rim of Eden Valley (33°39′1″N, 104°33′17″W), immediately east of highway U.S. 285, and approximately 29 km north of the county courthouse in Roswell, Chaves County, New Mexico. Arroyo del Macho (Cienega del Macho on certain maps) trends west-to-east and delimits the southern boundary of the hybridization site before opening into Eden Valley. A different major drainage channel named Arroyo del Macho is located east of the study area and carries precipitation runoff southward through Eden Valley to Salt Creek, a tributary of the Pecos River.

The general habitat of the hybridization site (fig. 1) is semidesert grassland (Brown, 1982), with terrain ranging from low rocky hills and slopes to intervening flats variously modified by livestock trails, a county road,

Fig. 1. **A.** A view to the east showing the principal gypsum outcrop and south-facing slope at the hybridization locality north of Roswell, Chaves County, New Mexico. Scattered dark shrubs in the center of the figure are mesquite (*Prosopis juliflora*) in a desert grassland; *C. exsanguis* and *C. inornatus* were

several unmaintained farm roads, and a short levee. The habitat is open and approximately 50% bare soil or rock. The balance is occupied by grasses, including Burro grass (*Scleropogon brevifolius*), Muhly (*Muhlenbergia repens*), and Blue gramma (*Bouteloua gracilis*), scattered cacti (*Opuntia* spp.), annual weeds, and a small assortment of shrubs. Mesquite (*Prosopis juliflora*) is an important microhabitat component at the center of the hybridization area 0.8 km east of highway U.S. 285. Scattered patches of creosote bush (*Larrea tridentata*) provided an alternative microhabitat that facilitates contacts between hybridizing species. Saltbush (*Atriplex canescens*) was represented by scattered individuals on hills and slopes, while stands of *Acacia* sp. delimited the eastern boundary of the study area. Although whiptail lizards were observed foraging around individual *Atriplex* and using basal burrows for cover, they were not regularly seen in *Acacia*.

Immediately north of Arroyo del Macho, the south-facing slope of an elevated promontory (fig. 1A) parallels the arroyo for approximately 1 km, providing a rather abrupt elevation gain of about 9 m (1119 to 1128 m elevation). We found the greatest numbers of *C. tesselatus* and hybrids in the vicinity of this salient topographic feature. In 1997, many individuals of *C. tesselatus* were also collected in mesquite and Russian thistle (*Salsola kali*) along the margins of Eden Valley Road within the hybridization area. We found a few representatives of *C. tesselatus* in stands of creosote bush, but they were more common around mesquite, on open slopes, and near small, weedy, erosion channels. In contrast, *C. tigris marmoratus* was predictably found only in patches of creosote bush. One patch at the base of the principal south-facing slope (fig. 1B) may have facilitated contacts between individuals of *C. tesselatus* and *C. tigris marmoratus* based on the numbers of hybrids and *C. tesselatus* collected in its vicinity. Representatives of *C. exsanguis* and *C. inornatus* were found throughout the study area, with the latter species using grass-dominated sites to a greater extent than its congeners. Daily contacts among members of all four species and the hybrid group could easily occur. Evidence of one such encounter consisted of an adult representative of *C. inornatus* that had been regurgitated by one of two hybrids in a collecting bag.

The *Cnemidophorus* Assemblage

Each of the four species of *Cnemidophorus* at Arroyo del Macho has a distinctive color pattern and can be reliably identified in the field prior to capture. Hybrids collected in 1996 and 1997 were initially identified as representatives of *C. tesselatus*, which they most closely resemble, prior to dissecting all individuals for an assessment of reproductive condition. Subsequently, consistent color pattern features of the hybrids were recognized that both diagnose their hybrid status and identify their parental species. While some hybrids can be approached closely enough for positive identification in the field, all hybrids can be identified as such subsequent to capture.

Cnemidophorus exsanguis

Parthenogenetic *C. exsanguis* is a medium-sized triploid species. Our sample of 11 representatives had a mean snout–vent length (in mm ± SE, range limits) of 74.1 ± 4.1, 58–98. The species is characterized by three pairs of pale longitudinal stripes (paravertebrals, dorsolaterals, and laterals), all with straight margins, alternating with brown or reddish-brown dark fields. Numerous pale spots are superimposed on the stripes and dark fields. Pale stripes of large individuals may become obscure, but conspicuous spots on a fundamentally brown dorsum is a per-

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common, *C. tesselatus* was rare, and *C. tigris marmoratus* was absent. The dark patch of shrubs partially cupped by the distant rocky outcrop is creosote bush (*Larrea tridentata*); a few individuals of *C. tigris marmoratus* were using this patch of habitat, and numerous *C. tesselatus* and hybrids were found in this area. **B.** A closer view of the patch of creosote bush (shown in A) surrounded by preferred microhabitat of *C. tesselatus* and hybrids. Other scattered patches of creosote bush were located north and east of this escarpment. Representatives of *C. tigris* were essentially restricted to the patches of creosote bush.

sistent feature of the pattern. A pale vertebral line (or its fragments) is missing in older adults, and the pale ventral surfaces are unspotted. Hybrids did not exhibit any of the basic color pattern features of *C. exsanguis*.

Cnemidophorus inornatus

This is the smallest of the four species at Arroyo del Macho; snout–vent length = 57.1 ±1.13, 50–65 (18). Males and females have similar body sizes (t_{16} = 0.876, P = 0.39). This species is distinguished by a sharply contrasting dorsal pattern of unspotted dark fields and pale stripes with straight margins. In the Arroyo del Macho population, the number of pale stripes may be 6 (no vertebral stripe; 2 of 18 specimens, 11%), 6.5 (vertebral stripe to midbody; 1 of 18 specimens, 6%), or 7 (a complete vertebral stripe; 15 of 18 specimens, 83%). The unspotted ventral surface is embellished with blue pigmentation of variable intensity, which is usually quite striking in males.

Cnemidophorus inornatus was a candidate for the paternal parent of the hybrids because of its abundance at Arroyo del Macho (thus opportunities for many contacts with individuals of *C. tesselatus*) and because of several attempted copulations in captivity between males of *C. inornatus* and individuals of *C. tesselatus* (Neaves, 1971). In addition, *C. inornatus* has hybridized in the past with species of both reproductive modes—*C. tigris marmoratus* (reviewed by Cole et al., 1988; Dessauer et al., 2000) and *C. neomexicanus* (reviewed by Taylor and Walker, 1996b).

Cnemidophorus tesselatus

This is the largest of the four species at Arroyo del Macho; snout–vent length = 88.6 ± 1.66, 59–109 (40). Zweifel (1965) provided the original descriptions of color pattern variation among populations presently assigned to pattern class E. This variation involved the degree to which the lateral stripes (a pair of pale stripes extending from dorsal edge of the ear opening to anterodorsal aspect of the thigh) were disrupted by vertical black bars. The lateral stripes were absent in some populations while other populations had a predominance of individuals with relatively intact lateral stripes, resembling pattern class C in this respect. This variation within pattern class E (as presently conceived) has been dealt with, consistently and conveniently, by using Zweifel's (1965) depiction of the geographic distributions of color pattern classes, rather than actual color pattern characteristics, to assign certain individuals and populations to pattern class. As an example of the problem, refer to Dessauer and Cole (1989: fig. 1A, 1B). Although they referred the individual in their figure 1A to pattern class C, this specimen would be identified as a representative of pattern class E (with relatively intact lateral stripes) based on its sampling locality in the northern Rio Grande drainage (Zweifel, 1965: fig. 1). This problem is currently under study.

The Arroyo del Macho population of *C. tesselatus* is distinguished from the other three sympatric species of *Cnemidophorus* by a sharply contrasting dorsal pattern of pale stripes and their transverse expansions on black dark fields (figs. 2A, 4). One color pattern element, evidence of lateral stripes, can be used to distinguish members of *C. tesselatus* from hybrids at this locality. Ontogenetic development can reduce definition of the lateral stripes in two ways. Fundamentally, dark fields subtending the lateral stripes can become subdivided into linear series of dark-field remnants through localized loss of melanin; this produces a series of transverse pale bars of the same hue as the stripes. Because pale bars and dark-field remnants can be in register above and below the stripes, there is loss of stripe definition where pale bars pass through the stripes. Similarly, stripes are disrupted by black bars where localized production of melanin in the pale stripes causes fusion of dark-field remnants above and below the stripes. However, the disruption of lateral stripes is reduced in the Arroyo del Macho population, and evidence of lateral stripes persists in all individuals of this population. Individual variation in dorsal color pattern is particularly evident in the vertebral dark field (fig. 4). A vertebral stripe is usually evident on the nape, extending posteriorly for variable distances before fragmenting into a series of pale elements. Lateral expansions of the vertebral stripe or its fragmented elements establish a sequence of contacts with the paravertebral stripes. These

expansions can be highly exaggerated in the final color pattern as a longitudinal series of transverse pale bars connecting the paravertebral stripes to form a ladderlike pattern. The ventral surfaces are immaculate or nearly so (fig. 3A); i.e., there are no black spots in the central gular region, although there may be one or two spots laterally. A few small black spots (often confined to single scales) may be scattered on the chest.

Cnemidophorus tigris marmoratus

There is sexual dimorphism in body size, with males being significantly larger than females in our sample ($t_{29} = 2.469$, $P = 0.02$); snout–vent length of males = 80.7 ± 1.81, 63–92 (21); snout–vent length of females = 73.3 ± 2.24, 57–80 (10). Individual variation in this subspecies can be observed in the relative prominence of pale stripes or their remnants in the dorsal pattern, ranging from clearly evident stripes to a loss of stripe identity in an irregular reticulum of pale and dark sectors. All stripes may be fragmented to various degrees. The double nature of the pale vertebral stripe (or its remnants) is usually evident, thus resembling its hybrid derivatives, pattern classes Colorado D and New Mexico D of *C. tesselatus,* in this feature. Pale stripes, or significant segments thereof, are retained in the dorsal pattern of many adult individuals of *C. tigris marmoratus* in the Pecos River drainage, and these populations were described as *C. marmoratus reticuloriens* by Hendricks and Dixon (1986). All individuals in our samples lack lateral stripes (fig. 2C). Pastel hues embellish the ventral pigmentation, with pink shades added to the gray throat and chest, shifting to orange or tan shades on the ventrolateral areas of the abdomen. Individuals larger than 78 mm snout–vent length have black spots and blotches on the gular and chest regions (fig. 3C), with considerable variation in their size and number. Although *C. tigris marmoratus* was uncommon at the hybridization site, it was a strong candidate for the paternal parent of the hybrids because of two shared color pattern features (see below).

Hybrids

Males and females had similar snout–vent lengths ($t_{18} = 0.194$, $P = 0.85$): males = 89.2 ± 3.15, 56–97 (12) and females = 88.4 ± 2.85, 71–97 (8). The dorsal color pattern (fig. 2B) is most similar to *C. tesselatus* (fig. 2A), but the hybrid pattern tends to be coarser, with larger dark and pale sectors (compare figs. 4–6). There is no vertebral stripe, and, from the shoulder region posteriorly, the vertebral dark field is either rather open or bridged by transverse pale elements. Ventrally, hybrids are intermediate to parental species in degree of black-spotting (fig. 3B). As currently understood (Lowe et al., 1970a; Cuellar and McKinney, 1976; Taylor et al., 1989; Walker et al., 1989a, 1989b), a lizard with male reproductive structures that has a color pattern resembling a normal parthenogenetic species is either a triploid or tetraploid hybrid—cytological states that are attained only through fertilization of unreduced eggs from diploid or triploid parthenogens by haploid, Y-bearing sperm from males of bisexual species.

Color Pattern and Identification of Hybrids

Although there is color pattern variation among the hybrids (figs. 5, 6), all bear a strong resemblance to their maternal parent, *C. tesselatus* (fig. 4). There are, however, several color pattern features that distinguish hybrids from *C. tesselatus* and implicate *C. tigris marmoratus* as their paternal parent. The most obvious difference is dichotomous—lateral stripes (or obvious remnants) are present in the Arroyo del Macho population of *C. tesselatus* and absent in hybrids and *C. tigris marmoratus* (fig. 2). In addition, some hybrids (particularly males) have subtle suffusions of pink on the throat and chest and yellowish-tan on the lateral abdominal surfaces. These are pigmentation features of *C. tigris marmoratus.* Certain hybrids also resemble *C. tigris marmoratus* in having small black spots in the central gular region and on the chest. However, the intensity of pastel hues and the size and number of black ventral spots are reduced in such hybrids compared to *C. tigris marmoratus* (figs. 2, 3, 5, 6).

As a group, hybrids differ from *C. tesselatus* in having a greater number of interruptions of the dorsolateral stripes (IDLS; $t_{26.747}$

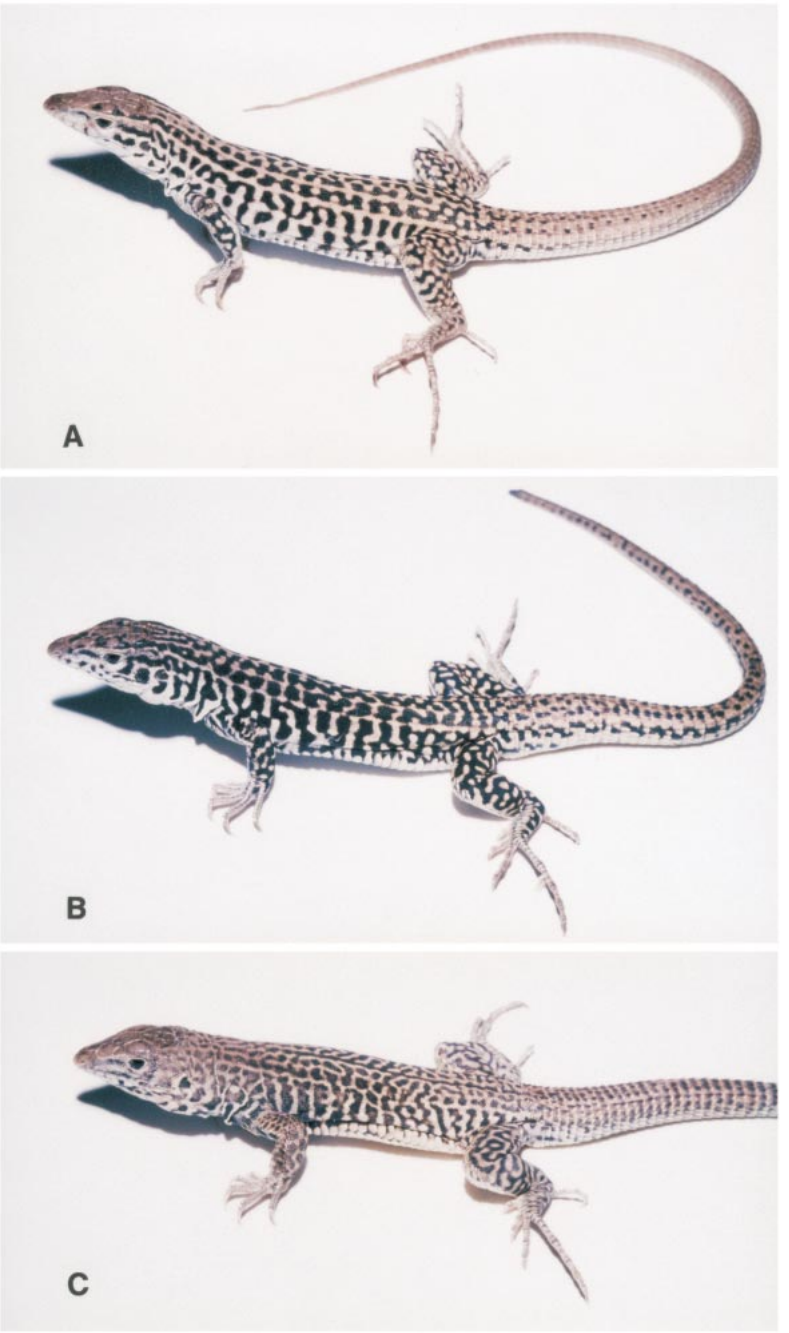


Fig. 2. Dorsolateral views of three representative whiptail lizards (*Cnemidophorus*) from Arroyo del Macho. **A.** Diploid unisexual *C. tesselatus,* AMNH R-146638, body length 97 mm. **B.** Triploid *C. tesselatus* × *C. tigris marmoratus* hybrid male, AMNH R-146693, body length 100 mm. **C.** Diploid bisexual *C. tigris marmoratus* male, AMNH R-146653, body length 94 mm. All photographed June 19, 1998.

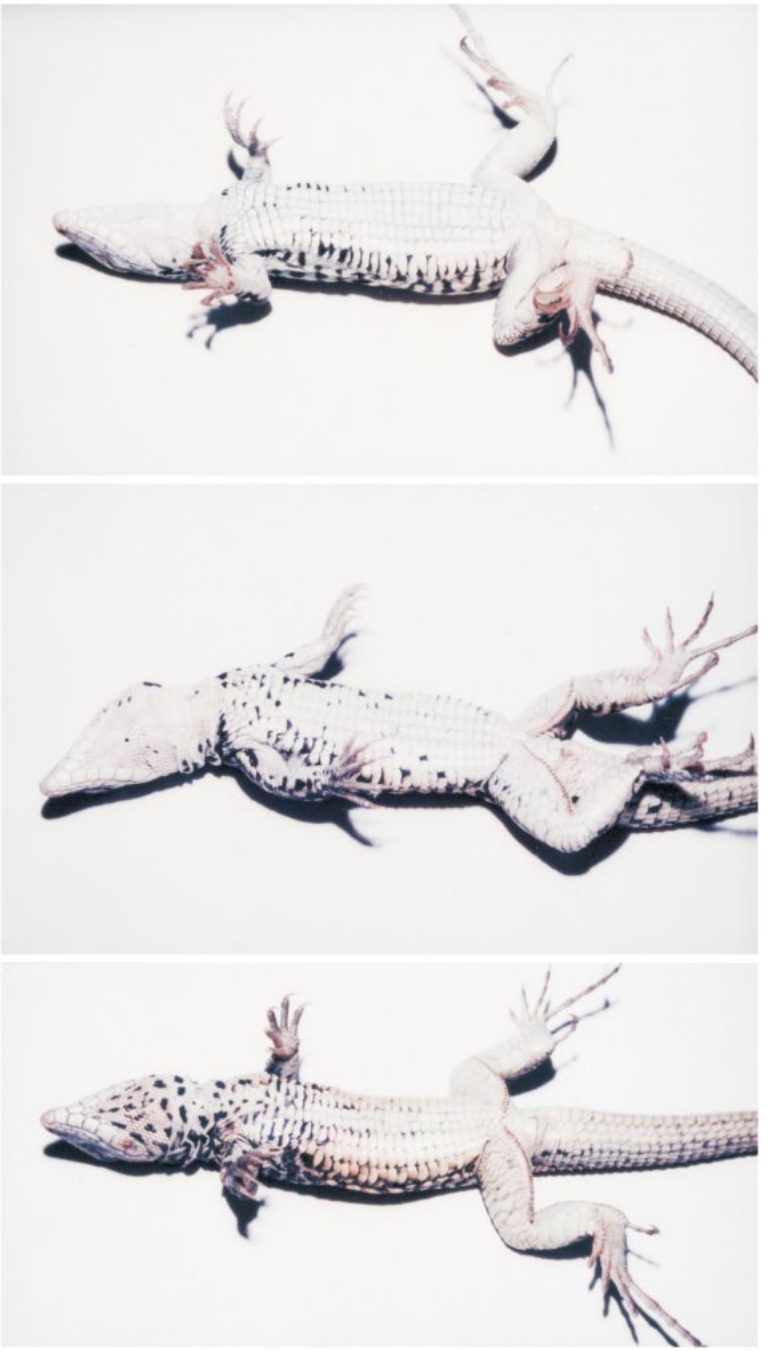


Fig. 3. Ventral view of the same lizards arranged in the same sequence as in figure 2.

= 5.438, $P < 0.0005$; table 1) and paravertebral stripes (IPVS; $t_{58} = 5.730$, $P < 0.0005$; table 1). The presence of a stripe or smaller pale segments in the vertebral dark field of *C. tesselatus* (fig. 4) and a more open vertebral dark field in hybrids (figs. 5, 6) will distinguish many individual hybrids from *C. tesselatus*. However, a quantification of this

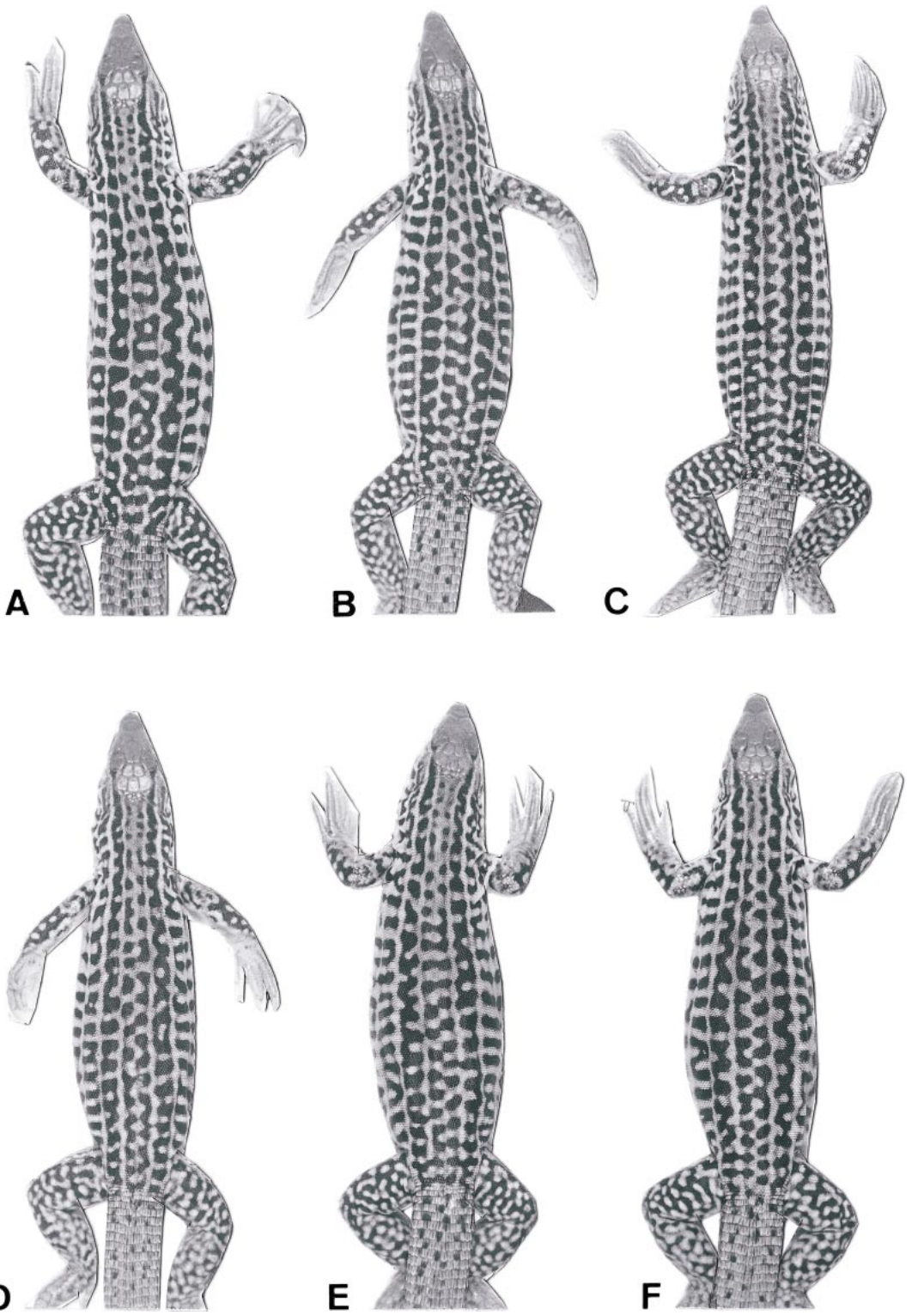


Fig. 4. Representatives of *Cnemidophorus tesselatus* from the Arroyo del Macho hybridization site. **A.** AMNH R-146615 (91 mm SVL); **B.** AMNH R-146618 (83 mm SVL); **C.** AMNH R-146619 (85 mm SVL); **D.** AMNH R-146623 (86 mm SVL); **E.** AMNH R-146627 (89 mm SVL); **F.** AMNH R-146626 (90 mm SVL).

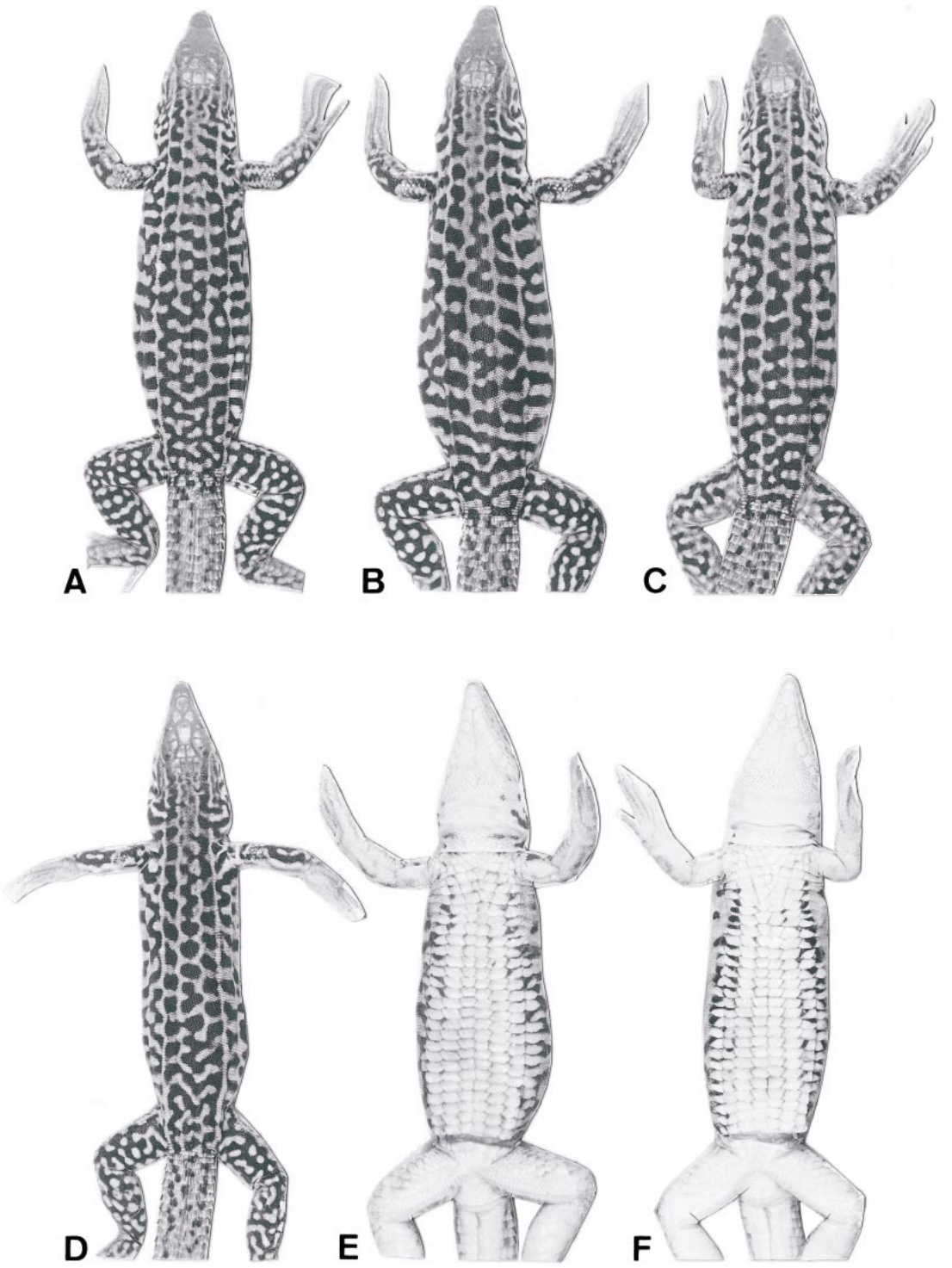
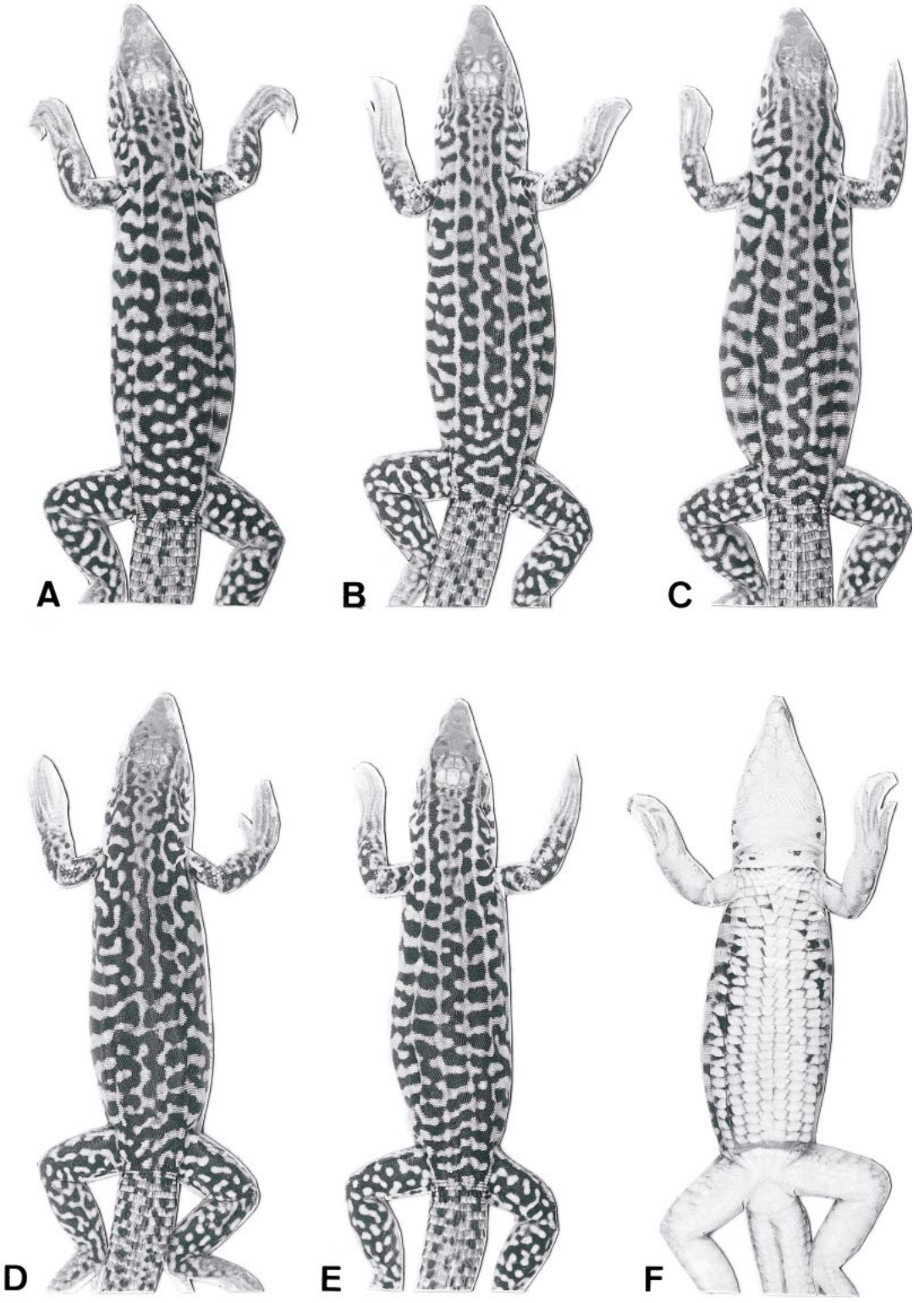


Fig. 5. Representative *Cnemidophorus tesselatus* × *C. tigris marmoratus* hybrid females from the Arroyo del Macho hybridization site. **A.** AMNH R-146689 (89 mm SVL); **B** and **E.** AMNH R-146687 (93 mm SVL); **C** and **F.** AMNH R-146688 (91 mm SVL); **D.** AMNH R-146681 (84 mm SVL).


A
B
C
D
E
F

feature (PSVF, number of midsagittal pale segments in the vertebral dark field) did not distinguish the two groups ($t_{55} = 1.754$, $P = 0.08$; table 1).

Univariate Morphological Comparisons

Sexual dimorphism was lacking in meristic characters of the hybrids (all Ps > 0.09) and representatives of *C. tigris marmoratus* (all Ps > 0.14) from the vicinity of Arroyo del Macho, permitting us to pool the sexes into single samples for each group. For seven of the eight scalation characters, there was one significant difference among the pairwise comparisons of hybrids, *C. tesselatus*, and *C. tigris marmoratus* (table 1). Hybrids differed significantly from both parental species in number of granules around midbody and in subdigital finger lamellae. However, the pattern of nonsignificant differences (resemblance) between hybrids and parental species was also informative. All 20 hybrids from Arroyo del Macho had abruptly enlarged mesoptychial scales (those bordering the gular fold anteriorly), thereby resembling *C. tesselatus* rather than *C. tigris marmoratus* in this character. Hybrids also resembled *C. tesselatus* in number of subdigital toe lamellae and gular scales, but resembled *C. tigris marmoratus* in number of femoral pores, circumorbital scales, and lateral supraocular granules (table 1). *Cnemidophorus tesselatus*, itself a product of past hybridization, also exhibited a mosaic pattern, resembling its maternal progenitor (*C. tigris marmoratus*) in some characters and its paternal progenitor (*C. gularis septemvittatus*) in others (Parker, 1979b).

Multivariate Morphological Analyses

Principal Components Analysis

The first three principal components summarized 64.1% of the variation found in eight meristic characters used in the PCA of *C. tesselatus*, *C. tigris marmoratus*, and their hybrids. Significant differences in principal components scores (treating these variables as univariate characters) among the three groups are shown in table 1. A comparison of component loadings (table 2) to characters with nonsignificant differences between hybrids and one of the two parental groups (table 1) indicated that principal component 1 was expressing similarities of hybrids to *C. tigris marmoratus* in number of femoral pores, circumorbital scales, and lateral supraocular granules. Principal component 2 expressed similarities of hybrids to *C. tesselatus* in number of subdigital toe lamellae and gular scales.

The pattern of variation was depicted in an ordination of principal components scores on the first two axes (fig. 7). There was considerable overlap in principal components scores of all three groups, presumably reflecting shared parentage; i.e., that *C. tigris marmoratus* was the maternal parental species of *C. tesselatus* and the paternal parental species of the hybrids from Arroyo del Macho. The third principal component reflected the distinctively low numbers of granules around midbody in hybrids compared to both parental species (table 1), and it effectively separated hybrids from parental species on this axis.

Phylogenetically (because *C. tesselatus* is also a hybrid derivative of *C. tigris marmoratus*), the hybrids at Arroyo del Macho have approximately 67% of their genes from *C. tigris marmoratus* and approximately 33% of their genes from *C. gularis septemvittatus*. Proximately, and more important in terms of phenotypic expression, the triploid hybrids have 100% of the genes of *C. tesselatus* and 50% of the genes of *C. tigris marmoratus*; the matriclinous nature of the multivariate expression of meristic characters is revealed by the canonical variate analysis that follows.

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Fig. 6. Representative *Cnemidophorus tesselatus* × *C. tigris marmoratus* hybrid males from the Arroyo del Macho hybridization site. **A.** AMNH R-146682 (93 mm SVL); **B** and **F.** AMNH R-146683 (89 mm SVL); **C.** AMNH R-146685 (93 mm SVL); **D.** AMNH R-146684 (87 mm SVL); **E.** AMNH R-146686 (88 mm SVL).

TABLE 1

Descriptive Statistics of Morphological Characters and Tests of Significance[a] Among the Parthenogenetic Species *Cnemidophorus tesselatus*, the Bisexual Species *C. tigris marmoratus*, and Their Hybrids

Character[b]	Sample [sample size] Mean ± SE (range limits)		
	C. tigris [31]	Hybrids [20]	*C. tesselatus* [39]
SDL-T4	32.2 ± 0.33 (26–36)	36.8 ± 0.32 (35–40)	37.0 ± 0.23 (35–42)
	C. tesselatus [40]	Hybrids [20]	*C. tigris* [31]
FP	41.4 ± 0.28 (37–46)	44.8 ± 0.44 (41–48)	45.4 ± 0.43 (41–50)
	C. tesselatus [39]	Hybrids [20]	*C. tigris* [30]
GS	19.5 ± 0.27 (16–23)	20.2 ± 0.52 (17–26)	22.1 ± 0.42 (17–29)
	C. tesselatus [40]	Hybrids [20]	*C. tigris* [28]
LSG	34.8 ± 0.75 (23–45)	40.3 ± 1.31 (33–53)	42.0 ± 2.04 (26–65)
	Hybrids [20]	*C. tigris* [28]	*C. tesselatus* [39]
GAB	84.8 ± 1.00 (76–90)	91.4 ± 0.86 (83–101)	93.5 ± 0.98 (87–109)
	C. tigris [31]	*C. tesselatus* [39]	Hybrids [20]
SDL-F4	16.6 ± 0.15 (15–18)	16.9 ± 0.10 (16–18)	17.6 ± 0.13 (17–19)
	C. tesselatus [40]	*C. tigris* [30]	Hybrids [20]
COS	18.0 ± 0.24 (14–20)	19.3 ± 0.63 (12–28)	19.9 ± 0.62 (13–25)
	Hybrids [20]	*C. tigris* [30]	*C. tesselatus* [40]
PSC	21.0 ± 0.25 (19–23)	21.0 ± 0.35 (18–25)	21.3 ± 0.25 (18–24)
	C. tesselatus [38]	Hybrids [20]	*C. tigris* [26]
PC1	−0.62 ± 0.108 (−1.83 to 0.82)	0.23 ± 0.166 (−1.12 to 1.60)	0.73 ± 0.201 (−1.27 to 2.78)
	C. tigris [26]	*C. tesselatus* [38]	Hybrids [20]
PC2	−1.03 ± 0.190 (−3.24 to 0.48)	0.46 ± 0.10 (−0.46 to 2.31)	0.47 ± 0.119 (−0.31 to 1.67)
	Hybrids [20]	*C. tigris* [26]	*C. tesselatus* [38]
PC3	−1.09 ± 0.157 (−2.23 to 0.42)	0.16 ± 0.153 (−1.80 to 1.58)	0.46 ± 0.135 (−0.87 to 2.22)
	C. tigris [26]	Hybrids [20]	*C. tesselatus* [38]
CV1	−2.96 ± 0.235 (−6.23 to −0.44)	0.58 ± 0.220 (−0.93 to 2.61)	1.72 ± 0.138 (0.20 to 3.93)
	Hybrids [20]	*C. tigris* [26]	*C. tesselatus* [38]
CV2	−2.50 ± 0.256 (−4.55 to −0.24)	0.47 ± 0.191 (−1.19 to 2.40)	1.00 ± 0.151 (−0.97 to 2.80)
	C. tesselatus [40]	Hybrids [20]	
PSVF	8.8 ± 0.57 (3–16)	10.3 ± 0.57 (6–15)	
	C. tesselatus [40]	Hybrids [20]	
IDLS	3.5 ± 0.81 (0–19)	14.4 ± 1.83 (2–28)	
	C. tesselatus [37]	Hybrids [20]	
IPVS	7.2 ± 0.78 (2–22)	15.6 ± 1.37 (6–23)	

[a] Underlined means are not significantly different at $\alpha = 0.05$ as determined from analyses of variance followed by Tukey's HSD or Tamhane's T2 multiple comparison tests.

[b] The characters are as follows: SDL-T4, number of subdigital lamellae on fourth toe of one foot; FP, total number of femoral pores; GS, number of gular scales; LSG, number of lateral supraocular granules; GAB, number of granules (scales) around midbody; SDL-F4, number of subdigital lamellae on fourth finger of one hand; COS, number of circumorbital scales; PSC, number of scales contacting the outer perimeter of parietal and interparietal scales; PC1, PC2, and PC3, scores on the first three principal components; CV1 and CV2, scores on the first two canonical axes; PSVF, number of midsagittal pale segments in the vertebral dark field; IDLS, total number of interruptions of the dorsolateral pale stripes; and IPVS, total number of interruptions of the paravertebral pale stripes.

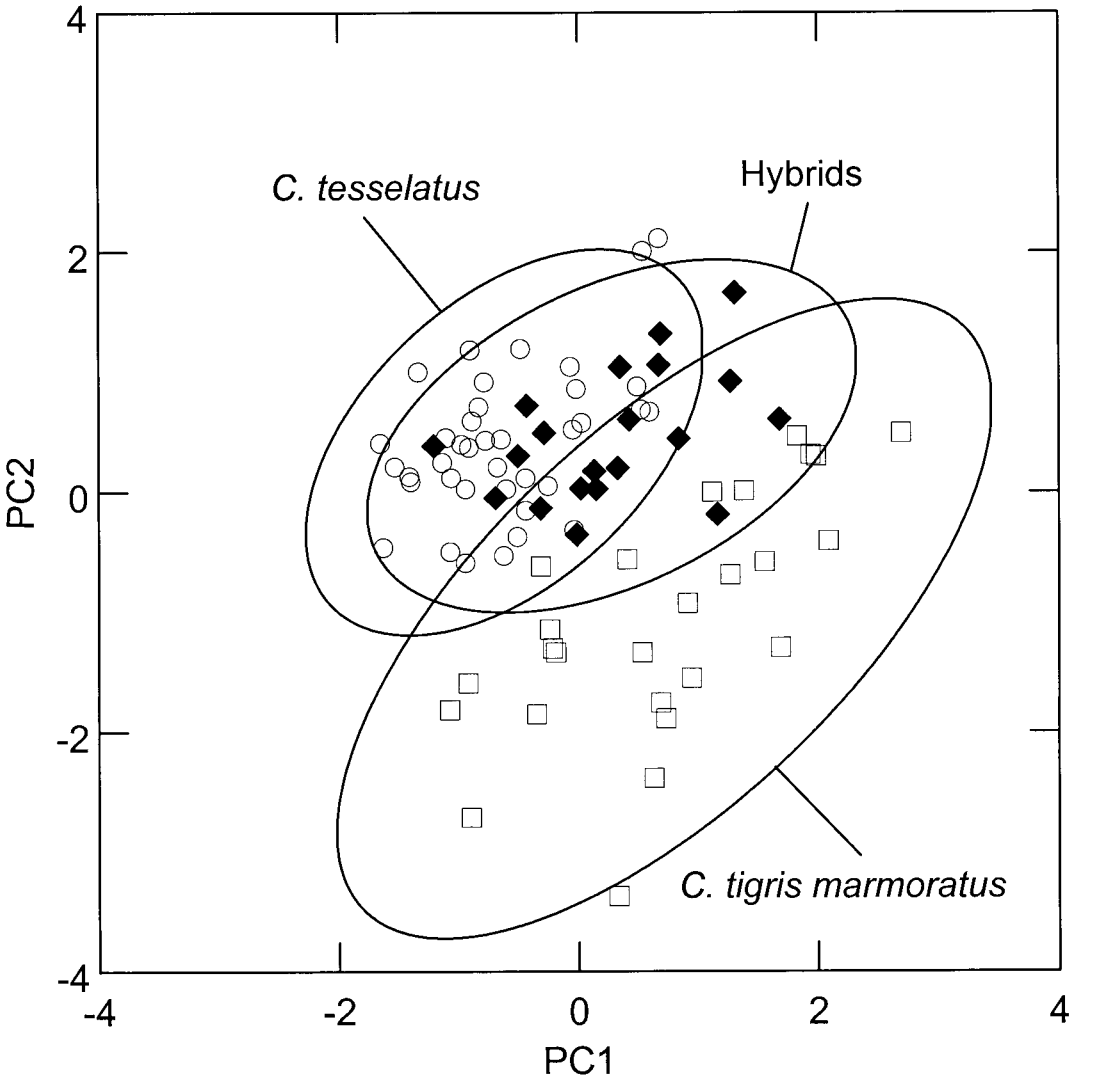

Fig. 7. Pattern of morphological variation expressed by the distribution of scores on the first two principal components extracted from a correlation matrix of eight meristic characters. Symbols depict scores of 84 individuals collected in the vicinity of Arroyo del Macho, Chaves Co., New Mexico: ○, 38 *C. tesselatus*; □, 26 *C. tigris marmoratus*; ◆, 20 hybrids. Ellipses define the 95% confidence limits of each group.

Canonical Variate Analyses

Each specimen was assigned to its correct a priori group in a quadratic canonical variate analysis of eight meristic characters, and hybrids were significantly different from both parental species in both canonical variates (table 1). The separation of the three groups on discriminant axes is depicted in figure 8. We ran a second canonical variate analysis (linear) with three characters excluded from the model (number of circumorbital scales, number of lateral supraocular granules, and number of gular scales) in order to achieve homogeneous covariance matrices (P = 0.06). This analysis provided squared Mahalanobis distances (D^2) among centroids of the three a priori groups for quantifying the degree of multivariate resemblance among

TABLE 2

Factor Loadings for Three Principal Components Derived from Meristic Variation Among the Parthenogenetic Species *Cnemidophorus tesselatus*, the Bisexual Species *C. tigris marmoratus*, and Their Hybrids

Character[a]	PC1	PC2	PC3
LSG	0.823	0.123	0.108
FP	0.771	−0.166	0.220
COS	0.712	0.212	0.252
SDL-T4	−0.301	0.826	−0.044
SDL-F4	0.355	0.606	0.232
GS	0.471	−0.536	−0.376
GAB	0.253	0.222	−0.763
PSC	0.323	0.329	−0.507
Eigenvalue	2.385	1.582	1.161
Total explained variation	29.8%	19.8%	14.5%

[a] Characters are defined in table 1.

hybrids and parental species. The hybrid group resembled maternal *C. tesselatus* more closely than paternal *C. tigris marmoratus*—D^2 values were 11.0 between hybrids and *C. tesselatus* and 19.9 between hybrids and *C. tigris marmoratus.* In comparison, the D^2 value between *C. tesselatus* and *C. tigris marmoratus* was 18.9. Because *C. tesselatus* has one set of chromosomes from *C. tigris marmoratus* and the hybrids have two sets of chromosomes from *C. tigris marmoratus,* one might assume that the resemblance to *C. tigris marmoratus* would increase (i.e., D^2 values would decrease) with each additional set of chromosomes inherited from this taxon. However, the distances of 18.9 (one set of *C. tigris marmoratus* chromosomes in *C. tesselatus*) and 19.9 (two sets of *C. tigris marmoratus* chromosomes in the hybrids) are evidence of an absence of significant additive interactions among the *tigris* genomes and substantiate the matriclinous nature of phenotypic expression in the hybrids; i.e., there is a disproportionate resemblance of hybrids to the female parent (see also Parker, 1979b).

The overall dorsal color pattern of the Arroyo del Macho hybrids most closely resembles that of the maternal parent, *C. tesselatus.* As an example of the unpredictability that seems to be associated with the genus *Cnemidophorus,* there is an example where the opposite is true. Hybrids between the diploid parthenogen *C. neomexicanus* and the bisexual *C. tigris* have the greatest color pattern resemblance to their paternal parent, *C. tigris,* in an area where *C. tigris* is highly polymorphic (Dessauer et al., 2000). These hybrids, like those from Arroyo del Macho, have two sets of *tigris* chromosomes, with *C. neomexicanus* rather than *C. tesselatus* contributing one of the two sets.

Karyotypic Analysis

Only one clearly resolved karyotype of the allodiploid *C. tesselatus* has been published (Dessauer and Cole, 1989: 57). However, additional studies of karyotypes (Wright and Lowe, 1967b; Lowe et al., 1970b) and of protein electrophoresis (Neaves and Gerald, 1968, 1969; Neaves, 1969; Parker and Selander, 1976; Dessauer and Cole, 1989) reveal that the diploid unisexual *C. tesselatus* had a hybrid origin involving *C. tigris marmoratus* × *C. gularis septemvittatus.*

Cnemidophorus tigris marmoratus and *C. gularis septemvittatus* belong to the *tigris* and *sexlineatus* species groups, respectively. Each species group has a diagnostically distinctive karyotype (Lowe et al., 1970b). The *C. tigris marmoratus* complement ($n = 23$) consists of three large set I biarmed macrochromosomes + eight smaller set II biarmed intermediate-sized macrochromosomes + 12 set III microchromosomes. The second largest chromosome in set I of *C. tigris* has a dotlike satellite on the end of one arm, which is often difficult to see, and the third largest chromosome is the sex chromosome (Cole et al., 1969; Bull, 1978), of which the X-chromosome is recognizable in the karyotype of *C. tesselatus.* The complement from *C. gularis septemvittatus* ($n = 23$) consists of only one large set I metacentric macrochromosome (with a subterminal secondary constriction on one arm followed by an elongate satellite) + 12 smaller set II intermediate-sized telocentric or subtelocentric macrochromosomes + 10 set III microchromosomes. The sex chromosomes of *C. gularis septemvittatus* are not morphologically recognizable. The secondary constrictions on the set I chromosomes are the nucleolar organizer regions (Ward and Cole, 1986).

As expected, three representatives of *C.*

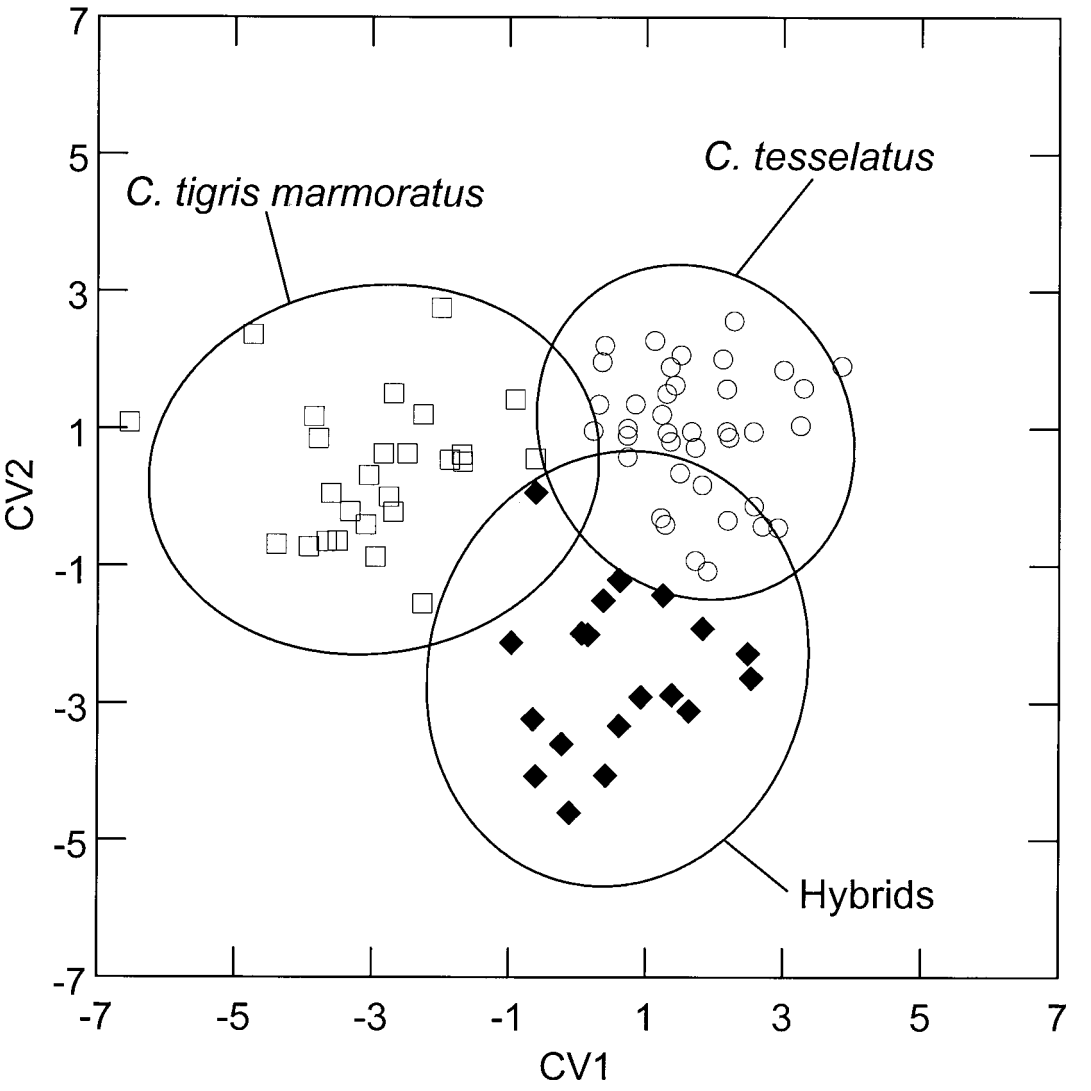

Fig. 8. Pattern of morphological distinctiveness expressed by the distribution of canonical variate scores derived from a quadratic canonical variate analysis of eight meristic characters. Symbols depict 84 individuals collected in the vicinity of Arroyo del Macho, Chaves Co., New Mexico: ○, 38 *C. tesselatus*; □, 26 *C. tigris marmoratus*; ♦, 20 hybrids. Ellipses define the 95% confidence limits of each group.

tesselatus from Arroyo del Macho had a diploid karyotype consisting of one normal *tigris* group haploid complement and one normal *sexlineatus* group haploid complement of chromosomes, or $2n = 46$ (fig. 9A). The other seven *C. tesselatus* from Arroyo del Macho that were karyotyped were all of a slightly derived karyotypic clone ($2n = 47$) in which the X-chromosome of *C. tigris* had apparently undergone centric fission, as it was represented by two telocentric chromosomes, each being the size of one of the arms of the ancestral X. Such karyotypic variants among parthenogenetic species are known to perpetuate themselves through cloning (Cole, 1979).

Nine suspected hybrids of *C. tesselatus* × *C. tigris marmoratus* from the Arroyo del

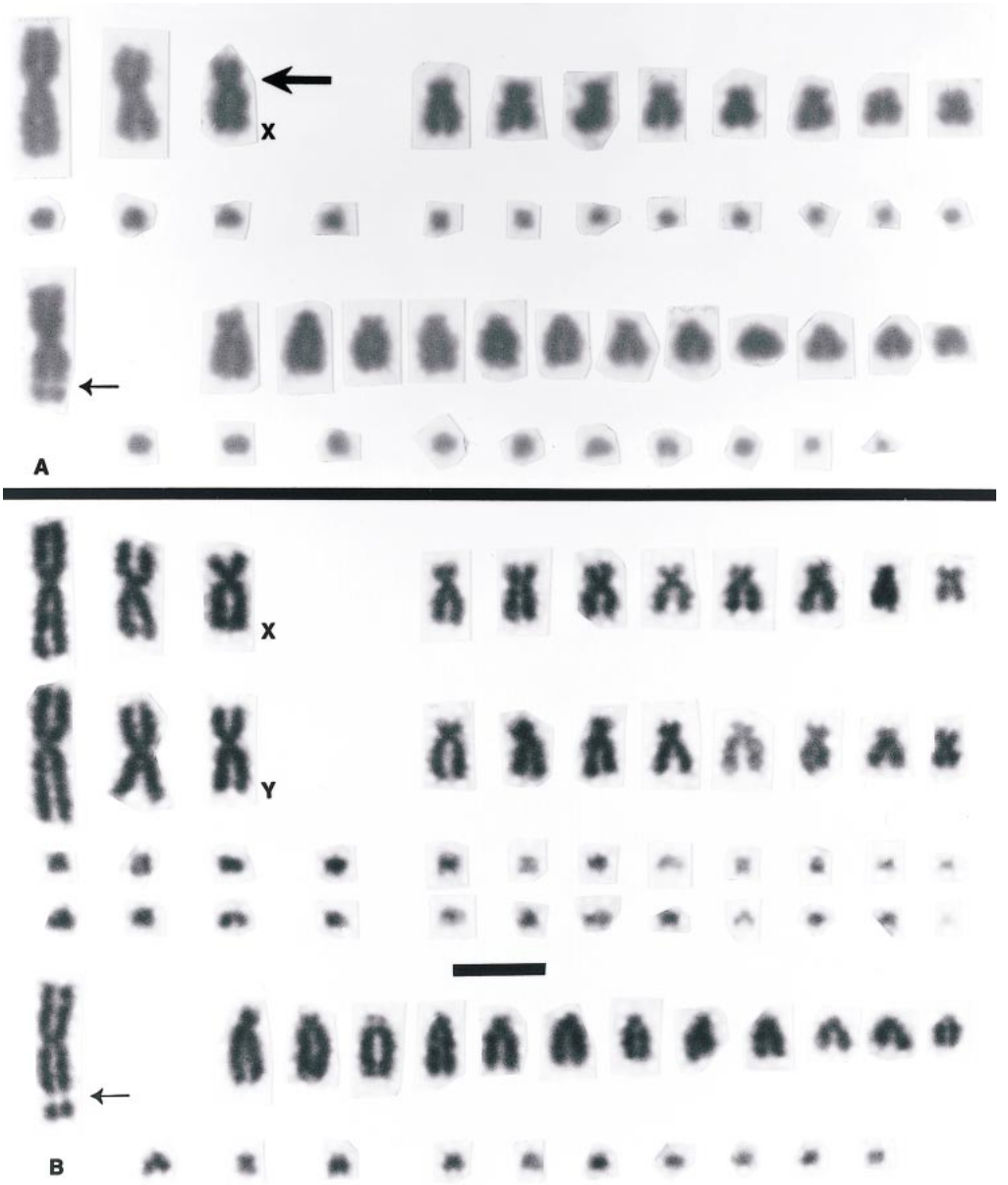

Fig. 9. Karyotypes of two whiptail lizards (*Cnemidophorus*) from Arroyo del Macho. **A.** Diploid unisexual *C. tesselatus,* AMNH R-146638 ($2n = 46$). This unisexual taxon is a clone with its ultimate ancestor being an F_1 hybrid between *C. tigris marmoratus* (haploid complement of chromosomes in the upper two rows) × *C. gularis septemvittatus* (haploid complement in the lower two rows). Large arrow points to the centromere of the X-chromosome ultimately inherited from *C. tigris marmoratus*; this chromosome apparently had undergone centric fission into two telocentric chromosomes in the other karyotypic clone of *C. tesselatus* found at the same locality ($2n = 47$). **B.** Triploid hybrid between *C. tesselatus* × *C. tigris marmoratus* (male), AMNH R-146690 ($3n = 69$). The four rows of chromosomes above the scale bar represent the two haploid complements inherited from *C. tigris marmoratus,* whereas the lower two rows represent the single haploid complement from *C. gularis septemvittatus.* The Y-chromosome was inherited from the most recent backcross hybridization between *C. tesselatus* and *C. tigris marmoratus.* Bar represents 10 μm. Small arrows in both A and B illustrate distinctive secondary constrictions (nucleolar organizer regions) from *C. gularis septemvittatus.*

Macho site were karyotyped. Eight of these were triploids having $3n = 69$ chromosomes, including the full diploid karyotype of *C. tesselatus* with the ancestral *tigris* X-chromosome unfissioned plus a second haploid complement of *tigris* chromosomes (fig. 9B). One individual was a modified triploid having $3n = 70$ chromosomes, with the fissioned *tigris* X-chromosome inherited from *C. tesselatus.* The four female hybrids had the *tigris* X-chromosome in both *tigris* complements, whereas the five male hybrids had the *tigris* Y-chromosome in the second *tigris* complement (fig. 9B). Clearly, these individuals all appeared to be F_1 hybrids between *C. tesselatus* × *C. tigris marmoratus.*

Relationship Between Karyotypic Cytotype and Morphology

There was an unequal distribution of the two cytotypes between *C. tesselatus* and the hybrids. The fissioned X-chromosome was found in a majority of *C. tesselatus* karyotyped (7 of 10), but only one hybrid had the fissioned X-chromosome (1 of 9). This disparity might reflect different effects of the fissioned X in the two groups. Although the type of X-chromosome inherited does not affect the expression of meristic characters in *C. tesselatus,* the presence of a fissioned X may exaggerate the expression of certain meristic characters in hybrids—with potentially negative effects. Evidence supporting this possibility comes from both univariate and multivariate analyses. At the univariate level, the two karyotypic clones in *C. tesselatus* ($2n = 46$ [intact X] and $2n = 47$ [fissioned X]) are similar (*t*-tests; *P*s > 0.38) in all 12 meristic characters (see Materials and Methods section for character descriptions), supporting a hypothesis that the two cytotypes confer equivalent reproductive success in *C. tesselatus.* Whether the 3:7 ratio reflects the true frequencies of the two cytotypes in the population of *C. tesselatus* is unknown; it could simply represent a deviation from a 1:1 ratio based on sampling variation (Chi-square = 1.600, 1 df, $P = 0.21$). Nevertheless, the ratio of 8 hybrids with intact X-chromosomes to 1 hybrid with a fissioned X-chromosome is a significant deviation from the 3:7 ratio in *C. tesselatus* (Chi-square [with Yate's correction for small samples] = 4.539, 1 df, $P = 0.03$).

To summarize the morphological differences associated with the two cytotypes, we used 11 meristic characters (table 3) in a principal components analysis to compare the hybrid with a fissioned X-chromosome to hybrids with intact X-chromosomes. Principal components scores for the only hybrid with a fissioned X-chromosome lie outside the 95% confidence ellipse for the sample of the other eight hybrids (fig. 10). All significant differences between the two hybrid cytotypes were conveyed by the first principal component ($t = 12.804$, 1 df, $P < 0.00005$; table 4). This component provided a contrast between the three color pattern characters and number of granules around midbody (all with positive loadings) and two characters associated with the hindlimb, number of subdigital lamellae on the fourth toe and number of femoral pores (both with negative loadings; table 3).

The disjunct position of the hybrid with a fissioned X-chromosome in the ordination of PC scores (fig. 10) was based on striking differences between the two cytotypes in univariate scores (table 4). We used one-sample *t*-tests on individual meristic characters to determine if the unusual hybrid was significantly different from the sample of hybrids with intact X-chromosomes. The hybrid with a fissioned X had fewer pale segments in the vertebral field ($t = 6.804$, 1 df, $P = 0.0003$), fewer interruptions in the dorsolateral stripes ($t = 6.065$, 1 df, $P = 0.0005$), fewer interruptions in the paravertebral stripes ($t = 6.174$, 1 df, $P = 0.0005$), fewer granular scales around midbody ($t = 12.477$, 1 df, $P < 0.0005$), more femoral pores ($t = 7.332$, 1 df, $P = 0.0002$), more circumorbital scales ($t = 4.314$, 1 df, $P = 0.0035$), more subdigital lamellae on the fourth toe ($t = 3.000$, 1 df, $P = 0.0199$), and more scales contacting the outer perimeter of the parietal and interparietal scales ($t = 7.483$, 1 df, $P = 0.0001$) (table 4). Whether these deviations, or others undetected by us, translate into higher mortality in hybrids inheriting a fissioned X-chromosome is unknown. The low frequency of the fissioned X cytotype in our sample of hybrids compared to its higher frequency in *C. tesselatus* indicates that this might be the

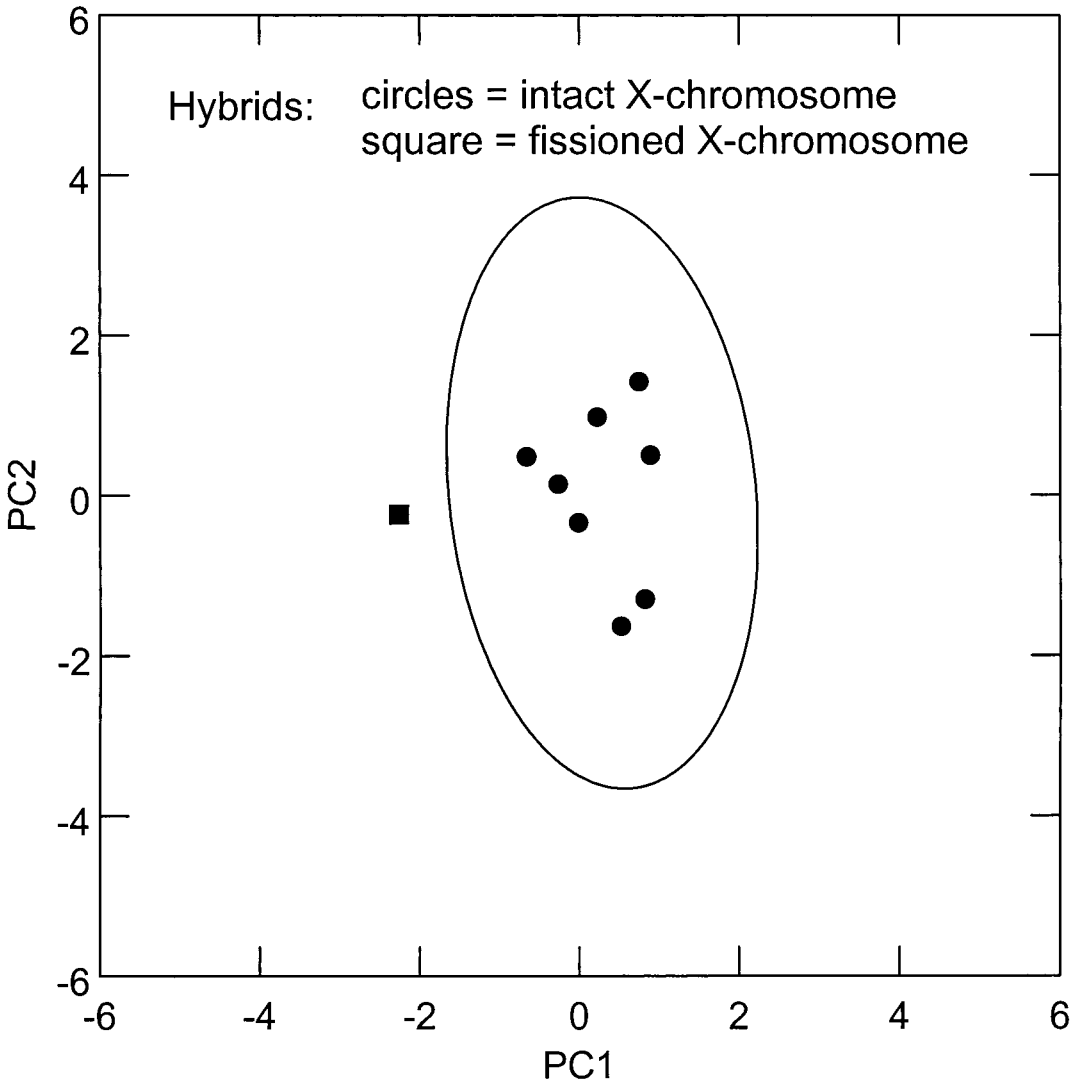

Fig. 10. Pattern of morphological variation expressed by the distribution of scores on the first two principal components extracted from a correlation matrix of 11 meristic characters. Symbols depict scores of nine karyotyped hybrids collected in the vicinity of Arroyo del Macho, Chaves Co., New Mexico: ●, 8 hybrids of $2n = 46$; ■, one hybrid of $2n = 47$ (fissioned X-chromosome). The ellipse defines the 95% confidence limits for the eight hybrids with intact X-chromosomes.

case. However, alternative hypotheses, such as a lower susceptibility of individuals of *C. tesselatus* with fissioned X- chromosomes to hybridization, cannot be tested with our evidence.

Evidence from Biochemical Genetics

Based on genotypes detected at 34 loci, we obtained evidence bearing on the following questions: (1) how many electrophoretically detected clones of *C. tesselatus* are present in our sample from the Roswell area; (2) were the suspected hybrids actually hybrids; (3) were the male parents of the hybrids representatives of *C. tigris marmoratus*; (4) was there evidence for separate fertilization events in the origin(s) of these hybrids; and (5) are the parental taxa at the hybridization

TABLE 3

Factor Loadings for Four Principal Components Derived from Meristic Variation Between Two Cytotypes of Hybrids Derived from the Parthenogenetic Species *Cnemidophorus tesselatus* × the Bisexual Species *C. tigris marmoratus*

Character[a]	PC1	PC2	PC3	PC4
GAB	0.966	−0.161	−0.068	0.064
PSVF	0.720	0.075	0.486	0.046
IDLS	0.817	0.458	−0.164	0.062
IPVS	0.887	0.148	−0.022	0.093
SDL-T4	−0.618	0.529	−0.326	0.205
FP	−0.561	0.161	0.395	−0.399
GS	0.216	0.714	0.404	0.408
LSG	0.203	−0.671	0.473	0.483
SDL-F4	0.064	−0.122	0.668	−0.545
COS	−0.577	0.411	0.594	0.332
PSC	−0.524	−0.458	−0.013	0.545
Eigenvalue	4.301	1.923	1.717	1.323
Total explained variation	39.1%	17.5%	15.6%	12.0%

[a] Characters are defined in table 1.

TABLE 4

Descriptive Statistics and Tests of Significance[a] for 11 Meristic Characters and Scores of Four Principal Components for Two Cytotypes in *Cnemidophorus tesselatus* × *C. tigris marmoratus* Hybrids

Scores for one hybrid with a fissioned X-chromosome are compared with scores (mean ± SE and range) from a sample of eight hybrids with intact X-chromosomes.

	Character scores	
Character[b]	Intact X	Fissioned X
PSVF	10.6 ± 0.68 (8–13)	6
IDLS	18.9 ± 2.45 (9–28)	4
IPVS	18.6 ± 1.72 (11–23)	8
GAB	87.1 ± 0.81 (84–90)	77
SDL-T4	36.2 ± 0.25 (35–37)	37
FP	45.6 ± 0.32 (44–47)	48
COS	21.1 ± 0.67 (19–25)	24
PSC	21.0 ± 0.27 (20–22)	23
PC1	0.28 ± 0.199 (−0.66 to 0.89)	−2.27
GS	20.4 ± 1.05 (18–26)	18
LSG	42.2 ± 2.76 (33–53)	38
SDL-F4	18.0 ± 0.19 (17–19)	18
PC2	0.03 ± 0.376 (−1.64 to 1.42)	−0.24
PC3	−0.02 ± 0.378 (−2.28 to 1.32)	0.13
PC4	0.01 ± 0.378 (−2.11 to 1.01)	−0.10

[a] Underlined scores and means are not significantly different at $\alpha = 0.05$ as determined from single-sample *t*-tests.

[b] Characters are defined in table 1.

site genetically similar to individuals representing the same taxa from other localities?

All 11 *C. tesselatus* examined electrophoretically, including a laboratory-reared offspring and her mother, had identical genotypes at each of the 34 loci (table 5). Seventeen loci (50%) had alleles in the heterozygous state, attesting to the origin of this taxon from a hybridization event, and the specific alleles at each locus were consistent with the parental taxa being *C. tigris marmoratus* and *C. gularis septemvittatus* (Neaves, 1969; Parker and Selander, 1976; Dessauer and Cole, 1989). The presence of identical genotypes in the laboratory-reared specimen, its mother, and every other individual of these *C. tesselatus* showed that they reproduce by parthenogenetic cloning, as do other individuals of different pattern classes of this taxon (Dessauer and Cole, 1986). Only one electrophoretic clone of *tesselatus* was detected at the hybridization site. Because individuals examined included both of the local karyotypic clones of *C. tesselatus* (see above), no molecular marker was correlated with the two cytotypes. In addition, these representatives of *C. tesselatus* were electrophoretically identical to other specimens of pattern class E from several other localities (work in progress), although more than one clone does exist among specimens examined from elsewhere (also see Parker and Selander, 1976).

The alleles found in the four specimens of *C. tigris marmoratus* from the hybridization site (table 5) were also the same ones commonly found in specimens of this taxon from other localities (Dessauer et al., 2000; however, we did not cross-correlate allele designations for the uncommon alleles found in their large samples from southwestern New Mexico). Despite our small sample from the Roswell area, four loci (IDDH, PEPA [by deduction from hybrids], PEPD, and GPI) showed local polymorphism in this bisexual species that reproduces with a Mendelian pattern of inheritance.

TABLE 5

Genotypes or Alleles[a] at 34 Gene Loci[b] in Samples of *Cnemidophorus* from the Hybridization Site

Locus	TES[c]	HYB[d]	MAR[e]
Oxidoreductases			
ADH	ab	aab	a
IDDH	aa	aab	b, a[f]
LDH1	ab	aab	a
sMDH	ab	aab	a
sMDHP	ab	aab	a
sSOD[g]	ab	abb	b
Transferases			
sAAT	ab	aab	a
mAAT	ab	abb	b
sALAT	ab	aab	a
Hydrolases			
EST2[h]	bc	bbc	b
PEPA[h]	cd	bcd, ccd[i]	b
PEPD	cc	bcc, ccc[j]	c[j], b
ADA[k]	ac	aac	a
Lyase			
sACOH[h]	bc	bcc	c
Isomerases			
MPI	ab	aab	a
GPI	ab	abb	b, a[f]
PGM2	ab	aab	a
PGM3	ab	aab	a
Blood protein			
ALB	ab	aab	a

[a] Alleles only are presented for *C. tigris marmoratus.* If one allele is given, all specimens examined were homozygous for that allele. If two alleles are given, their frequencies are noted. For *C. tesselatus,* all individuals were identical to each other (one electrophoretic clone); their genotypes are presented. Genotypes are presented for the hybrids also, and frequencies are given for genotypes that varied among hybrids (PEPA and PEPD).

[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. Abbreviations and 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; G3PDH, glycerol-3-phosphate dehydrogenase; IDDH, L-iditol dehydrogenase; LDH, L-lactate dehydrogenase; MDH, malate dehydrogenase; MDHP, malate enzyme; IDH, isocitrate dehydrogenase; PGDH, phosphogluconate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase; SOD, superoxide dismutase; AAT, aspartate aminotransferase; ALAT, alanine aminotransferase; CK, creatine kinase; AK, adenylate kinase; EST, esterase; PEP, peptidase; ADA, adenosine deaminase; ACOH, aconitase hydratase; MPI, mannose-6-phosphate isomerase; GPI, glucose-6-phosphate isomerase; PGM, phosphoglucomutase; ALB, albumin; TF, transferrin; and HB, hemoglobin. The following 15 loci showed no variation among all individuals compared: G3PDH, LDH2, mMDH, sIDH, mIDH, PGDH, G6PDH, mSOD, CK1, AK, ESTD, PEPB, PEPE, TF, and HB.

[c] *Cnemidophorus tesselatus* pattern class E; 11 specimens examined, all females, including one laboratory-reared offspring and its mother, all of which were identical.

[d] Hybrids between *C. tesselatus* × *C. tigris marmoratus*; 10 specimens examined (6 males, 4 females).

[e] *Cnemidophorus tigris marmoratus*; 4 specimens examined (3 males, 1 female).

[f] At IDDH and GPI in *C. t. marmoratus,* frequency of the b-allele was 0.75, the a-allele, 0.25. All hybrids inherited the more common b-allele at both of these loci.

[g] The b-allele at sSOD was not resolved in earlier studies (Dessauer and Cole, 1986, 1989). Resolution was achieved in using phosphate/citrate buffer at pH 6.5.

[h] For EST2, PEPA, and sACOH we have found the a-allele in other relatives, including *C. sexlineatus* and *C. neotesselatus.*

[i] At PEPA in the four *C. tigris marmoratus* from the Roswell area, frequency of the b-allele was 1.0. However, in large samples from other localities, frequency of the c-allele was 0.08 (Dessauer et al., 2000). Only one hybrid (AMNH R-145146) inherited the c-allele (fig. 13).

[j] At PEPD in *C. tigris marmoratus,* frequency of the c-allele was 0.75, the b-allele was 0.25. Six of the hybrids inherited the b-allele, and four (AMNH R-145149, R-146690, R-146691, and R-146692) inherited the c-allele. We have found the a-allele in another member of the "*tesselatus* complex."

[k] The b-allele at ADA was found in other species of *Cnemidophorus.*

Of the 34 loci analyzed, 15 showed no variation among all individuals of each taxon and the hybrids examined (the same alleles were shared universally), but 19 loci were particularly informative for identifying hybrids and their parental species (table 5). For each locus, all 10 suspected hybrids (based on morphology and karyotypes) had electrophoretic banding patterns consistent with triploids bearing a combination of alleles that included the two alleles found in the diploid *C. tesselatus* plus a third allele from the local *C. tigris marmoratus.* This is consistent with a cloned *tesselatus* ovum having been fertilized by a haploid *marmoratus* spermatozoan (table 5).

Although we did not examine, electrophoretically, representatives of *C. inornatus* from the hybridization locality, this was not necessary. Previous studies (e.g., the review by Cole et al., 1988) have shown that *C. tigris marmoratus* and *C. inornatus* are electrophoretically distinguished from each other at many loci, including the following eight in table 5: ADH, LDH1, sSOD, sAAT, mAAT, PEPE, ADA, and TF. In addition to the third haploid set of chromosomes being diagnostic of *marmoratus* in the hybrids reported here (see above), the alleles detected at these loci were also those specifically of *marmoratus,* not *inornatus.*

The presence of the extra *marmoratus* allele in hybrids was detected at most loci based on allele dosage effects on band densities (isozyme activities) in electrophoretic phenotypes. For example, at GPI, specimens of the diploid *C. tesselatus,* which are heterozygotes, show a three-banded pattern. Hybrids show the same three bands, but the relative band densities differ from those of *tesselatus.* Phenotypes (gel patterns) predicted for the ab genotype of this dimeric enzyme by the expansion of $(a + b)^2$, which equals $a^2 + 2ab + b^2$, consist of three isozymes with a ratio of activities (band densities on gels) approximating 1:2:1, as observed for *tesselatus* (fig. 11). Phenotypes predicted for the abb genotype predicted by the expansion of $(a + 2b)^2$, which equals $a^2 + 4ab + 4b^2$, consist of the same three isozymes but with a ratio of activities approximating 1:4:4, as observed in triploid hybrids (fig. 11). Consequently, *tesselatus* and the hybrids had three-banded patterns for GPI, but the ratio of the band densities differed between them.

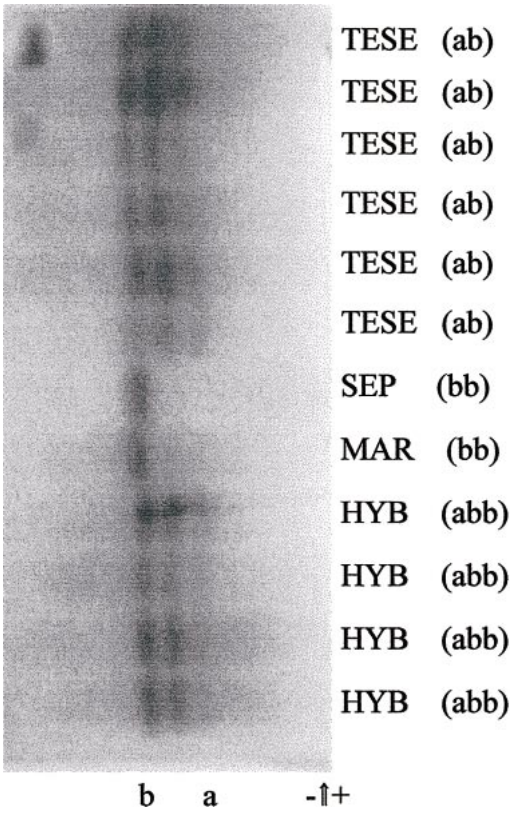

Fig. 11. Electrophoretic phenotypes of GPI, a dimeric enzyme, from erythrocytes of 12 lizards. Letters below gel identify allozymes based on alleles present (table 5). Lanes for individual lizards are labeled beside the gel (with genotype) as follows: TESE, *C. tesselatus* pattern class E; SEP, *C. gularis septemvittatus*; MAR, *C. tigris marmoratus*; and HYB, *C. tesselatus* × *C. tigris marmoratus* hybrid. Although the SEP and MAR are identical on this gel (bb), the a-allele occurs in other individuals of MAR. All specimens except the SEP are from the hybridization site near Roswell. Anode is to the right; arrow indicates position of sample applications.

Banding patterns for IDDH and PEPA, however, involved differences in both the number of isozymes and the band densities present in *C. tesselatus* versus hybrids. All hybrid genotypes at IDDH included the b-allele of *marmoratus* (fig. 12). Phenotypes predicted for the aab genotype of this tetrameric enzyme by the expansion of $(2a + b)^4$, which equals $16a^4 + 32a^3b + 24a^2b^2 + 8ab^3$

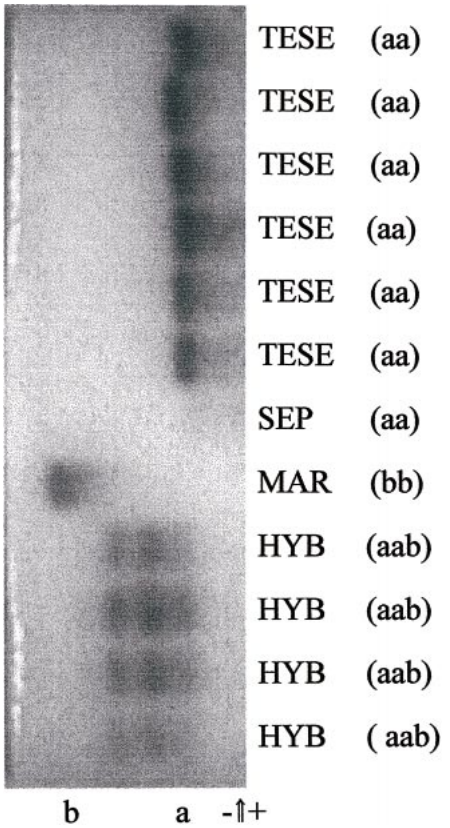

Fig. 12. Electrophoretic phenotypes of IDDH, a tetrameric enzyme, for 12 lizards; letters and labeling as in figure 11, except the SEP on this gel was actually an individual of *C. gularis scalaris.* The a-allele occurs in other individuals of MAR. Activity in SEP was too weak to appear clearly in this photograph. Anode is to the right; arrow indicates position of sample applications.

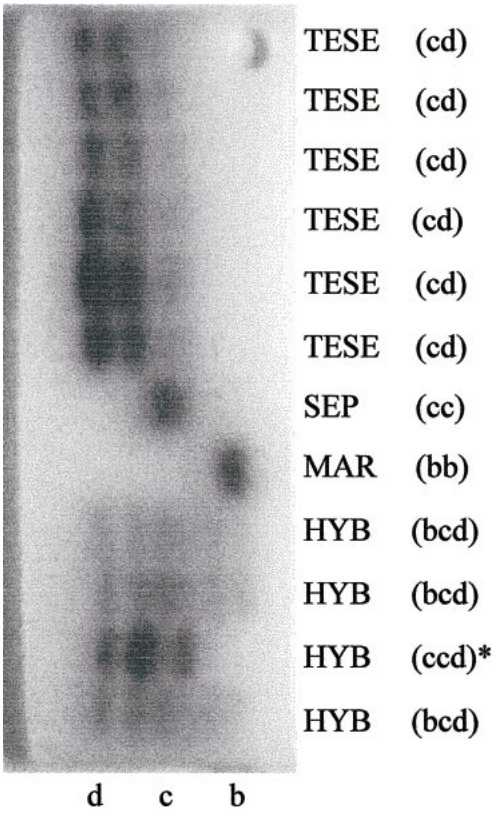

Fig. 13. Electrophoretic phenotypes of PEPA, a dimeric enzyme, for 12 lizards; letters and labeling as in figure 11, except asterisk marks hybrid (AMNH R-145146) that received the c-allele from MAR instead of the b-allele (coincidentally, this is the only hybrid karyotyped that has a fissioned X-chromosome). The c-allele occurs in other individuals of MAR, the d-allele in others of both MAR and SEP. Anode is to the right.

+ b^4, consist of five isozymes with a ratio of activities approximating 16:32:24:8:1. The least active isozyme (b^4) is not visible in the hybrids in figure 12.

Although dosage effects were not as clear for PEPA as for IDDH, comparison of the *tesselatus* and hybrid patterns in figure 13 clearly shows that the banding pattern of the hybrids varies according to which allele was inherited from *marmoratus.* Nine of the 10 hybrids had phenotypes of five isozymes. Dosage effects for the triple heterozygote bcd of this dimeric enzyme predict a six-banded phenotype, estimated by the expansion of $(b + c + d)^2$, which is $b^2 + 2bc + c^2 + 2bd + 2cd + d^2$, with band intensities approximating the ratio of 1:2:1:2:2:1. Five of the six isozymes were resolved in these triple heterozygotes (fig. 13). Note that the middle band of the triple heterozygotes is the most dense, suggesting that the c^2 and 2bd bands are superimposed to produce a five-banded pattern with a ratio of 1:2:3:2:1.

One would expect a sample of 10 hybrids from the Roswell area to include individuals with different genotypes at IDDH, PEPD, and GPI because the local population of *marmoratus* is polymorphic at these loci (table 5). For both IDDH and GPI, all 10 hybrids inherited the b-allele, which has a frequency of 0.75 in the local *marmoratus.* For PEPD,

six hybrids inherited the b-allele and four inherited the c-allele, which have frequencies as follows in the local *marmoratus*: b = 0.25, c = 0.75. In addition, for PEPA, nine hybrids inherited the b-allele (bcd genotype), whereas one inherited the c-allele (ccd genotype) from *marmoratus*. The *marmoratus* in our small sample from the Roswell area expressed only the b-allele at PEPA, but the c-allele is present in *marmoratus* from other sites (frequency of about 0.08; Dessauer et al., 2000).

Finally, the apparent sterility of the triploid female hybrids (see Histological Analysis, below) also is consistent with hypothesizing that a new triploid parthenogenetic clone has not been generated from the triploid hybrids at this site. If such a new clone were present, our sample of 10 triploids would have included a preponderance of females bearing identical genotypes, which is not the case. In fact, considering the combination of genotypes together with the presence of the Y-chromosomes observed, we have evidence for nine separate fertilization events (nine different combinations of eggs and sperm) among the 10 hybrids examined electrophoretically.

Histological Analysis

Cnemidophorus tesselatus

Sexually mature and reproductive adults of *C. tesselatus* usually exhibit the following characteristics: oocytes are present, the ovarian follicle cell layer is complete and well organized, connective tissue is not conspicuous, vacuoles are few, and the follicle is well vascularized. The distal oviduct contains a thin mucosa with poorly developed folds. The middle oviduct is ciliated and contains well-developed alveolar glands, which are restricted to this section of the oviduct. The proximal oviduct has a thick (normal) mucosa and well-developed folds. The mesonephric tubules are 20–30 μm in diameter (figs. 15C, 18A–D).

The ovary of a normal, reproductive *C. tesselatus* contains developing follicles with a well-developed tunica granulosa that includes active pyriform cells (fig. 14E, F). The zona radiata (formed by microvilli) is usually evident and probably indicates the active transport of components (such as phosvitin and lippovittelin) for the later synthesis of yolk granules (fig. 14G). The increase in the surface area that results from the presence of the microvilli facilitates transport of substances between the oocyte and the follicle cells (Balinsky, 1975; Anderson and Beams, 1960). The cytoplasm is granular but homogeneous in distribution in young oocytes and becomes layered in older oocytes (fig. 14I, K). Yolk deposition begins when the oocyte is 2.3 mm (largest nonyolked oocyte measured) to 3.1 mm (smallest yolked oocyte measured) in diameter (table 6, fig. 14G, H); as vitellogenesis progresses the theca granulosa regresses with the loss of pyriform cells but retention of the small granulosa cells (fig. 16G). Atretic oocytes have stopped development for some unknown reason. A previtellogenic oocyte that is atretic shows irregular or vacuolated cytoplasm (fig. 14A, B). The theca granulosa is often hypertrophied, and enlarged granulosa cells move through the disrupted zona radiata or zona pellucida into the oocyte cytoplasm (fig. 14C, D). As atresia progresses the basement lamina of the theca granulosa becomes a hyaline layer known as the glassy membrane (fig. 14C, D). At ovulation the follicle wall appears thicker because the thin, stretched wall of the mature follicle is now shrunken due to the collapse of the follicle; cellular debris may be present in the lumen, but neither oocyte nor yolk will be present (fig. 14J). As the corpus luteum ages the lumen becomes filled with luteal cells (fig. 14K, L). The fully developed corpus luteum is easily distinguished from an advanced atretic follicle by the absence of yolk, absence of the glassy membrane, and the presence of vacuolated luteal cells (fig. 14L). Corpora lutea regress rapidly to a distinctive, triangular collapsed structure, composed primarily of connective tissue and usually located between other follicles (fig. 14I). Perhaps this is typical of lizard corpora lutea (Miller, 1948: pl. 13d, e for *Xantusia*; Goldberg, 1970: fig. 12 for *Sceloporus*).

Two individuals exhibited gonadal abnormalities. One of these (AMNH R-146636), a large individual (95 mm snout–vent length), had very small ovaries lacking enlarged oocytes; no sperm were seen in the reproduc-

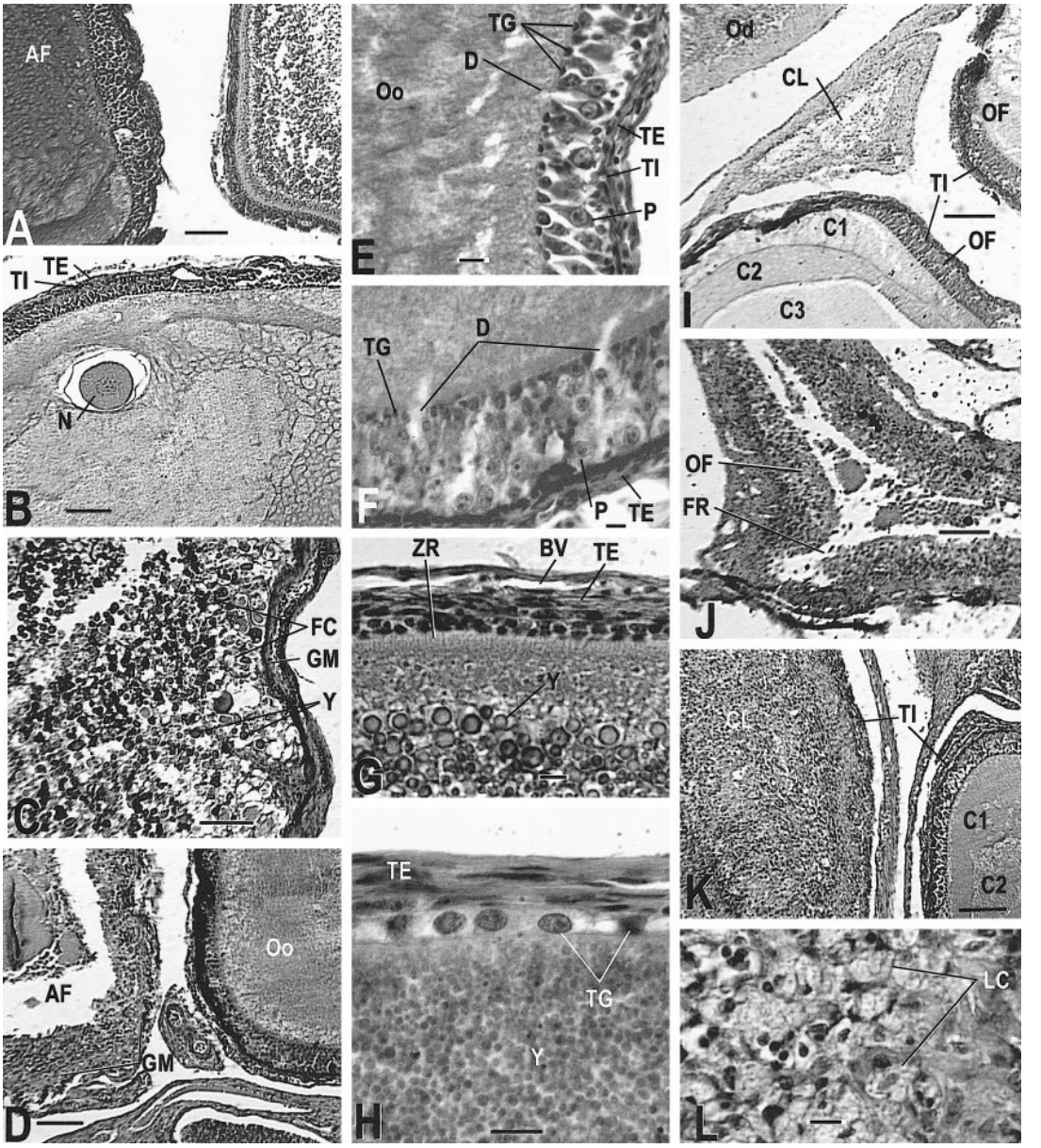

Fig. 14. Atretic and normal follicles and corpora lutea of *C. tesselatus*. **A.** Developing oocyte during vitellogenesis (right) and atretic follicle (left; AMNH R-145143, slide 19, row 1, section 1). **B.** Atretic follicle with disorganized cytoplasm but intact nucleus (AMNH R-145143, slide 19, row 1, section 1). **C.** Atretic follicle after vitellogenesis had begun; notice the glassy membrane (AMNH R-146620, slide 4, row 2, section 5). **D.** Atretic follicle (left) and normal follicle (right), both before vitellogenesis (AMNH R-146621, slide 4, row 1, section 2). **E.** A normal follicle showing the structure of the follicle wall and discharging pyriform cells (AMNH R-145142, slide 106, row 1, section 3). **F.** Detail of the tunica interna showing discharging pyriform cells prior to the formation of yolk granules (AMNH R-146637, slide 41, row 1, section 4). **G.** Detail of a follicle during vitellogenesis, showing a well-developed zona radiata, vascularization, and the tunica granulosa (AMNH R-145143, slide 28, row 1 section 2). **H.** Near the end of vitellogenesis the tunica interna is reduced to the tunica granulosa (AMNH R-145142, slide 49, row 1, section 2). **I.** Older corpora lutea showing the triangular shape and absence

tive tract. The second (AMNH R-146640) was the smallest *C. tesselatus* in the sample (snout–vent length = 65 mm) and contained a few abnormal follicles (many irregular spaces and an incomplete theca granulosa); no sperm were found in her reproductive tract. All other representatives of *C. tesselatus* contained 3–22 follicles; seven individuals were undergoing vitellogenesis in follicles 1.4–8.5 mm in diameter (table 6).

Salient features of the reproductive systems of representative individuals of *C. tesselatus,* all *C. tesselatus* × *C. tigris marmoratus* hybrids, and all male *C. tigris marmoratus* collected at the hybridization site are described below with date of collection in parentheses. The first series are representatives of *C. tesselatus.*

AMNH R-146629 (June 11, 1998): The left ovary was 3 × 7 mm. Both oviducts were swollen; the left was 16 × 3.5 mm, the right was 14 × 4 mm. The largest oocyte had not begun vitellogenesis but the cytoplasm is differentiated into at least three zones, and the granulosa and zona radiata are well developed (as in fig. 14A, I). Several oocytes show pyriform cells (fig. 16H), which indicate the onset of yolk deposition in *Sceloporus* (Goldberg, 1970). At least two corpora lutea were present (fig. 14I). The small size and triangular shape of the corpora lutea suggest that ovulation and oviposition occurred from at least two weeks (Miller, 1948: pl. 12 for the viviparous *Xantusia*) to several months earlier (Goldberg, 1970, for the ovoviviparous *Sceloporus jarrovi*); however, the sequence and timing of corpus luteum degeneration in *Cnemidophorus* is not well known. Two atretic follicles were present. No sperm were found.

AMNH R-146636 (June 12, 1998): The left ovary and oviduct were smaller than those of the other *C. tesselatus* examined; however, this individual is 95 mm snout–vent length, clearly of adult size; the mean snout–vent length for the other 25 specimens examined is 89.6 (65–109 mm). The mesonephric kidney and adrenal were well developed and vascularized. The ovarian epithelium (cortex) was very thin and contained some oocytes and a well-developed lip (fig. 15E). The ovary consisted of a thin layer (3–4 cells thick) over a thinner layer of smooth muscle, all of which were on the surface of the adrenal away from the mesonephros (fig. 15E). Follicles were not present, the ovary was probably just beyond the indifferent stage of development, and the oviduct was tiny. This animal, although of large size for a mature female, is equivalent to a hatchling in its reproductive anatomy. The karyotype and protein electrophoresis confirmed the identity of this specimen. No sperm were found.

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of an oocyte; note the layered cytoplasm of the adjacent oocyte (AMNH R-146629, slide 28, row 1, section 4). **J.** A recently ovulated follicle that will become a corpus luteum; note triangular shape and point of rupture (AMNH R-146620, slide 31, row 1, section 1). **K.** Corpus luteum (left) and normal follicle (right; AMNH R-146618, slide 1, row 2, section 13). **L.** Detail of corpus luteum (AMNH R-146621, slide 1, row 2, section 9). Slides stained with Mallory triple connective tissue technique are designated MT; all other slides were stained with Harris hematoxylin and eosin. Abbreviations for this and the following figures are as follows: A, adrenal gland; AC, adrenal cells; AF, atretic follicle; Al, alveolar gland; BGE, boundary of germinal epithelium; BV, blood vessel; CL, corpus luteum; CT, connective tissue; C1, outermost layer of pre-yolk cytoplasm of oocyte; C2, middle layer of pre-yolk cytoplasm of oocyte; C3, innermost layer of preyolk cytoplasm of oocyte; D, discharging pyriform cell; Dod, distal oviduct; E, epididymis; FC, follicle cell; FR, site of follicle rupture due to ovulation; GM, glassy membrane of an atretic follicle; Gr, granulosa cells of follicle; I, interrenal cells; L, lumen; LC, luteal cells; Le, Leydig cell layer; Lip, lip of germinal epithelium; LRV, left renal vein; M, mesonephros; ML, mesonephros, large tubules; Mod, middle oviduct; MS, mesonephros, small tubules; Mu, mucosa; Mus, muscularis; N, nucleus; O, ovary; Od, oviduct; OF, ovarian follicle; Og, oogonia of germinal epithelium; Oo, oocyte; P, pyriform cell in theca granulosa; S, serosa; Sp, spermatozoa; ST, seminiferous tubules; TE, theca externa of ovarian follicle; TG, theca granulosa of ovarian follicle; TI, theca interna of ovarian follicle; V, vas deferens; Y, yolk of oocyte; ZR, zona radiata. Scale bar, 0.1 mm, except E–H and L, which are 0.01 mm.

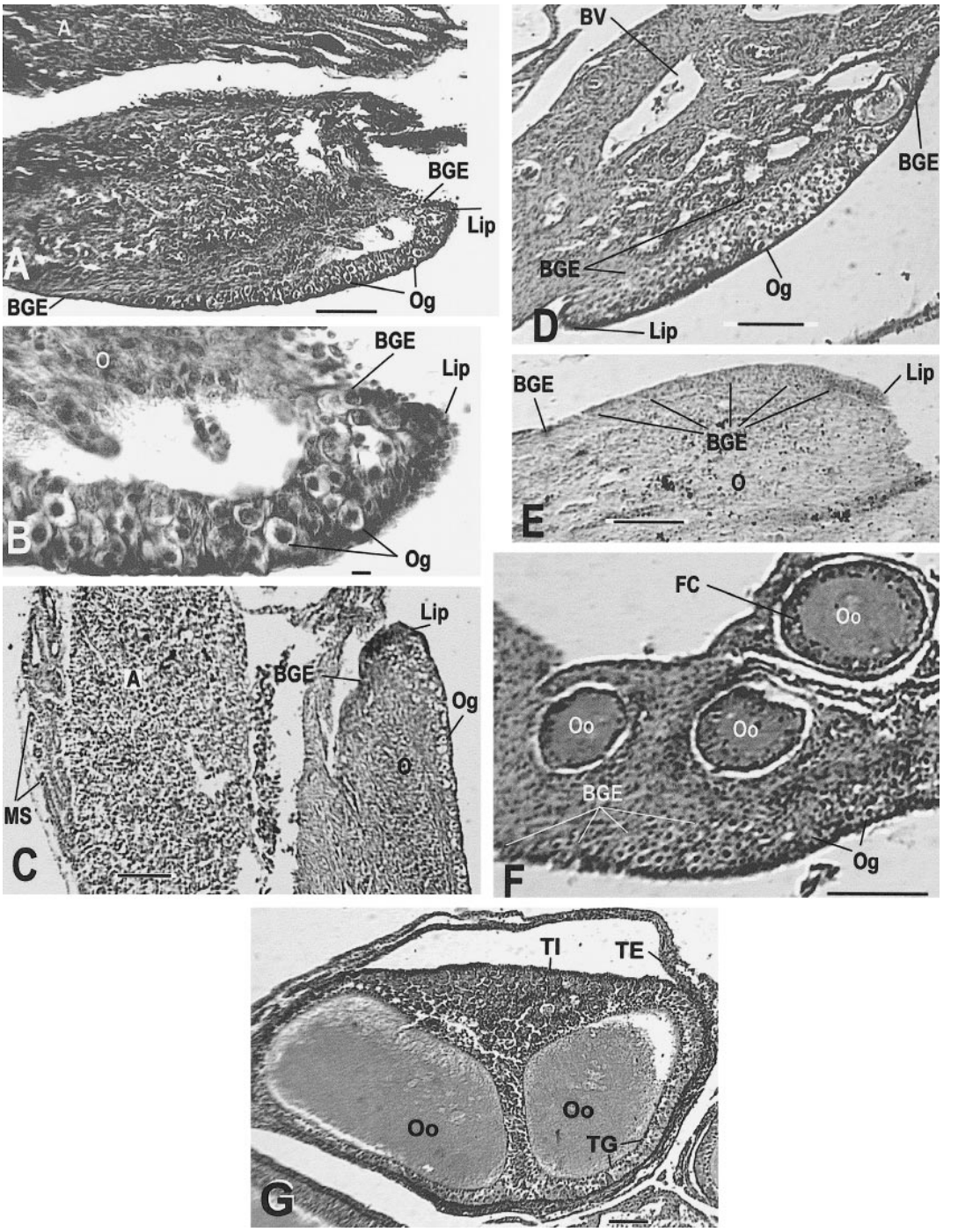

Fig. 15. Germinal epithelium and adjacent adrenal gland of hybrid females (A–C) and *C. tesselatus* females (D–F). **A.** Germinal epithelium with well-defined lip, margin, and oogonia (AMNH R-146689, slide 3, row 2, section 6, MT). **B.** Detail of epithelium in figure 15A (AMNH R-146689, slide 3, row 2, section 6, MT). **C.** Germinal epithelium, adrenal gland, and mesonephros (AMNH R-146691, slide 13, row 2, section 9). **D.** Germinal epithelium showing boundary and lip (AMNH R-146637, slide 41,

TABLE 6

Reproductive Characteristics of *Cnemidophorus tesselatus* and Female *C. tesselatus* × *C. tigris marmoratus* Hybrids

Specimen (AMNH R)	Date collected	SVL (mm)	Developing follicles			Atretic follicles		Corpora lutea	
			No.	Max. diam. (mm)	Yolk present[a]	No.	Max. diam. (mm)	No.	Max. diam. (mm)
C. tesselatus									
145142	5/12/99	97	13	8.5 × 5.9	Yes (2)	0	—	0	—
145144	5/14/99	89	18	8.3	Yes (2)	2	5.1	0	—
146612	6/10/96	98	NC	6.7 × 6.4	Yes (1)	0	—	1	0.5 × 0.5
146638	6/12/98	95	11	6.3	Yes (1)	—	—	—	—
146613	6/10/96	86	22	4.8 × 3.1	Yes (1)	2	2.6 × 2.2	1	1.5 × 0.4
145143	5/14/99	93	11	3.6	Yes (3)	1	3.8	0	—
146639	6/12/98	89	16	3.5	Yes (3)	0	—	4	1.8
146620	7/21/97	109	12	2.3 × 0.7	No	1	7.0 × 3.7	1	1.5 × 0.7
146617	7/20/97	85	15	2.0 × 1.3	No	0	—	1	1.0 × 0.7
146627	7/22/97	89	14	2.0 × 1.0	No	0	—	0	—
146624	7/21/97	79	21	1.7 × 1.3	No	0	—	0	—
146626	7/22/97	90	21	1.7 × 1.1	No	0	—	0	—
146625	7/21/97	82	9	1.7 × 0.9	No	1	3.0 × 2.0	0	—
146619	7/20/97	85	13	1.5 × 1.5	No	0	—	0	—
146623	7/21/97	86	19	1.5 × 1.1	No	0	—	0	—
146615	7/20/97	91	10	1.5 × 0.9	No	1	1.0 × 0.6	3	1.0 × 0.5
146629	6/11/98	97	13	1.5	No	2	1.0	2	0.3
146637	6/12/98	93	14	1.4	No	0	—	1	1.2
146628	7/22/97	88	14	1.4 × 0.7	No	0	—	0	—
146616	7/20/97	84	12	1.4 × 0.6	No	2	1.3 × 0.8	0	—
146622	7/21/97	86	8	1.1 × 0.9	No	0	—	2	1.4 × 1.0
146618	7/20/97	83	15	1.0 × 0.5	No	0	—	1	1.8 × 0.7
146621	7/21/97	100	22	0.9 × 0.9	No	1	1.1 × 0.6	2	0.9 × 0.5
146614	7/20/97	100	3	0.9 × 0.5	No	0	—	2	0.7 × 0.3
146640	6/12/98	65	10	0.5 × 0.3	No	0	—	0	—
146636	6/12/98	95	0	—	No	0	—	0	—
♀ hybrid									
145147	5/14/99	97	2	0.4 × 0.2	No	0	—	0	—
146681	6/10/96	84	1	0.3 × 0.2	No	0	—	0	—
145146	5/12/99	94	1	0.4	No	0	—	0	—
146691	6/12/98	88	0	—	No	0	—	0	—
146687	7/22/97	93	0	—	No	0	—	0	—
146688	7/22/97	91	0	—	No	0	—	0	—
146689	7/22/97	89	0	—	No	0	—	0	—
146694	6/15/98	71	0	—	No	0	—	0	—

[a] Number in parentheses is number of yolked oocytes present.

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row 1, section 5). **E.** Germinal epithelium with lip (AMNH R-146636, slide 11, row 1, section 3). **F.** Germinal epithelium with developing oocytes; section did not pass through lip (AMNH R-146618, slide 1, row 1, section 15). **G.** One follicle containing two oocytes. The theca interna completely separates the two oocytes and a single theca externa encompasses the follicle (AMNH R-146618, slide 4, row 2, section 2). Scale bar, 0.1 mm, except B, which is 0.01 mm.

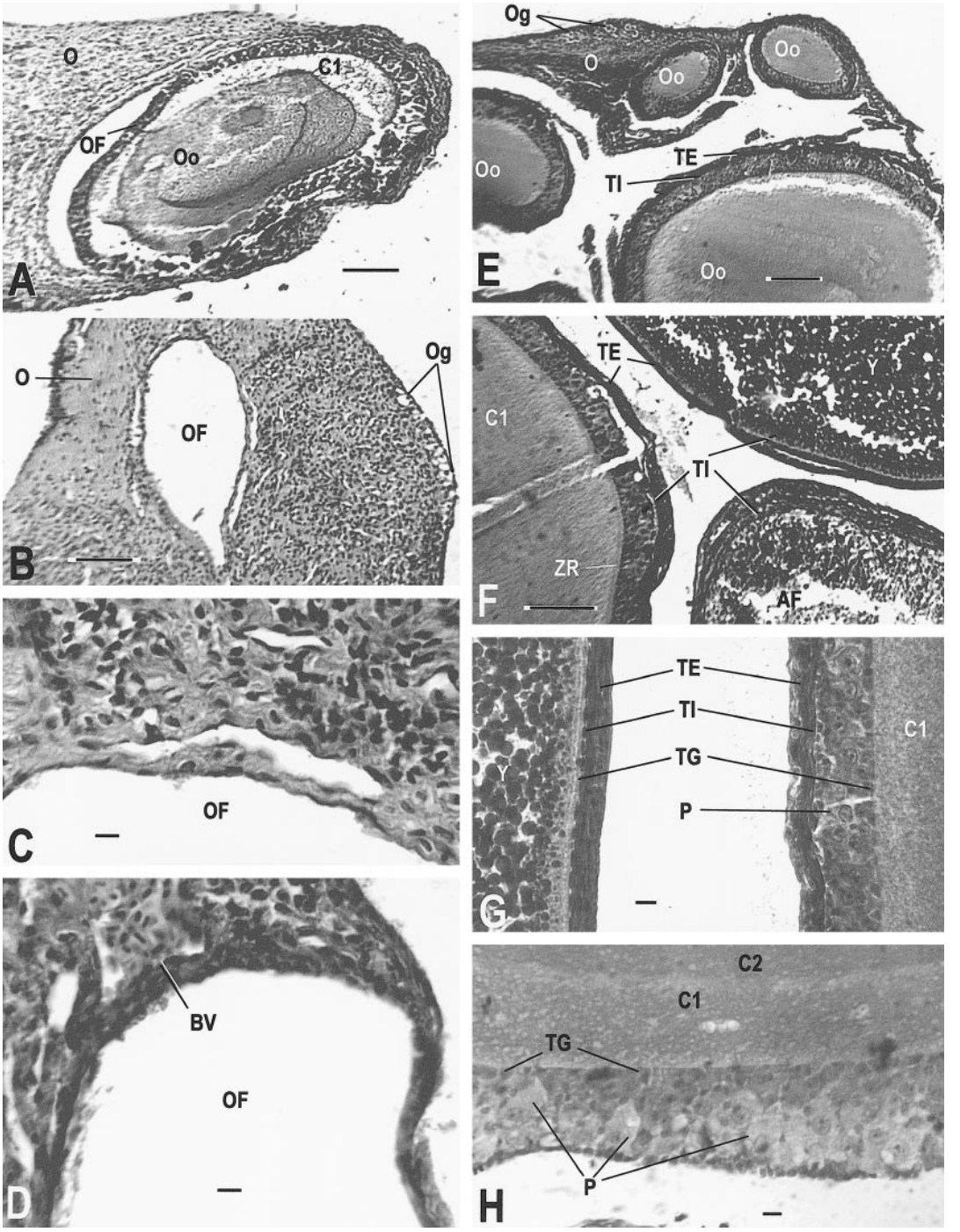

Fig. 16. Ovary structure of hybrid females (A–D) and *C. tesselatus* females (E–H). **A.** Ovary with abnormal follicle (AMNH R-145146, slide 5, row 1, section 4). **B.** Ovary with empty follicle and abnormal follicular epithelia (AMNH R-145147, slide 16, row 1, section 1). **C.** Detail of follicle wall of empty follicle (AMNH R-145147, slide 16, row 1, section 1). **D.** Empty follicle with blood supply (AMNH R-146681, slide 4, row 1, section 2). **E.** Ovary with oogonia and several sizes of developing

AMNH R-146637 (June 12, 1998): The left oviduct was enlarged but lacked eggs. The left ovary contained 14 enlarged follicles (fig. 14F) up to 1.4 mm in diameter and one corpus luteum. Macroscopically, the ovary showed one reddish spot, possibly the corpus luteum detected later by histological examination. The germinal epithelium contained oogonia and a distinctive lip (fig. 15D). The two largest oocytes had not begun vitellogenesis. No sperm were found.

AMNH R-146638 (June 12, 1998): The left ovary and oviduct were enlarged and the largest oocyte was yolked. No other oocytes were yolked and no recognizable corpora lutea were visible. No sperm were present in the oviduct.

AMNH R-146639 (June 12, 1998): The left ovary and oviduct were enlarged and the ovary contained three enlarged and yolked oocytes. The largest nonyolked oocyte was 1.9 mm in diameter, almost as large as those containing yolk, and larger by 0.5 mm than a yolked oocyte in AMNH R-146637. There were at least four corpora lutea present, all flattened or triangular, and up to 1.4 mm wide. No sperm were found.

AMNH R-145142 (May 12, 1999): Two oocytes 8.5 mm in diameter were depositing yolk when preserved. Several previtellogenic follicles exhibited secretory activity of the theca granulosa and conspicuous pyriform cells (fig. 14E) similar to those seen in *Ctenosaura* (del Carmen et al., 1996). Near the end of vitellogenesis, the granulosa was still evident as a distinctive cell layer (fig. 14H). The distal oviduct had a very thin mucosa and very thin folds near the infundibulum, but near the junction with the middle oviduct the mucosa was thickened (fig. 17F). The middle oviduct contained normal alveolar glands (fig. 17G, H). No sperm were found.

AMNH R-145143 (May 14, 1999): This specimen had an early atretic follicle with disorganized cytoplasm (fig. 14A, B) and follicles that were undergoing vitellogenesis (fig. 14G). The oviducts were enlarged; the folds of the distal oviduct were shallow nearer the infundibulum (fig. 17E, left) and deeper away from the infundibulum (fig. 17E, right). No sperm were found.

AMNH R-146612 (June 10, 1996): This specimen contained a yolked follicle (6.4 × 6.7 mm) and a corpus lutuem (0.5 mm diam.). The mesonephric tubules were small (0.02 mm diam.). Other ovarian and oviductal structures appeared normal. No sperm were found.

AMNH R-146613 (June 10, 1996): The ovary contained follicles of several sizes (as in fig. 16E), including yolked and atretic follicles (fig. 16F). No sperm were found.

AMNH R-146614 (July 20, 1997): This specimen contained two small corpora lutea and very small follicles (table 6). Like all others collected in July 1997 (fig. 21, table 6), this individual was postreproductive.

AMNH R-146618 (July 20, 1997): This specimen contained a recently ovulated follicle (fig. 14K), several normal follicles with germinal epithelium (fig. 15F), and two oocytes in one follicle (future twins that might share the same eggshell; fig. 15G). This follicle would have been available for the 1998 reproductive season. No sperm were found.

AMNH R-146620 (July 21, 1997): A large (7.0 × 3.7 mm) yolked atretic follicle (fig. 14C) and a recent corpus luteum with visible rupture site (fig. 14J) suggest that one of the two large oocytes ovulated and the other aborted, perhaps within two weeks (Miller, 1948) prior to collection. No sperm were found.

AMNH R-146621 (July 21, 1997): An atretic follicle (fig. 14D) and two corpora lutea (fig. 14L) were present. No sperm were found.

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follicles containing oocytes (AMNH R-146619, slide 3, row 1, section 3, MT). **F.** Follicle edges of a normal yolked follicle (upper right), atretic yolked follicle (lower right), and normal nonyolked follicle (left; AMNH R-146613, slide 6, row 2, section 9, MT). **G.** Previtellogenic follicle (right) and vitellogenic follicle (left) showing differences in cytoplasm, yolk, and theca development (AMNH R-146613, slide 6, row 2, section 6, MT). **H.** Theca granulosa of normal developing oocyte (AMNH R-146629, slide 19, row 1, section 3). Scale bar, 0.1 mm, except C, D, G, H, which are 0.01 mm.

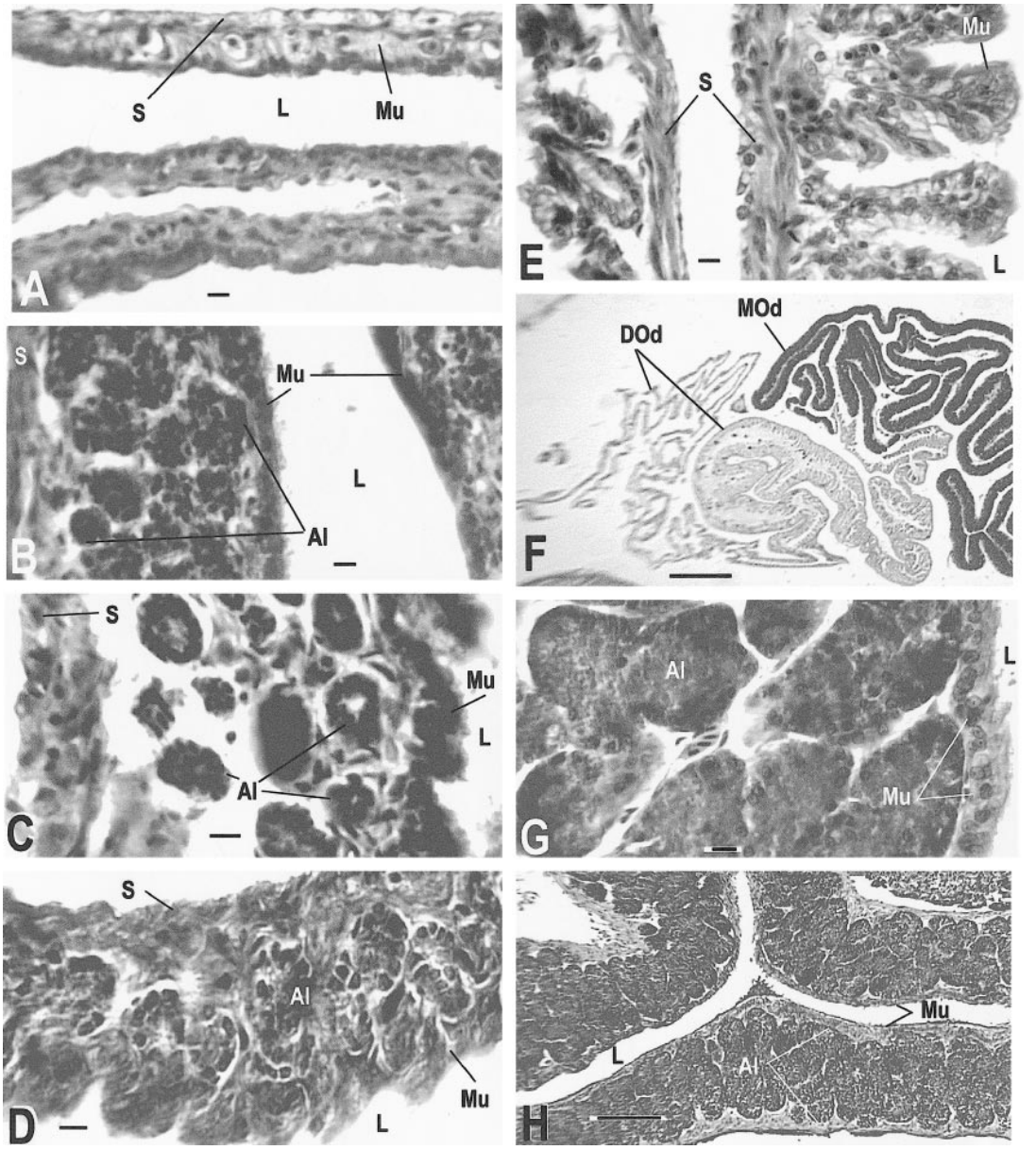

Fig. 17. Oviduct structure of hybrid females (A–D) and *C. tesselatus* females (E–H). **A.** Distal oviduct showing thin mucosa and absence of folds (AMNH R-145147, slide 8, row 1, section 1). **B.** Middle oviduct with alveolar glands (AMNH R-145147, slide 8, row 1, section 1). **C.** Middle oviduct showing poorly developed alveolar glands (AMNH R-145146, slide 11, row 2, section 2). **D.** Middle oviduct (AMNH R-146691, slide 9, row 2, section 6, MT). **E.** Distal oviduct showing mucosa and variation in fold development (AMNH R-145143, slide 5, row 2, section 6). **F.** Distal and middle oviducts; note the swollen (active) posterior part of the distal oviduct and the distinctive appearance of the middle oviduct. The proximal oviduct is not visible (AMNH R-145142, slide 136, row 1, section 2). **G.** Middle oviduct with mucosa and well-developed alveolar glands (AMNH R-145142, slide 80, row 1, section 2). **H.** Middle oviduct showing serosa, mucosa, and organization of the alveolar glands (AMNH R-145142, slide 85, row 1, section 4). Scale bar, 0.01 mm, except F, which is 1 mm, and H, which is 0.1 mm.

AMNH R-146640 (June 12, 1998): This specimen contained enlarged oviducts but tiny (unrecognizable macroscopically) ovaries. Many spaces were present around follicle cells and the theca granulosa was almost absent; however, there were no follicle cells in the cytoplasm of the oocytes, which appeared normal (fig. 18C). The adrenal and mesonephric tubules appeared normal (not enlarged) for a female (fig. 18C). No sperm were found.

Other specimens examined did not vary conspicuously from the above descriptions. Important to the question of hybridization frequency, none of the specimens of *C. tesselatus* had sperm in the oviducts.

During development the ovary is attached to the surface of the adrenal. In its earliest stages (during and soon after the indifferent stage) the ovary appears as a thickened epithelium, almost always with a distinctive lip on one edge. This ovarian lip makes the extremely young ovary more easily recognizable (see Hardy and Cole, 1981: fig. 3, top right corner, fig. 4, lower right corner, and fig. 6, lower right corner; Hardy and Cole, 1998: fig. 3B, top center, and fig. 2). In some samples the ovary is so small that only a few oocytes are present. The scarcity of oocytes (figs. 15F, 16B, E) and small size of the immature ovary make it a difficult organ to recognize. Thus, one must focus on the shape of the ovarian lip, plus the presence of oocytes, in order to recognize the small ovary (fig. 15A–E).

Vitellogenesis in *C. tesselatus* begins when the follicles are between 3.1 mm (smallest yolked oocyte measured) and 2.3 mm (largest nonyolked oocyte measured) in diameter (using largest dimension of oocyte [table 6]); however, if vitellogenesis is well under way for a normal complement of eggs (2–3 per ovary), then vitellogenesis might be delayed for other oocytes even though they are within the minimum size range for initiation of vitellogenesis.

The two oocytes in one follicle (fig. 15G) could have been independently derived from two different oogonia; a mutation in one oogonium but not in the other would result in these two oocytes being genetically different (one preserving the mutation). Alternatively, they could be the daughter cells, following endomitotic duplication, of a single oogonium, and mutation in the oogonium would be contained in both daughter oocytes.

Rupture of the follicle during ovulation would probably result in both oocytes moving as a unit, held together by the theca interna, into the oviduct and thence becoming incorporated within a single eggshell. Development of each oocyte to hatching would produce two hatchlings from a single shell—apparent twinning. However, if the oocytes separated after ovulation but before eggshell formation so that each had its own eggshell, then the potential twinning event would never be detected.

The number of normal yolks produced by a mature female in a season (about five, see below) is probably determined by the energy required for yolk synthesis and the available food supply. How the yolk is packaged is independent of the food supply and reflects the anatomical constraints of the lizard (is the egg too large to be laid?). Containment of both embryos in the same eggshell would probably result in a much larger egg (if both had a normal yolk reserve); this could be detrimental to the mother—the only significant evolutionary consequence of the apparent twinning.

All except 1 of the 26 adult *C. tesselatus* females had developing follicles and oocytes, and 4 were nearly ready for oviposition (oocyte diameters greater than 6 mm). One female (AMNH R-146636; snout–vent length = 95 mm) was nonreproductive, evidently because of developmental failure. This specimen had a snout–vent length large enough that one would expect it to contain enlarged oocytes, but it had no follicles, only a thin ovarian epithelium (almost an indifferent stage of embryonic development; fig. 15E), no corpora lutea, no increase in collagen, no evidence of atretic follicles, and a tiny oviduct, whereas the smallest female (AMNH R-146640; snout–vent length = 65 mm) had at least 10 enlarged oocytes (maximum size = 0.3 × 0.5 mm) in follicles that appeared normal (fig. 18C). With the exception of AMNH R-146636, these specimens of *C. tesselatus* appeared to be typical females in all details.

Cnemidophorus tesselatus × *C. tigris marmoratus* Females

AMNH R-146691 (June 12, 1998): The left ovary was 2.0 × 0.5 mm; the left oviduct was very small (despite the snout–vent length of 88 mm) but did contain alveolar glands (fig. 17D). The ovarian epithelium was thin but contained oogonia (fig. 15C). The mesonephros contained enlarged tubules and the adrenal was well developed. The middle oviduct contained a smooth muscle band, but the wall seemed thinner than normal (fig. 17D). No sperm were found.

AMNH R-146694 (June 15, 1998): The left oviduct was folded but not enlarged. The left ovary was 2.4 × 0.7 mm. No oocytes were recognizable. The stroma of the ovary was poorly defined and the cortex was not recognizable; however, this individual was only 71 mm snout–vent length (table 6). No sperm were found.

AMNH R-145146 (May 12, 1999): The ovaries were very small (unrecognizable macroscopically); one oocyte was enlarged, but the ooplasm layers and the follicle were abnormal (fig. 16A). The adrenal appeared normal and contained small and enlarged mesonephric tubules (fig. 18B). The oviducts were enlarged; the middle oviduct had shallow folds and small alveolar glands (fig. 17C). No sperm were found.

AMNH R-145147 (May 14, 1999): The very small ovary was unrecognizable macroscopically but contained loosely organized, empty follicles that contained an incomplete follicle cell layer (fig. 16B, C). The oviducts were enlarged and wrinkled. The distal oviduct had a thin epithelium and very low folds (fig. 17A) and the alveolar glands of the middle oviduct were small and poorly organized (fig. 17B). The mesonephric tubules were of normal diameter or they were enlarged (fig. 18A). No sperm were found.

AMNH R-146681 (June 10, 1996): The ovarian follicles were empty and lined with irregular and abnormal epithelium (fig. 16D). The mesonephric tubules were not enlarged. No sperm were found.

AMNH R-146689 (July 22, 1997): The adrenal contained germinal epithelium and small or slightly enlarged mesonephric tubules. No sperm were found.

AMNH R-146687 (July 22, 1997): The adrenal contained germinal epithelium (as in fig. 15A) with oogonia (as in fig. 15B) and small mesonephric tubules. The oviduct was small. No sperm were found.

AMNH R-146688 (July 22, 1997): The adrenal contained only a trace of germinal epithelium and small mesonephric tubules. No sperm were found.

There is no evidence that hybrid females can produce viable and fertile eggs. All of the ovaries examined were either undeveloped (without developing oocytes), or the follicles were empty, or the single developing oocyte (AMNH R-145146) was abnormal (see above). Empty ovarian follicles were also seen in a sterile, tetraploid hybrid (*C. sonorae* × *C. tigris*; Hardy and Cole, 1998: fig. 3B).

The eight hybrid females (table 6) were not reproductive, although seven of them were of adult size. The abnormal development of the ovary and lack of oocytes in three hybrids (AMNH R-145146, R-145147, and R-146681) suggest that they were sterile. The other five female hybrids (table 6, fig. 15A–C) did not contain any developing follicles (oogonia were present), were not reproductive for that breeding season, and were probably sterile. Although female hybrids could alleviate the impact of hybridization on *C. tesselatus* by being available to mate with hybrid males or with males of *C. tigris marmoratus*, there was no evidence that this takes place (all hybrid oviducts lacked sperm).

Cnemidophorus tigris marmoratus Males

AMNH R-146650 (June 11, 1998): The left testis was 3.7 × 2.9 mm. The seminiferous tubules contained sperm and spermatids (fig. 19D), and meiotic divisions were visible. A thick tunica vasculosa and thinner tunica albuginea were present. The Leydig cell layer was thin.

AMNH R-146652 (June 13, 1998): The left testis was 2.6 × 1.9 mm. The seminiferous tubules had poorly developed lumina that contained large spermatogonia-like cells with darkly stained nuclei (fig. 19E). The tubule walls also contained large vacuolated cells; spermatocytes were present but no

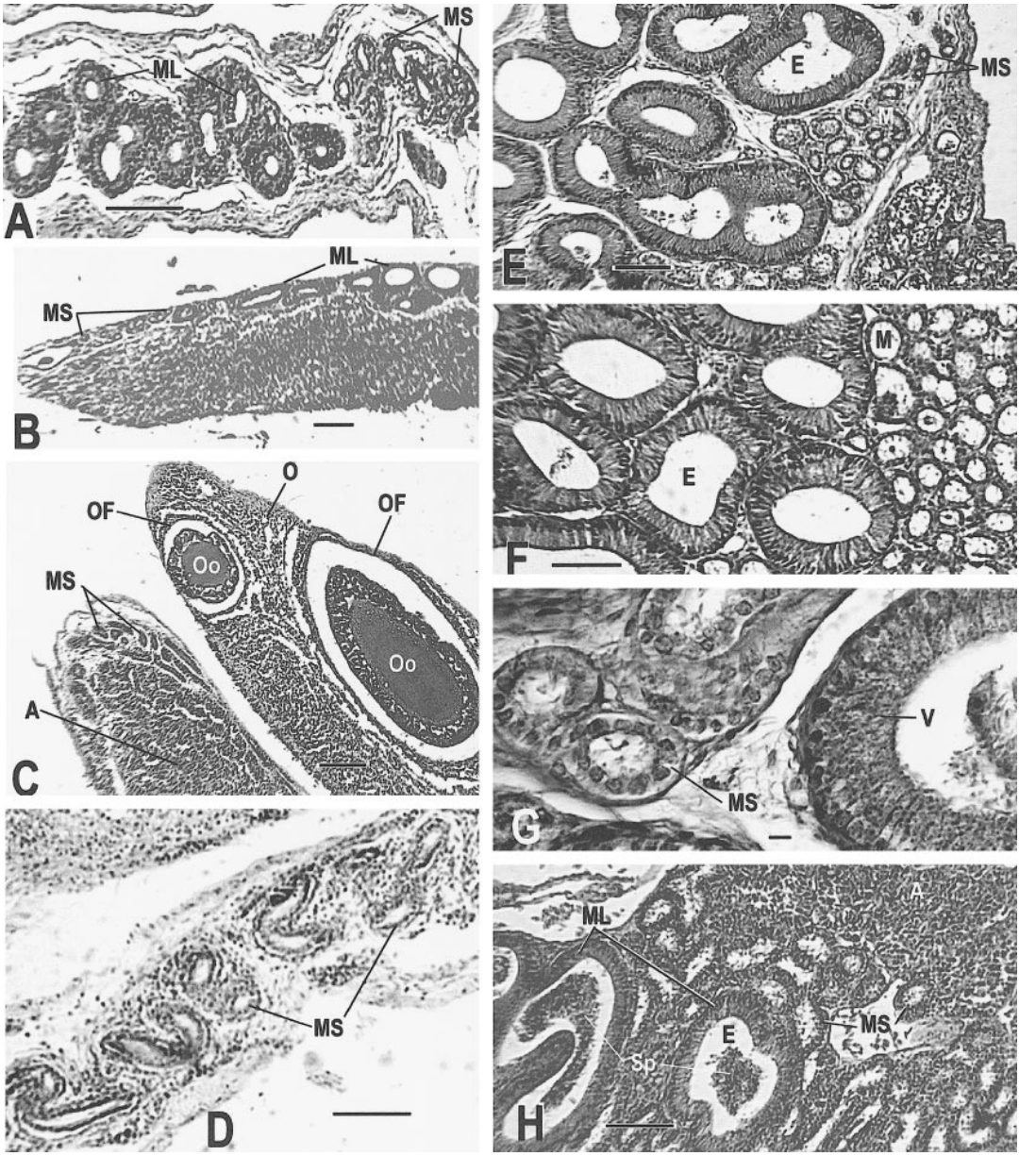

Fig. 18. Mesonephros of hybrid females (A, B), *C. tesselatus* (C, D), hybrid males (E–G), and a *C. tigris* male (H). **A.** Mesonephros showing small and large tubules (AMNH R-145147, slide 8, row 2, section 6). **B.** Adrenal gland with large and small mesonephric tubules (AMNH R-145146, slide 11, row 1, section 2). **C.** Ovary and adrenal with small mesonephric tubules; no large tubules present (AMNH R-146640, slide 4, row 1, section 1). **D.** Mesonephros with small tubules; no large tubules present (AMNH R-146629, slide 10, row 1, section 4). **E.** Large tubules (= epididymis) and small mesonephric tubules; debris does not include sperm (AMNH R-145148, slide 8, row 1, section 6). **F.** Epididymis and small mesonephric tubules (AMNH R-146695, slide 25, row 3, section 6). **G.** Vas deferens and small mesonephric tubules (AMNH R-146692, slide 12, row 2, section 6, MT). **H.** Large mesonephric tubules and epididymis (packed with sperm) and small mesonephric tubules (AMNH R-146653, slide 10, row 2, section 4). Scale bar, 0.1 mm, except G, which is 0.01 mm.

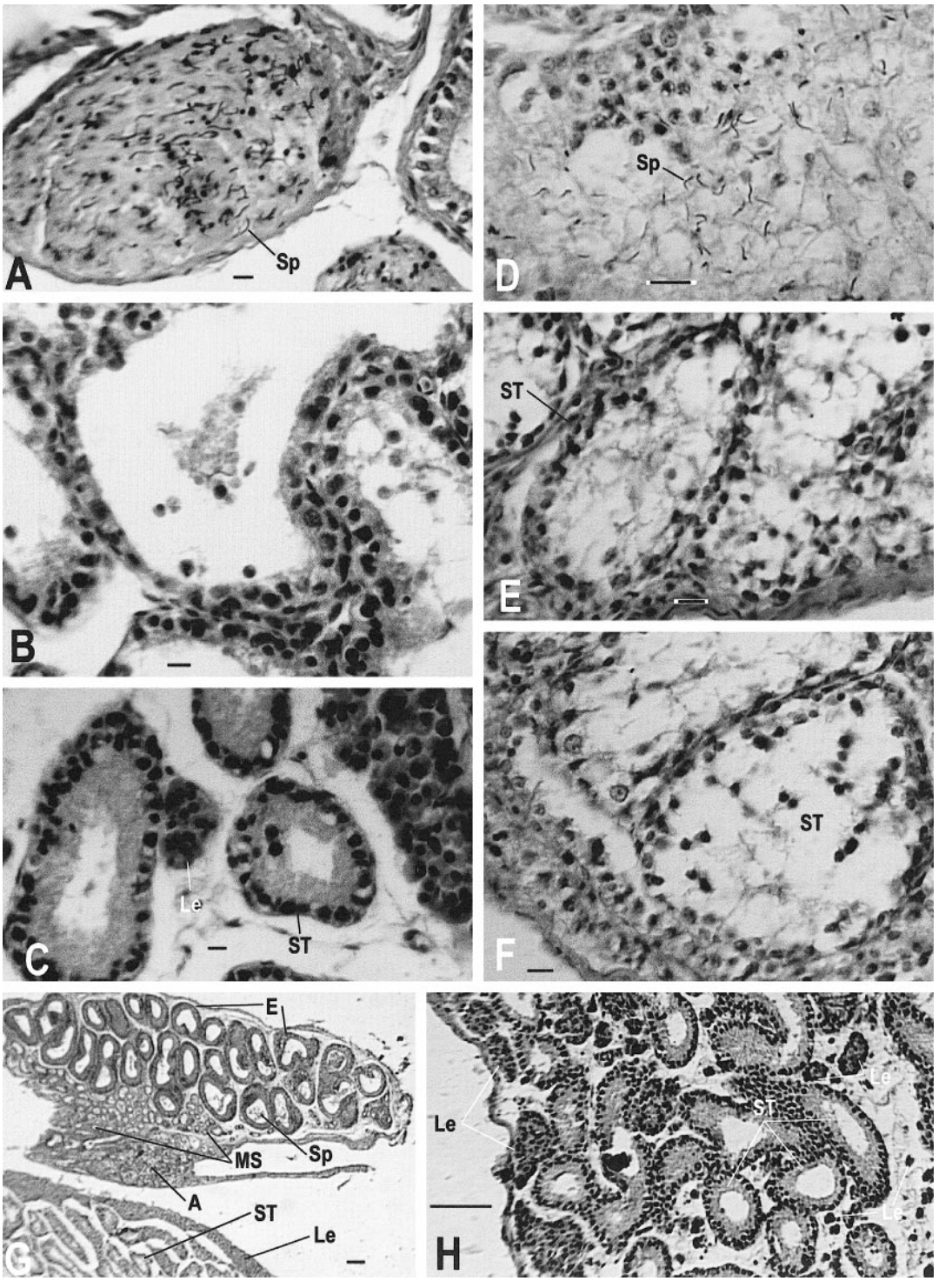

Fig. 19. Testes of hybrid males (A–C, G, H) and *C. tigris* males (D–F). Note that G and H are out of sequence because of size. **A.** Testis with sperm (AMNH R-145145, slide 5, row 2, section 3). **B.** Testis with debris and few sperm (AMNH R-146683, slide 10, row 1, section 4). **C.** Seminiferous tubules

sperm were visible (fig. 19F). The thin wall contained smooth muscle. The large lumen of the vas deferens contained irregular cells, almost like debris; no sperm were present. The tunica albuginea and tunica vasculosa were as described by Goldberg and Lowe (1966). The Leydig cell layer of the tunica vasculosa was thin and incomplete. The seminiferous tubules were occluded with fibrous material and some spermatids, and only a few spermatogonia and primary spermatocytes were present. Since the specimen (snout–vent length = 65 mm) was at the minimum size for sexual maturity (snout–vent length = 64 mm; Vitt and Breitenbach, 1993), it is likely that limited spermatogenesis had begun, but that sperm were not yet being moved from the seminiferous tubules to the epididymis. Sertoli cells were not obvious (fig. 19E, F). The mesonephric tubules were of normal (20–30 μm) or large (75–100 μm) size.

AMNH R-146653 (June 13, 1998): The left testis was 5.4 × 3.3 mm. Most of the seminiferous tubules were almost empty but all contained a few sperm; however, masses of sperm were present in a few tubules in the center of the testis and also in the vas deferens and epididymis (fig. 18H). Those tubules without masses of sperm were occluded with fibrous material and a few spermatids as in AMNH R-146652. Reproductive activity was probably ending because the seminiferous tubules had a poorly defined lumen, occluded with nonreproductive material, and spermatids were less abundant. Since the epididymis contained sperm (fig. 18H), a recent copulation probably had not occurred, if empty vasa deferentia are true indicators of copulation (Goldberg and Lowe, 1966).

Of the three male *C. tigris marmoratus* examined, one small individual of 65 mm snout–vent length (AMNH R-146652) appeared to be in the process of acquiring reproductive maturity, and the other two (each 92 mm snout–vent length) were reproductively competent.

Cnemidophorus tesselatus × *C. tigris marmoratus* Males

Cytological preparations of a testis from one triploid male hybrid (AMNH R-146690) showed many cells undergoing mitosis but few in meiosis. A few cells at diakinesis of meiosis I were recognizable, some with clear trivalents. Considering that this male was collected during the mating season of the bisexual species (June 11, 1998), in which meiotic activity should have been high, this male appeared to be unable to produce viable spermatozoa. However, histological sections did reveal spermatozoa in this individual (see below).

AMNH R-145145 (May 12, 1999): Small amounts of sperm were present in the testis (fig. 19A), epididymis, and vas deferens. The epididymis tubules contained debris and few sperm.

AMNH R-145149 (May 15, 1999): The epididymis contained both large and small mesonephric tubules. The seminiferous tubules of the testis were poorly defined; most contained cellular and noncellular debris. Even though some cell divisions were present, sperm or spermatids were not recognizable.

AMNH R-146682 (July 20, 1997): Sperm were present in the large mesonephric tubules of the epididymis and in the seminiferous tubules of the testis.

AMNH R-146683 (July 20, 1997): Some sperm were present in the testis. Most of the seminiferous tubules had only one cell layer between the basement membrane and the lumen (fig. 19B). The epididymis tubules were either slightly enlarged (up to 0.13 mm) and usually contained cellular debris and few

←

with isolated masses of Leydig cells (AMNH R-146686, slide 7, row 1, section 7). **D.** Testis with sperm (AMNH R-146650, slide 1, row 2, section 4). **E.** Seminiferous tubules (AMNH R-146652, slide 4, row 1, section 5). **F.** Seminiferous tubules (AMNH R-146652, slide 2, row 3, section 5). **G.** Testis, adrenal, mesonephros, and epididymis (AMNH R-145148, slide 8, row 1, section 3). **H.** Seminiferous tubules, clusters of Leydig cells, and incomplete Leydig cell layer (AMNH R-146686, slide 7, row 1, section 6). Scale bar, 0.01 mm, except G and H, which are 0.1 mm.

sperm or they were of normal size (0.02 mm) and without sperm.

AMNH R-146684 (July 20, 1997): The small testis (3.2 × 1.9 mm) and large tubules of the epididymis contained a few sperm.

AMNH R-146685 (July 21, 1997): Masses of sperm and cellular debris were in some tubules of the epididymis; small mesonephric tubules were present but did not contain sperm. Sperm were also in the seminiferous tubules.

AMNH R-146686 (July 21, 1997): The seminiferous tubules contained debris, but no sperm; most tubule walls consisted of a single layer of cells, including spermatogonia and a few spermatocytes. Approximately two-thirds of the seminiferous tubule wall was fine granular material adjacent to the lumen (fig. 19C). The granular material did not stain blue with Mallory triple stain, so the material was not collagen, mucus, hyaline substance, or connective tissue. Cell membranes were not obvious, but some indistinct boundaries were visible, and thus the material might be partially coalesced cytoplasm. Seminiferous tubules were very loosely organized with much space between tubules (fig. 19C, H). The Leydig cell layer was incomplete and there were clusters of Leydig cells in the interior of the testis (fig. 19H). No sperm were present in the testis or vas deferens.

AMNH R-146690 (June 11, 1998): The left testis was 6.2 × 5.4 mm. The right testis was abnormal in appearance; it was elongated, almost triangular in shape, and seemed to be composed of an anterior and posterior part. The left testis was lobular in shape and contained tailed sperm and spermatids in the seminiferous tubules and tailed sperm in the epididymis (as in fig. 19G). The Leydig cell layer was thin (approximately one half or less the thickness of the layer in normal *C. tigris* testes measured by us). Lowe and Goldberg (1966) reported the Leydig cell layer thickness for adult *C. tigris* to average 6.7 cells.

AMNH R-146692 (June 12, 1998): The left testis (3.2 × 3.8) contained a Leydig cell layer. Only a few spermatids and spermatozoa were present in some seminiferous tubules. Many primary spermatocytes were present and there were several spermatogonia-like cells. The seminiferous tubules had a very thin epithelium, little more than one cell layer of cuboidal cells, but the lumen was well defined. The largest cells (probably primary spermatocytes) had large, less dense nuclei containing nucleoli and heterochromatin, clear cytoplasm, and were in contact with the basement membrane. The seminiferous tubules contained heterogeneous debris and a few spermatids but few (in some tubules) or no sperm. The vas deferens and mesonephric tubules contained debris and a few spermatids (fig. 18G).

AMNH R-146693 (June 15, 1998): Macroscopically, the left kidney had a white arbor vitaelike pattern and the left epididymis was white. The left testis was sectioned; the right testis measured 5.6 × 4.5 mm. The seminiferous tubule walls were 2–3 cells in thickness and the lumina lacked sperm; however, there were some spermatogonia and spermatids present but spermatocytes were rare (some primary spermatocytes were recognizable). The vas deferens was empty. Part of the mesonephros looked like an epididymis, but the lumina lacked sperm.

AMNH R-145148 (May 14, 1999): The testis and epididymis (fig. 18E) contained sperm.

AMNH R-146695 (May 12, 1999): The testis and epididymis (fig. 18F) contained sperm.

One of the hybrid males (AMNH R-146690) was reproductive and had produced sperm; however, the sperm-packed epididymis suggests that recent copulation had not occurred. Seven other hybrid males contained sperm in the testis and epididymis; one contained sperm in the testis but none in the epididymis. Three hybrid males lacked sperm in the testis and in the epididymis, but there were spermatocytes and some spermatids present in the testis (table 7). Sperm was absent from the small mesonephric tubules of all individuals. The hybrid males were capable of producing sperm that appeared normal, and the variable presence of sperm in the epididymis suggested that they were mating with females. However, it is not known which females would receive their attention (hybrid, *C. tesselatus*, or *C. tigris marmoratus*) or if the males were fertile.

Mating of a male *C. tesselatus* × *C. tigris*

TABLE 7
Reproductive Characteristics of Male *Cnemidophorus tigris marmoratus* and Male *C. tesselatus* × *C. tigris marmoratus* Hybrids

Specimen (AMNH R)	Date collected	SVL (mm)	Testis size (mm)	Spermato-gonia	Spermato-cytes	Sperm in testis	Sperm in epididymis	Repro-ductive
C. tigris marmoratus								
146653	6/13/98	92	5.4 × 3.3	Yes	Yes	Yes	Yes	Yes
146650	6/11/98	92	3.7 × 2.9	Yes	Yes	Yes	—[a]	Yes
146652	6/13/98	63	2.6 × 1.9	Yes	Yes	No	No	No
♂ hybrid								
146695	5/12/99	96	7.3 × 5.5	Yes	Yes	Yes	Yes	Yes
146690	6/11/98	93	6.2 × 5.4	Yes	Yes	Yes	Yes	Yes
146693	6/15/98	97	5.6 × 4.5[b]	Yes	Yes	No	No	No
145148	5/14/99	92	5.2 × 4.5	Yes	Yes	Yes	Yes	Yes
146692	6/12/98	92	3.8 × 3.2	Yes	Yes	Yes	No	No
146685	7/21/97	93	3.4 × 2.3	Yes	Yes	Yes	Yes	Yes
145145	5/12/99	95	3.0	Yes	Yes	Yes	Yes	Yes
146682	7/20/97	93	2.9 × 2.0	Yes	Yes	Yes	Yes	Yes
146686	7/21/97	88	2.8 × 1.5	Yes	Yes	No	No	No
145149	5/15/99	56	2.0 × 0.8	Yes	Yes	No	No	No
146683	7/20/97	89	—	Yes	Yes	Yes	Yes	Yes
146684	7/20/97	87	—	Yes	Yes	Yes	Yes	Yes

[a] Epididymis not sectioned.
[b] Right side.

marmoratus hybrid has probably been witnessed in a laboratory setting (Saxon, 1968), where copulation between a male (identified as a male *C. tesselatus*) and a small parthenogenetic female of *C. tesselatus* was documented on five different days. However, it is likely that this male was a *C. tesselatus* × *C. tigris marmoratus* hybrid based on its color pattern and collecting locality. Saxon's male had the same bold contrast between relatively large pale and dark sectors that characterize the color pattern of the majority of hybrids from Arroyo del Macho. Compare figure 1 in Saxon (1968) to our figures 2B and 6C. Note similarities in the absence of pale elements in the midsagittal region of the vertebral dark field, the short transverse pale bars extending from the paravertebral stripes into the adjacent dark fields, and the high contrast between dark and pale elements in the pattern. In addition, Saxon's male came from the same locality as another male (TCWC 22324) reported as a male *C. tesselatus* by Saxon et al. (1967). Specimen TCWC 22324 was one of five males from the Presidio, Texas area that we have identified, in this report, as *C. tesselatus* × *C. tigris marmoratus* hybrids (see below). The male reported by Saxon (1968) was not one of these five specimens, and it is not in the TCWC collections (L. Fitzgerald, personal commun.).

Mesonephric tubules in *C. tesselatus* were small (20–30 μm in diameter; fig. 18C, D). During embryonic development of a normal male the mesonephric tubules become the tubules of the epididymis and the vas deferens, as seen in normal males of *C. tigris marmoratus* (fig. 18H). In males of *C. tigris* (AMNH R-146650, R-146652, R-146653; LSUS 971, 4135) and *C. sexlineatus* (LSUS 1014, 1018, 1019) examined thoroughly or spot-checked, the epididymis consisted of both larger and smaller tubules. Both groups of tubules are probably derived from the embryonic mesonephric kidney. The smaller tubules (20–30 μm in diameter) are remnants of the mesonephros. The larger tubules (usually more than 75 μm in diameter) are probably remnants of the mesonephros and become the functional epididymis of adult males of bisexual species. The hybrids (*C. tesselatus* × *C. tigris marmoratus*) of both sexes contained both smaller (mesonephric)

and larger (epididymis) tubules. The mesonephric tubules (smaller size) never contained sperm in hybrids or in nonhybrid males. The function of the mesonephric tubules in adults is not known (the lumen is always open, suggesting a secretory duct). Sperm were never seen in the small tubules of the mesonephros. The structural and functional relationship between the mesonephric tubules and the epididymis in adults is not known (if they are connected, why are sperm not seen in the mesonephric tubules?).

This study confirms the distinctive histological nature of some aspects of the reproductive tract of female hybrids between parthenogenetic species and bisexual, gonochoristic species (Hardy and Cole, 1998). Four significant histological characteristics were defined by Hardy and Cole (1998) as indicating sterility in a tetraploid hybrid female between *C. exsanguis* and *C. tigris*: (1) poorly defined follicular epithelium with little vascularization in very small ovaries, (2) empty or fluid-filled follicles without oocytes, (3) numerous abnormally large mesonephric tubules, and (4) few or no cilia in the middle oviduct. The seven hybrid females in this study were also sterile, and all had very small ovaries. Some lacked any developing oocytes or follicles, others contained empty follicles, and one contained a follicle with an abnormal oocyte. In every recognizable follicle the epithelium was thin and lacked pyriform cells in the granulosa. In those specimens with only germinal epithelium the mesonephric tubules were of normal size (small); however, in most of those specimens with follicles the mesonephric tubules were enlarged. One specimen (AMNH R-146681) had empty follicles with abnormal epithelia and small mesonephric tubules. The extent of cilia development in the middle oviduct was difficult to quantify; however, in all specimens for which the middle oviduct was examined the wall was thin, with shallow mucosal folds, small alveolar glands, and few cilia. The presence of small ovaries, empty follicles, abnormal follicular epithelia, and enlarged mesonephric tubules provide additional evidence for the identification of female museum specimens of suspected hybrids between parthenogenetic and bisexual species.

TABLE 8

Comparison[a] of Reproductive Characteristics in *Cnemidophorus tesselatus* and *C. tigris marmoratus* Collected June 11–15, 1998, in Chaves County, New Mexico

Character	*C. tesselatus*	*C. tigris marmoratus*
SVL of gravid females	93.7 ± 1.45	76.0 ± 1.34
	87–103	70–80
	N = 13	N = 8
Clutch size	5.5 ± 0.35	2.2 ± 0.25
	3–7	1–3
	N = 13	N = 8

[a] Mean ± SE, range, and sample size are shown.

Reproductive Characteristics and Size Distributions

All reproductively mature females of *C. tigris marmoratus* are probably inseminated each reproductive season because of their concentration in a specific, limited microhabitat at Arroyo del Macho. The relatively large number of hybrids indicates that males of *C. tigris marmoratus* readily extend their attention to individuals of *C. tesselatus,* which may be encountered more often than females of their own species. This may be particularly true of young adult males if they are displaced by older males into marginally preferred habitats. Reproductively mature females of *C. tesselatus* are significantly larger than those of *C. tigris marmoratus* (snout–vent length: $t_{19} = 8.308$, $P < 0.0005$). Because of a positive relationship between clutch size and body size in both species (adjusted $R^2 = 0.86$), *C. tesselatus* has a significantly larger clutch size ($t_{19} = 6.534$, $P < 0.0005$). Comparisons are provided in table 8.

Two clutches per year may be the usual reproductive output by *C. tesselatus* at the hybridization site based on the following evidence. Each of two representatives of *C. tesselatus* collected June 10, 1996 (AMNH R-146612, 98 mm snout–vent length, and R-146613, 86 mm snout–vent length) had a corpus luteum and yolking follicles for a second clutch, and another individual collected June 12, 1998 (AMNH R-146639, 89 mm snout–vent length) had four corpora lutea and yolking follicles (table 6). In addition,

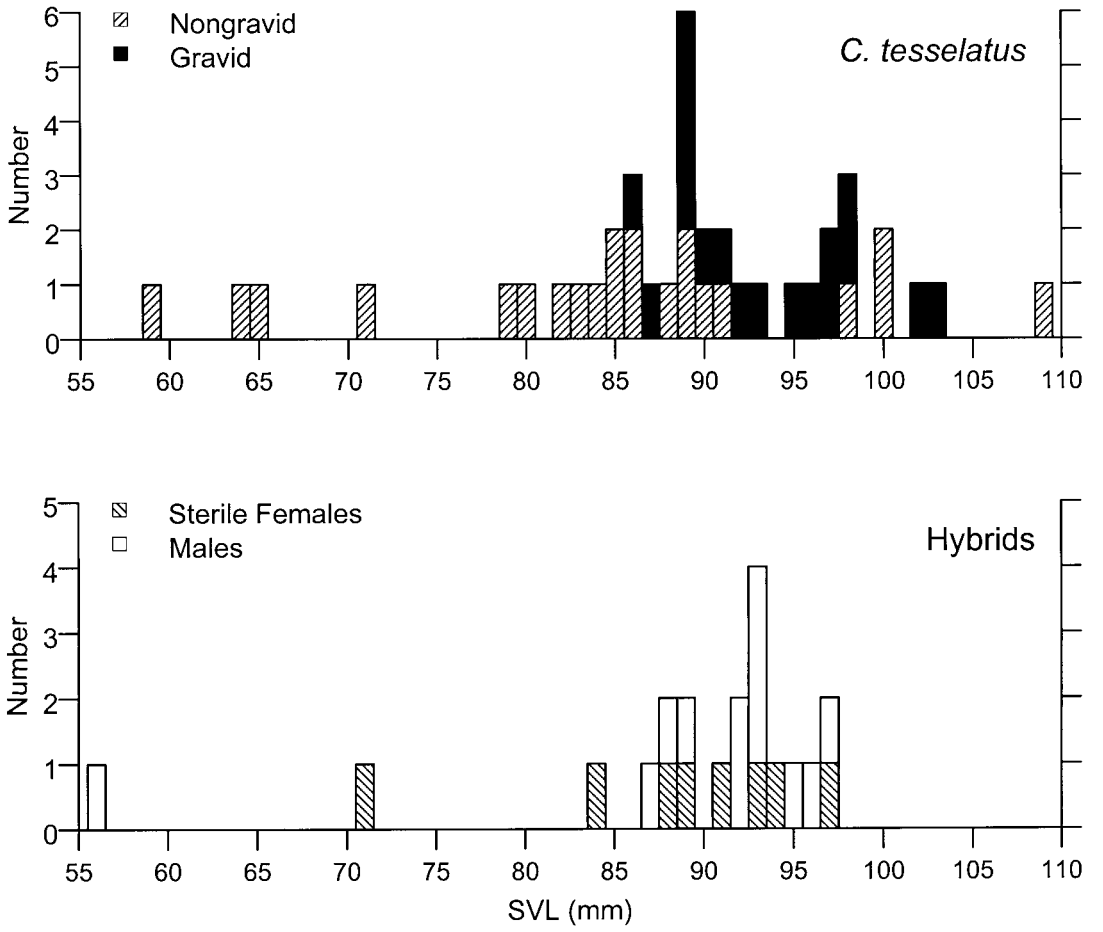

Fig. 20. A comparison of body size (snout–vent length) and gravid status of female individuals of *Cnemidophorus tesselatus* and body size of male and female *C. tesselatus* × *C. tigris marmoratus* hybrids collected from the Arroyo del Macho hybridization site in 1996–1999.

two successive bouts of reproduction, one in May and another in June, appear to be depicted by the distribution of gravid individuals in figure 21.

On the other hand, the reproductive success of individuals of *C. tesselatus* is occasionally compromised by the production of sterile individuals. For example, AMNH R-146636, a large individual (95 mm snout–vent length), had experienced developmental failure of both ovaries and oviducts (see Histological Analysis section above). This was 1 individual out of 26 *C. tesselatus* examined.

Seven individuals of *C. tesselatus* maintained in the *Cnemidophorus* colony at the American Museum of Natural History for short periods subsequent to capture produced five clutches containing 5, 5, 5, 6, and 7 eggs. Of the six hatchlings derived from these clutches, none was a hybrid. It is curious that the remaining 22 eggs failed to hatch. Seventeen of the 22 eggs were entire clutches of 5, 5, and 7 eggs, all of which appeared to be developing normally for two weeks or more before failing. The unexpected high proportion of failures could be explained if these clutches consisted of hybrid embryos that failed to develop, although we have no way of testing this hypothesis now. If this hypothesis were correct, the number of living hybrid lizards observed in the field would represent an incomplete estimate of the amount of hybridization occurring at this

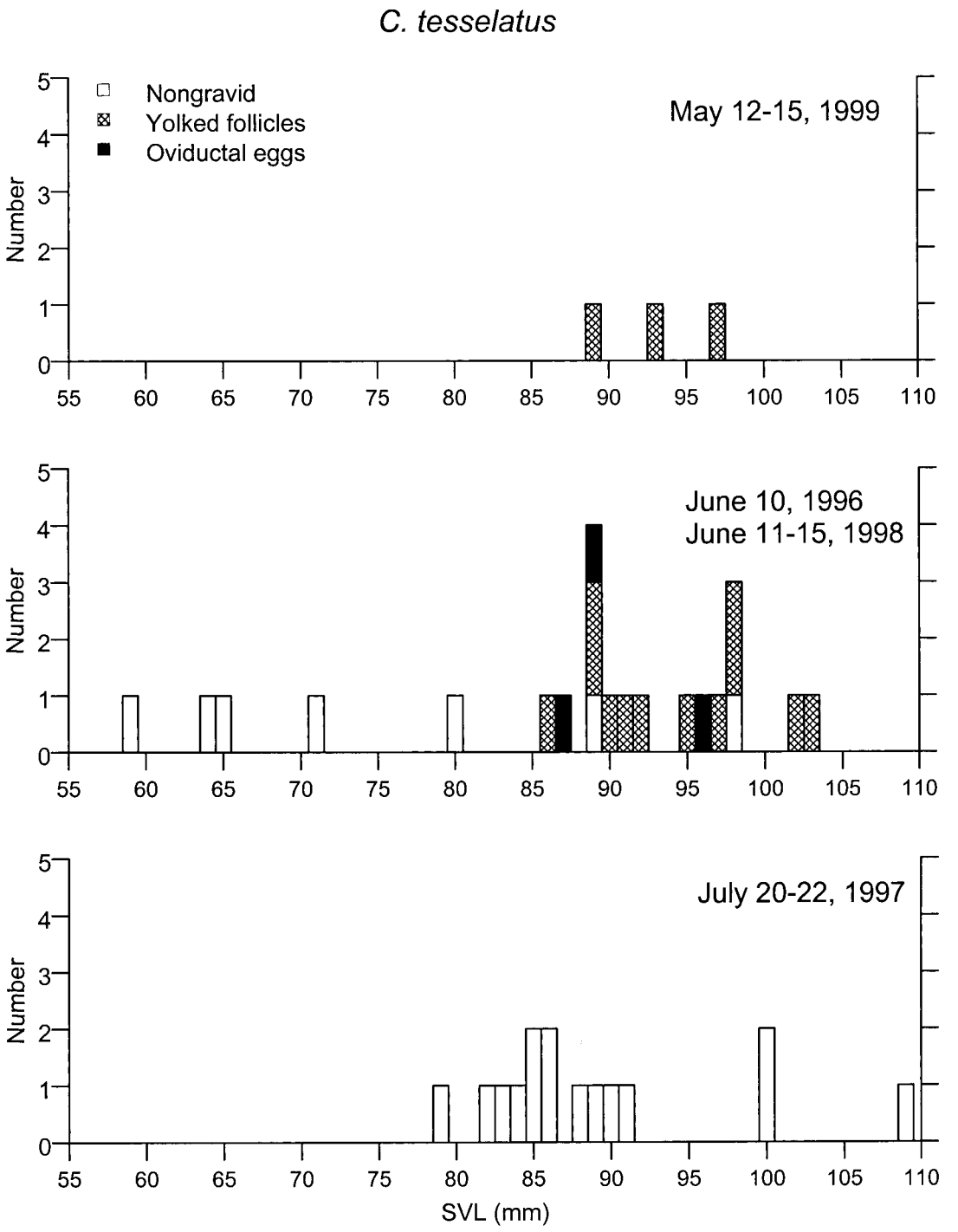

Fig. 21. A monthly comparison of size distributions and reproductive status of individuals of *Cnemidophorus tesselatus* collected at the Arroyo del Macho hybridization site in 1996–1999.

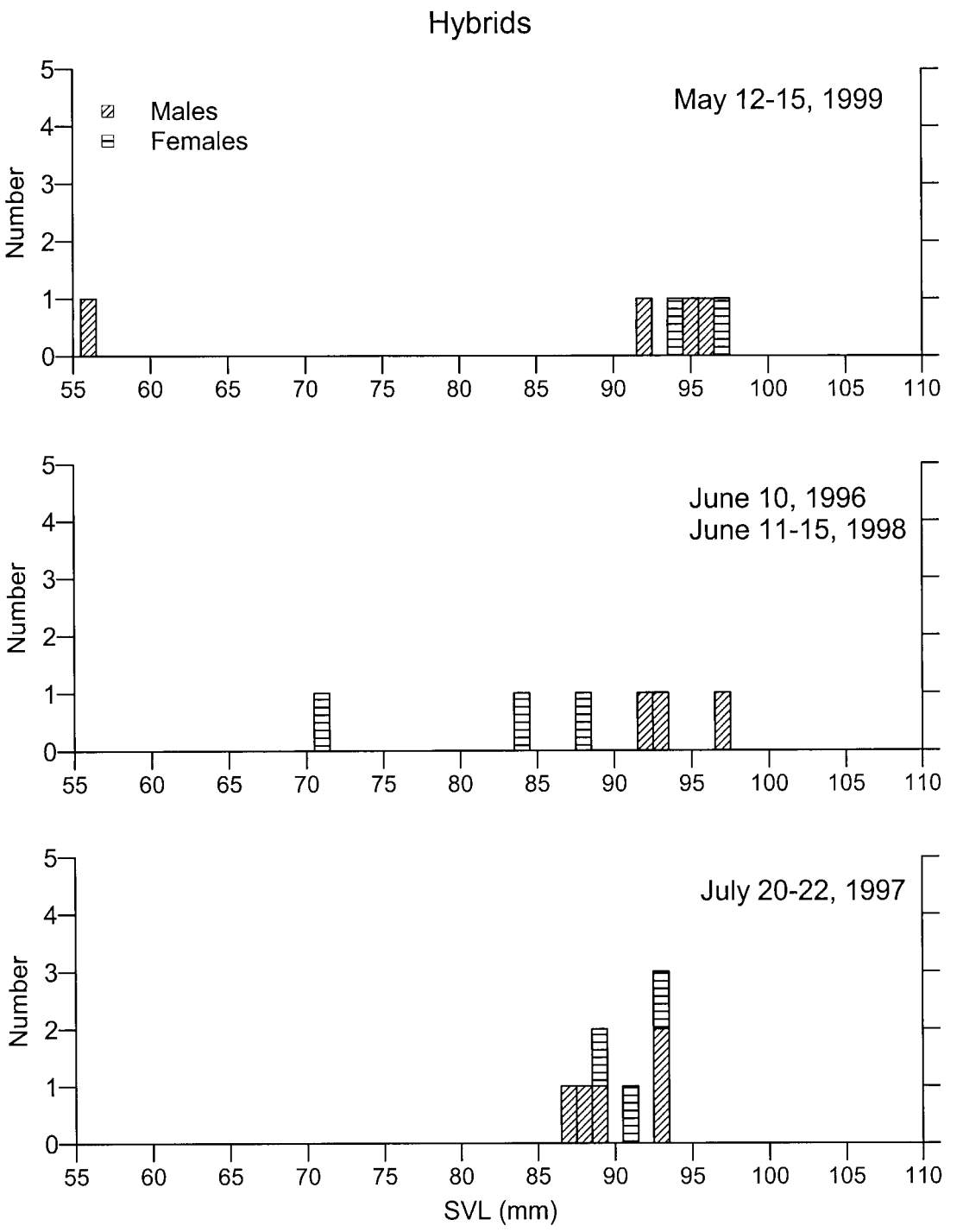

Fig. 22. A monthly comparison of size distributions of *Cnemidophorus tesselatus* × *C. tigris marmoratus* hybrids collected at the Arroyo del Macho hybridization site in 1996–1999.

site. However, another explanation for this developmental failure lies in the difficulty of successfully incubating *C. tesselatus* eggs in the laboratory (Maslin, 1966; Townsend, 1979). In addition, whether or not entire clutches of parthenogenetic eggs are typically fertilized by male congeners is problematic. For example, Darevsky and Danielyan (1968) showed that only a portion of a clutch of eggs was fertilized following mating between individuals of parthenogenetic *Lacerta armeniaca* or *L. unisexualis* and males of the bisexual species *L. saxicola valentini* (= *L. valentini*). Diploid and triploid offspring were both produced from the same clutch of eggs. Absence of sperm in the reproductive tracts of 11 individuals of *C. tesselatus* sampled at the height of reproductive activity in May and June indicates that many individuals escape being fertilized by males of *C. tigris marmoratus* (see Histological Analysis section above).

Body-size distributions (snout–vent length) were similar for 40 *C. tesselatus* and 20 hybrids collected in 1996–1999 (fig. 20; Mann-Whitney $U = 368.5$, $P = 0.62$). Mean snout–vent length was also nonsignificantly different for these groups (*C. tesselatus*: 88.6 $\pm$ 1.65; hybrids: 88.9 $\pm$ 2.16; $t_{58} = 0.107$, $P = 0.92$). Excluding samples collected in 1999 (when collection pressure on *C. tesselatus* was intentionally relaxed), data from 1996–1998 (figs. 20–22) indicate that between two and three individuals of *C. tesselatus* were being produced for each hybrid generated. A finer discrimination scale is provided by using snout–vent length as an index to the age of individuals. This approach indicated that our samples contained five separate generations of *C. tesselatus* and hybrids produced in five different years (fig. 23). However, only 2 of 20 hybrids were in their second year of life (12 were in year 3, and 6 were in year 4), indicating that younger hybrids are underrepresented in our sample. Because our sample of hybrids is made up of individuals that had accumulated at the site across several years, the likely impact that an annual production of hybrids has on the survival of this population of *C. tesselatus* would seem to be ameliorated. A second factor contributing to the local success of *C. tesselatus*, despite the annual production of sterile hybrids, is related to the distribution of the preferred microhabitats of the two hybridizing species (see below).

Ecological Differences Between the Hybridizing Species

Representatives of *C. tesselatus* were approximately twice as abundant as either hybrids or representatives of *C. tigris marmoratus* at the Arroyo del Macho hybridization site (table 9). The estimate for *C. tigris marmoratus* is potentially biased because a disproportionate amount of our time was spent in areas yielding the greatest numbers of *C. tesselatus* and hybrids. In general, these areas were peripheral to the creosote bush stands favored by *C. tigris marmoratus.* Nevertheless, we are certain that the density of *C. tigris marmoratus* was very low at the hybridization site, and that the density of *C. tesselatus* was significantly higher. This was similar to the situation found by Paulissen et al. (1992) for other species in Texas where a marked imbalance in the numbers of either the parthenogenetic species or the sympatric bisexual species was a condition for predictable hybridization between them.

Two contrasting hypotheses can serve as entrees to studies of the relative merits of parthenogenetic and sexual reproduction and to the evolutionary prospects of parthenogenetic and bisexual species. We can use the ecological relationships between *C. tesselatus* and *C. tigris marmoratus* at Arroyo del Macho to address each hypothesis. The first hypothesis is the ''tangled-bank'' model advanced by Bell (1982). This idea posits that bisexual species (with phenotypic variation generated by recombination and exposed to selection each generation) should have advantages over their parthenogenetic derivatives (with heritable phenotypic variation constrained by clonal reproduction) in capturing varied niches provided by heterogeneous habitats. According to the ''tangled-bank'' model, bisexual species (with alleged adaptive superiority) should eventually relegate parthenogenetic species to marginal habitats wherever they coexist, an idea earlier espoused by Cuellar (1977). Price (1992) gave credence to the hypothesis by concluding that parthenogenetic *Cnemidophorus*

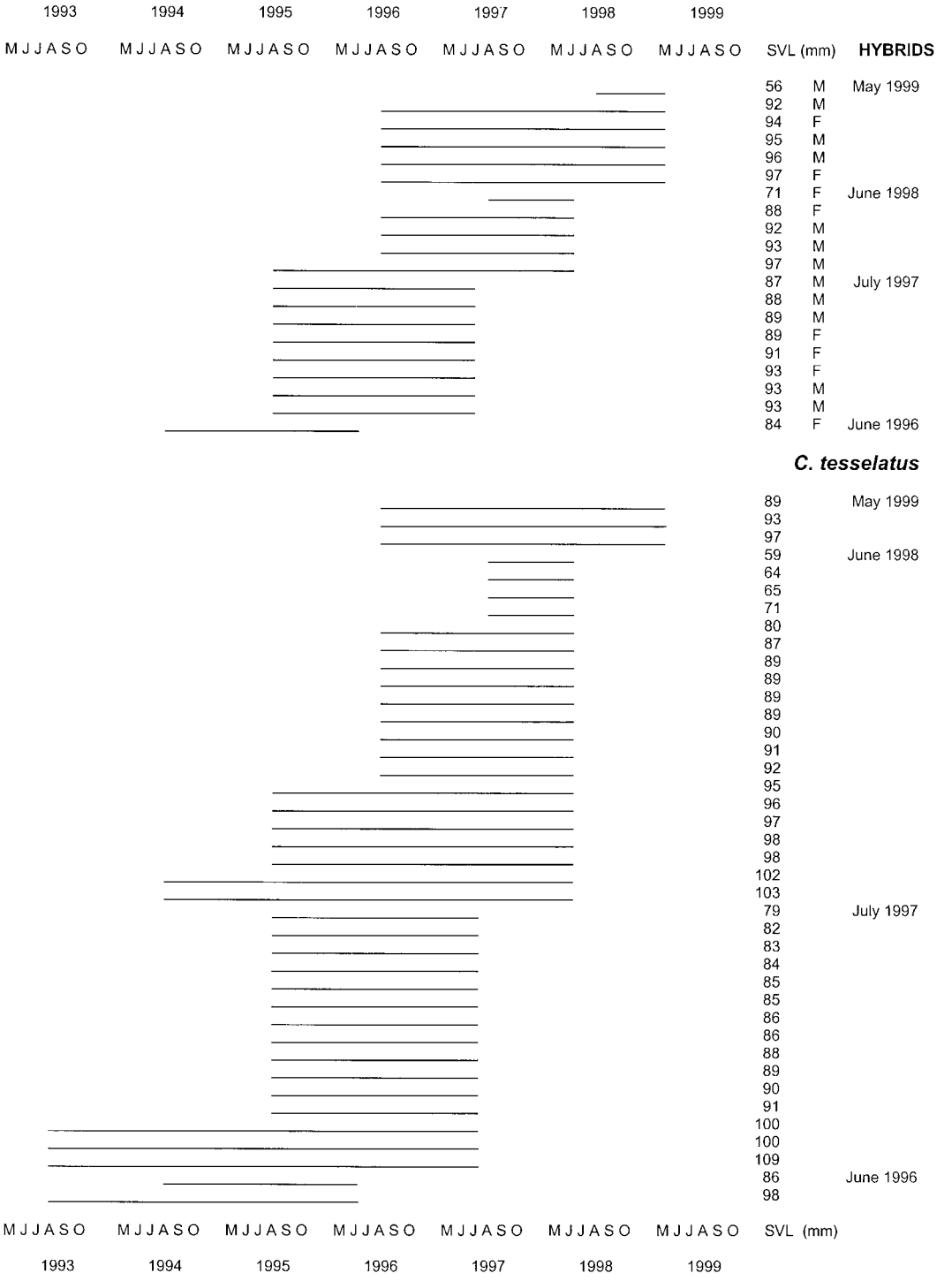

Fig. 23. Generations of *Cnemidophorus tesselatus* × *C. tigris marmoratus* hybrids and of *C. tesselatus* inferred from snout–vent length data (mm) in samples collected at Arroyo del Macho in 1996–1999. For each year depicted, the May–October activity period is included, and each horizontal line representing a lizard extends from the estimated year and month of hatching to the year and month of collection. Sex of each hybrid (M or F) is indicated.

TABLE 9

Numbers of *Cnemidophorus tesselatus*, *C. tigris marmoratus*, and Their Hybrids Collected or Observed at Arroyo del Macho

Date	*C. tesselatus*	*C. tigris*	Female hybrids	Male hybrids
June 10, 1996	2	0	1	0
July 20, 1997	6	1[a]	0	3
July 21, 1997	6	2[a]	0	2
July 22, 1997	3	0	3	0
June 11, 1998	4	2	0	2[b]
June 12, 1998	6	4[a]	1	1
June 13, 1998	8	2	0	0
June 15, 1998	2[c]	1[a]	1	1
May 12, 1999	1	1[a]	1	2
May 13, 1999	0	0	0	0
May 14, 1999	5[d]	2[a]	1	1
May 15, 1999	1[a]	2[a]	0	1
Total	44	17	8	13

[a] Individuals were observed but not collected. To avoid double counts, individuals were counted once for each patch of occupied habitat.

[b] One male hybrid could not be preserved and had to be discarded.

[c] One was captured and released; one was observed but not captured.

[d] Two were observed but not captured.

were persisting in spite of parthenogenesis rather than expressing advantages derived from it. However, Paulissen et al. (1992) found that the ''tangled-bank'' hypothesis was not supported in a study of sympatric *C. laredoensis* (parthenogenetic) and *C. gularis* (its paternal progenitor), and the hypothesis was not supported at Arroyo del Macho, where *C. tesselatus* was utilizing a range of microhabitats including the very restricted microhabitat to which the bisexual *C. tigris marmoratus* was largely confined.

The ecological flexibility exhibited by *C. tesselatus* at Arroyo del Macho does appear to fit expectations of the ''generalized genotype'' hypothesis as expressed by Lynch (1984). According to this model, generalized genotypes are a natural consequence of parthenogenetic species having inherited genomes from each of two ecologically successful progenitor species. Theoretically, these novel sets of genetic information expand ecological opportunities for parthenogenetic species and preadapt the parthenogens to a range of microhabitats, possibilities discussed also by Parker and Selander (1976), Parker (1979a), Cole (1980), and Dessauer and Cole (1984). An ability to utilize microhabitats avoided by bisexual congeners would enhance the chance of parthenogens escaping extermination from destabilizing hybridization. In fact, many populations of *C. tesselatus* are sympatric with one or more bisexual congeners (Taylor et al., 2000). Paulissen et al. (1992) hypothesized that populations of parthenogenetic and bisexual species could coexist only if competition and backcrossing were not occurring or, if they were occurring, other factors ameliorated their detrimental effects on one or both species. Because backcrossing between *C. tesselatus* and *C. tigris marmoratus* occurs frequently at Arroyo del Macho, what ''other factors'' permit their coexistence and enable *C. tesselatus* to achieve a higher population density than does *C. tigris marmoratus* despite an annual loss of eggs to the production of sterile hybrids? In addition to the relatively large clutch size of *C. tesselatus* (previously discussed), individuals of *C. tesselatus* and *C. tigris marmoratus* confine most of their activities to different microhabitats. The extremely limited and patchy distribution of creosote bush at Arroyo del Macho is a factor that reduces contacts between *C. tesselatus* and *C. tigris marmoratus*. A few individuals of *C. tesselatus* were found in creosote bush associations, but the majority of individuals were found in open microhabitats that included some form of scattered plant cover (annual or perennial) and an element (often quite modest) of vertical topography (e.g., rock outcrops, slopes, erosion cuts, and elevated road-sides). In contrast, representatives of *C. tigris marmoratus* were essentially restricted to the small patches of creosote bush.

The northern range limit of creosote bush in southeastern New Mexico is depicted as being south of Arroyo del Macho by Benson and Darrow (1981: 122, map 3.38). Therefore, the sparse, patchy distribution of *Larrea tridentata* in the vicinity of Arroyo del Macho may reflect a suboptimal environment at the northern periphery of its range in the Pecos River drainage. Creosote bush was unmistakably the preferred habitat of *C. tigris*

marmoratus at Arroyo del Macho; although mesquite was available, few individuals of this species were seen in its vicinity. Paradoxically, populations of *C. tigris marmoratus* thrive in mesquite-dominated habitats in other parts of its range. One such example is the population of *C. tigris marmoratus* located only 18 km southeast of the hybridization locality. Despite the absence of creosote bush, *C. tigris marmoratus* occurred there in large numbers on a sandy substrate stabilized into hummocks by mesquite. In keeping with the unpredictable nature of its distribution (Zweifel, 1965), *Cnemidophorus tesselatus* was not found with *C. tigris marmoratus* at this site. Although the two localities were only 18 km apart, the substrates were different; therefore, edaphic factors might be of greater importance than a preference for creosote bush in excluding *C. tigris marmoratus* from the broader range of microhabitats available to *C. tesselatus* at Arroyo del Macho. However, Cuellar (1979) reported an example at another locality where *C. tigris marmoratus* was the ecological specialist and a different parthenogenetic species, *C. uniparens*, was the ecological generalist, thus resembling the differential use of habitats at Arroyo del Macho. A similar situation prevails between *C. tigris* and *C. uniparens* in the vicinity of Lordsburg, Hidalgo County, New Mexico, in the study area of Dessauer et al. (2000).

Other Possible *C. tesselatus* × *C. tigris marmoratus* Hybrids

In addition to the hybrids from Arroyo del Macho, a number of unusual specimens with male reproductive structures and the fundamental color pattern features of *C. tesselatus* pattern class E have been discovered at diverse localities in New Mexico and Texas. It is virtually certain that these males are also hybrids between *C. tesselatus* of pattern class E and *C. tigris marmoratus*. Nine of these suspected hybrids were compared to the hybrids from Arroyo del Macho: University of Colorado Museum 10367 and 23377 (illustrated in Taylor et al., 1967, with evidence of their hybrid status provided by Taylor et al., 1989); Texas Cooperative Wildlife Collection 22324 (illustrated in Saxon et al., 1967), 27884, 27885, 29405, and 30503; and Museum of Southwestern Biology 15039 and 15098. All individuals came from sites of sympatry between *C. tesselatus* and *C. tigris marmoratus* (see Specimens Examined, appendix, for collecting localities). However, unlike the population of *C. tesselatus* at Arroyo del Macho, these populations of *C. tesselatus* exhibit an inconspicuous, extensively disrupted lateral stripe and prominent lateral barring—the pattern generally associated with the designation ''pattern class E'' (Zweifel, 1965). Therefore, the absence of lateral stripes in these nine unusual males does not automatically implicate *C. tigris marmoratus* as the paternal parental species, but their hybrid status is supported by canonical variate analysis of meristic characters (fig. 24). All of the nine suspected hybrids were assigned to the hybrid a priori group—one with a probability of 0.88, eight with probabilities of 1.0.

The Prospect of Speciation

All parthenogenetic species of *Cnemidophorus* presently known are either diploid or triploid. Species belonging to each ploidy level can be further subdivided based on the phylogenetic relationships of their progenitor species. Some parthenogenetic species originated from hybridization between members of the same species group (groups of species that presumably reflect phylogenetic relationship; see Lowe et al., 1970b and Wright, 1993 for summaries), while other parthenogenetic species originated from hybridization between members of different species groups.

Although the specific combinations of genetic information necessary to trigger and maintain parthenogenetic reproduction (e.g., the balance hypothesis of Moritz et al., 1989) presently elude prediction, the hybridization taking place at Arroyo del Macho can be placed in the general context of the sources of genetic information in currently established triploid species. There are two classes of triploid species characterized by the specific (taxonomic) source of their three sets of chromosomes. In one class, each of the three sets of chromosomes can be traced to a different bisexual species. *Cnemidophorus neo-*

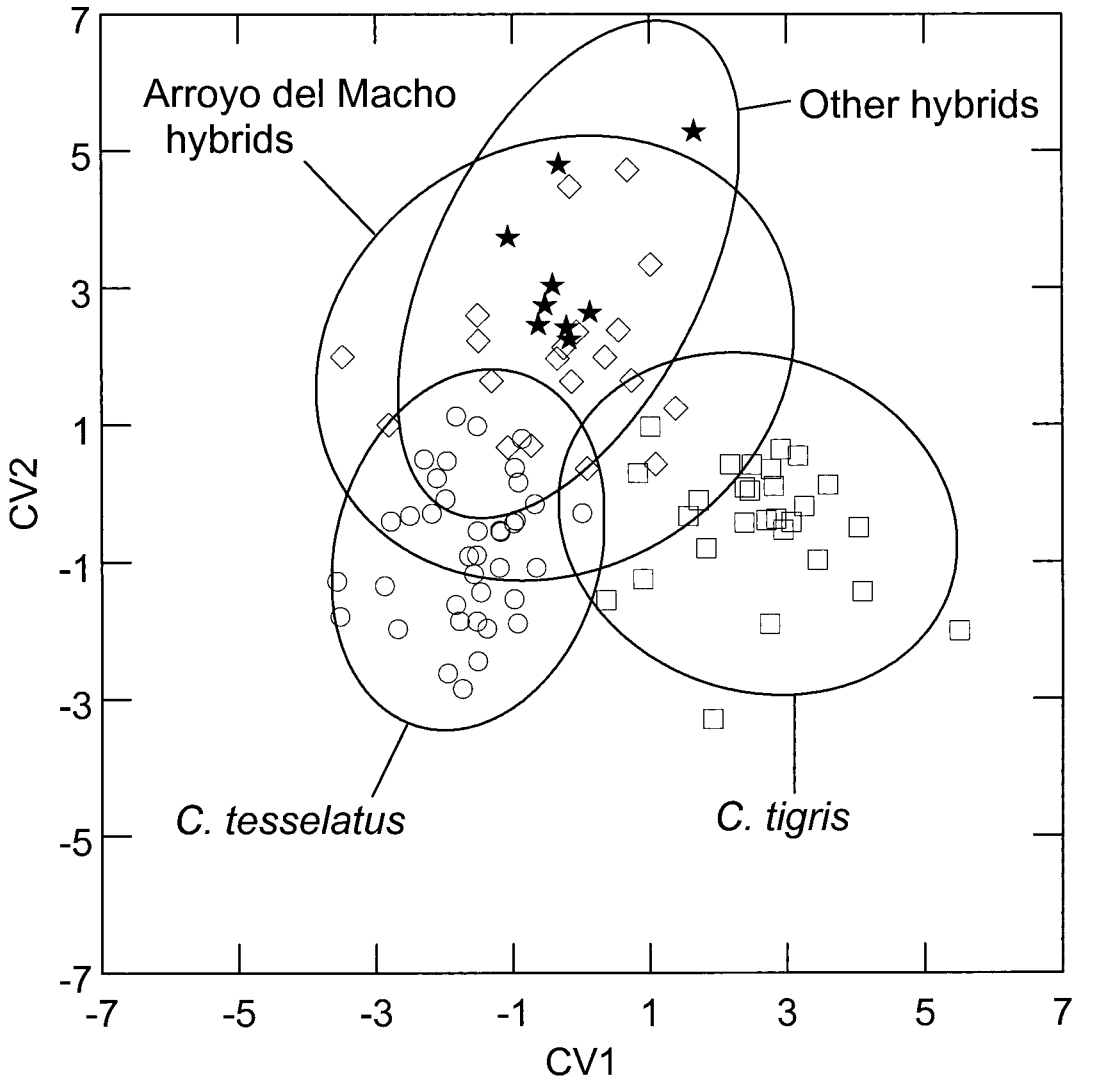

Fig. 24. Multivariate association of nine putative male *C. tesselatus* × *C. tigris marmoratus* hybrids from several disparate localities with hybrids from Arroyo del Macho. Canonical variate scores were derived from a quadratic canonical variate analysis of three meristic characters (FP, GAB, and SDL-T4—those characters with complete data for all nine putative hybrids). Open symbols depict 84 individuals collected in the vicinity of Arroyo del Macho, Chaves Co., New Mexico: ○, 38 *C. tesselatus*; □, 26 *C. tigris marmoratus*; ◇, 20 hybrids; ★, 9 suspected hybrids from other localities. Ellipses define the 95% confidence limits of each group. Compare with figure 8.

tesselatus (Walker et al., 1997a) is an example, with its three sets of chromosomes ultimately derived from *C. tigris marmoratus* and *C. gularis septemvittatus* (contributed by its maternal parent *C. tesselatus*) and *C. sexlineatus*, its paternal parent (Wright and Lowe, 1967b; Parker and Selander, 1976; Dessauer and Cole, 1989). In this example, the progenitor species belong to two different (*tigris* and *sexlineatus*) species groups (Lowe et al., 1970b). Members of the second class have three sets of chromosomes derived from two, rather than three, bisexual species. *Cnemidophorus uniparens* and *C. velox* are examples. Each of these parthenogenetic species has one set of chromosomes from *C.*

burti (paternally derived in *C. uniparens*, maternally derived in *C. velox*) and two sets of chromosomes from *C. inornatus* (Dessauer and Cole, 1989; Moritz et al., 1989). In the latter examples, the progenitor species belong to the same (*sexlineatus*) species group.

If a new triploid species were to originate at Arroyo del Macho, it would have genomes from two species belonging to two species groups, with sets of chromosomes from *C. gularis septemvittatus* and *C. tigris marmoratus* donated by *C. tesselatus* combining with a second set of *C. tigris marmoratus* chromosomes donated by the hybridizing male. Our data indicate that this speciation event is not happening. Although one haploid set of chromosomes from *C. tigris marmoratus* is found in two diploid parthenogenetic species, *C. neomexicanus* and *C. tesselatus* (and thus in the triploid *C. neotesselatus*), none of the parthenogenetic species presently known has two sets of chromosomes from *C. tigris* (Dessauer and Cole, 1989; Vrijenhoek et al., 1989; Wright, 1993). Because the eight female hybrids from Arroyo del Macho were sterile, this particular combination of genetic information—two sets of chromosomes from *C. tigris marmoratus* and one set from *C. gularis septemvittatus*—appears to be ineffective in generating a new triploid parthenogenetic species. The second genome from *C. tigris* is evidently responsible for the inability to establish a reproductively functional triploid, and this might be generally true. For example, two female *C. neomexicanus* × *C. tigris* hybrids were recently reported from field sites in southwestern New Mexico (Dessauer et al., 2000). Their maternal parent, diploid parthenogenetic *C. neomexicanus*, was derived from a mating between individuals of *C. tigris* and *C. inornatus* (reviewed by Cole et al., 1988). Therefore, these hybrids also inherited two sets of *tigris* chromosomes, and they, too, were sterile.

However, the number of hybridizations that occurred prior to achieving the successful combinations of genetic information found in contemporary triploid species of *Cnemidophorus* is not known. If many hybridizations are typically necessary before a fortuitous combination of genetic information leads to continued parthenogenetic reproduction at a higher ploidy level, then the annual hybridization taking place at the Arroyo del Macho site should be periodically monitored for the possibility of witnessing the origin of a new species.

SUMMARY AND CONCLUSIONS

1. An assemblage of whiptail lizards utilizing desert grassland habitats at Arroyo del Macho, Chaves County, New Mexico, is composed of one triploid parthenogenetic species, *Cnemidophorus exsanguis*, one diploid parthenogenetic species, *C. tesselatus*, and two diploid bisexual species, *C. inornatus* and *C. tigris marmoratus*.

2. Samples collected in 1996 and 1997 from this locality contained 17 individuals of *C. tesselatus* and 9 individuals with the fundamental color pattern of *C. tesselatus* but having either male reproductive systems or abnormally small ovaries and oviducts. These unusual individuals were hypothesized to be hybrids derived from *C. tesselatus* and males of one of the two syntopic bisexual species.

3. Additional sampling was conducted in 1998 and 1999 to collect males of the two bisexual species, additional hybrid suspects, and representatives of *C. tesselatus* for definitive morphological, cytogenetic, genetic, and histological analyses. Twenty-one hybrids were collected in 1996–1999, with 12 males and 8 females available for study.

4. Certain color pattern features implicate *Cnemidophorus tigris marmoratus* as the paternal parental species of the hybrids. Unlike *C. inornatus* (and the population of *C. tesselatus* from this locality), all hybrids and representatives of *C. tigris marmoratus* lack lateral stripes. In addition, several hybrids had the pink-to-orange ventral pigmentation seen in individuals of *C. tigris marmoratus* but absent from the other three species at the locality.

5. Although hybrids were phenotypically intermediate to the parental species in four of eight univariate characters (table 1), they are distinguished statistically from both parental species by the low number of granular scales around midbody. There is also a statistical resemblance of hybrids to *C. tesselatus* in number of subdigital toe lamellae,

number of gular scales, and size of mesoptychial scales, and to *C. tigris marmoratus* in number of femoral pores, number of circumorbital scales, and number of lateral supraocular granules.

6. The pattern of meristic resemblance of hybrids to parental species was resolved by multivariate analyses. A principal components analysis summarized 64% of the variation in eight meristic characters in PC1–PC3. The first component expressed the resemblance of hybrids to *C. tigris marmoratus* while the second component expressed hybrid resemblance to *C. tesselatus.* The third component reflected the distinctiveness of the hybrids from both parental species.

7. Mahalanobis D^2 distances, derived from a linear canonical variate analysis, quantify the resemblance among hybrids and parental species: D^2 between hybrids and *C. tesselatus* = 11.0; D^2 between *C. tesselatus* and *C. tigris marmoratus* = 18.9; and D^2 between hybrids and *C. tigris marmoratus* = 19.9. Therefore, the overall meristic affinity of hybrids was matriclinous, and the progression from one *C. tigris marmoratus* genome in *C. tesselatus* to two *C. tigris marmoratus* genomes in the hybrids did not increase the resemblance of hybrids to the paternal species.

8. A quadratic canonical variate analysis of meristic characters provided 100% discrimination (with a jackknifed classification to minimize bias) among the samples of *C. tesselatus, C. tigris marmoratus,* and hybrids.

9. Karyotypic analysis revealed the presence of two diploid cytotypes of *C. tesselatus* at Arroyo del Macho. One cytotype ($2n$ = 46) had the original ancestral X-chromosome that had been ultimately inherited from *C. tigris,* whereas the other cytotype had the X-chromosome apparently fissioned at the centromere ($2n$ = 47).

10. Karyotypes clearly revealed that the presumed hybrids are triploids of *C. tesselatus* × *C. tigris marmoratus,* and hybridization occurs with both cytotypes of *C. tesselatus.*

11. Seven of 10 representatives of *C. tesselatus* had a fissioned X-chromosome while 3 had intact X-chromosomes. In contrast, 8 of 9 karyotyped hybrids had the intact X-chromosome. It is unlikely that this 8:1 ratio is based on chance. For example, the hybrid with the fissioned X-chromosome was significantly different in 12 meristic characters from the sample of hybrids with intact X-chromosomes. In addition, principal components scores for this individual fell outside the 95% confidence ellipse surrounding scores of the other eight hybrids. Therefore, hybrids inheriting fissioned X-chromosomes might be at a selective disadvantage.

12. Electrophoretic analyses of tissue proteins representing 34 nuclear gene loci revealed one clone of *C. tesselatus* at Arroyo del Macho. The protein data are consistent with the karyotypes, revealing that the hybrids are triploids of *C. tesselatus* × *C. tigris marmoratus.* Considering the local allozyme variation in *C. tigris marmoratus* together with the Y-chromosome inherited in male hybrids, clearly at least 9 of the 10 hybrids examined electrophoretically were products of separate fertilization events, not a product of triploids cloning themselves.

13. Sexually mature and reproductive adults of *C. tesselatus* usually have oocytes in the ovary, complete and well-organized ovarian follicle walls, inconspicuous connective tissue and fewer vacuoles in the well-vascularized ovary, a thin mucosa in the distal oviduct, well-developed alveolar glands restricted to the middle oviduct, a proximal oviduct with a thick mucosa and well-developed folds, and small mesonephric tubules. Oocyte cytoplasm is homogeneous in young oocytes and becomes organized in layers in older oocytes; yolk deposition starts in oocytes 2.3–3.1 mm in diameter.

14. There is no evidence that *Cnemidophorus tesselatus* × *C. tigris marmoratus* hybrid females can produce viable and fertile eggs. All of the ovaries examined were undeveloped, had empty follicles, or contained abnormal oocytes. No sperm were found in the female reproductive tracts of either hybrids or *C. tesselatus.*

15. Although *Cnemidophorus tesselatus* × *C. tigris marmoratus* hybrid males are capable of producing sperm that appear normal, balanced sets of genetic information may be precluded by the allotriploidy of these males. The variable presence of sperm in the epididymides suggests that hybrid males could be successful at courtship and

mating with females. Sperm were never seen in the small mesonephric tubules of males.

16. Small mesonephric tubules (20–30 μm in diameter) are remnants of the mesonephric kidney. Larger mesonephric tubules (usually more than 75 μm in diameter) become the functional epididymides of adult males of bisexual species. Hybrids (*C. tesselatus* × *C. tigris marmoratus*) of both sexes contain both smaller (mesonephric) and larger (epididymis) tubules.

17. Poorly defined follicular epithelium with little vascularization in small ovaries, empty or fluid-filled follicles without oocytes, numerous abnormally large mesonephric tubules, and few or no cilia in the middle oviduct are histological characteristics suggesting that a female may be a sterile hybrid rather than a nonhybrid of a parthenogenetic species.

18. Based on samples collected on June 11–15, 1998, individuals of *C. tesselatus* are significantly larger than females of *C. tigris marmoratus* in minimum and maximum sizes of gravid individuals (87–103 vs. 70–80 mm snout–vent length, respectively), mean size of gravid individuals (93.7 vs. 76.0 mm snout–vent length, respectively), and mean clutch size (5.5 vs. 2.2 eggs, respectively). These differences favor the survival of this population of *C. tesselatus* despite the reduced reproductive potential associated with an annual production of sterile hybrids.

19. *Cnemidophorus tigris marmoratus* is inextricably linked to creosote bush stands at the Arroyo del Macho locality, and its low density is based on the sparse and patchy distribution of this shrub at the hybridization site. Although representatives of *C. tesselatus* unhesitatingly enter these patches of creosote bush, more time is spent in the more extensive intervening open areas, particularly on rocky slopes. Therefore, although encounters between the two species take place on a regular basis, the low density of *C. tigris marmoratus* precludes insemination of all individuals of *C. tesselatus.* This is evidenced histologically by an absence of sperm in the reproductive tracts of 11 individuals of *C. tesselatus* collected during the prime reproductive seasons of three different years. Based on sampling results and field observations, we estimate the relative abundance of hybrids and parental species to be 1 hybrid : 2 *C. tesselatus* : 1 *C. tigris marmoratus.*

20. Additional evidence for a low impact of hybridization on the reproductive success of individuals of *C. tesselatus* comes from age estimates based on the snout–vent length of individuals in May, June, and July samples. These samples apparently comprise five generations of *C. tesselatus* and hybrids produced in five different years. Although 20 hybrids suggest an inordinate amount of hybridization, they could have been produced by a moderate amount of annual hybridization.

21. Canonical variate analysis of meristic characters can be used to test hypotheses that candidate museum specimens might be *C. tesselatus* × *C. tigris marmoratus* hybrids. Nine males, similar to the Arroyo del Macho hybrids in color pattern but collected from other localities, were entered in a canonical variate analysis model as unknowns, with the samples of *C. tesselatus, C. tigris marmoratus,* and hybrids from Arroyo del Macho serving as a priori groups. All nine individuals were assigned to the hybrid group.

22. All triploid parthenogenetic species of *Cnemidophorus* were derived from F_1 hybrids produced by the insemination of females of diploid parthenogenetic species (such as *C. tesselatus*) by males of sympatric bisexual species. Therefore, the predictable hybridization at the Arroyo del Macho locality provides an exceptional opportunity to evaluate the possibility of speciation through hybridization. The ultimate genetic makeup of the hybrids consists of two genomes from *C. tigris marmoratus* (one from *C. tesselatus,* one from a male *C. tigris* at the hybridization site) and one genome from *C. gularis septemvittatus* (through *C. tesselatus*). All female hybrids collected (8) were sterile; therefore, this particular genetic combination may be ineffective in perpetuating parthenogenetic reproduction in triploids.

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APPENDIX: SPECIMENS EXAMINED

Primary Material

The specimens referred to by their individual catalog numbers and initials for their collections are as follows: AMNH (American Museum of Natural History), LSUS (Louisiana State University in Shreveport, Museum of Life Sciences), MSB (Museum of Southwestern Biology), TCWC (Texas Cooperative Wildlife Collection), and UCM (University of Colorado Museum). Morphological data were recorded for each specimen. Numbers followed by K and/or P denote specimens from which karyotypic and/or protein data were obtained, respectively; X-I and X-F refer to two karyotypic cytotypes in hybrids and *C. tesselatus* that are distinguished by either an intact X-chromosome or a fissioned X-chromosome. Sex of individuals is provided for hybrids and individuals of *C. tigris marmoratus* from Arroyo del Macho, and dates of collection are shown in brackets [month/day/year]. All specimens of *C. tesselatus* are females.

Cnemidophorus tesselatus: New Mexico, Chaves Cty., N side Arroyo del Macho (33°39′1″N, 104°33′17″W), 22 km N on Hwy. U.S. 285 from junction with Hwy. U.S. 70 N of Roswell, New Mexico, then 0.8 km E on Eden Valley Road. AMNH R-145142 (K, P, X-F) [5/12/99], R-145143 (K, P, X-F) [5/14/99], R-145144 (K, P, X-I) [5/14/99], R-146612–146613 [6/10/96], R-146614–146619 [7/20/97], R-146620–146625 [7/21/97], R-146626–146628 [7/22/97], R-146629 (K, P, X-F) [6/11/98], R-146630 offspring of either AMNH R-146629 or R-146631 (P), R-146631 (K, P, X-F) [6/11/98], R-146632 (K, P, X-I) [6/11/98], R-146633 (K, P, X-F) [6/11/98], R-146634 and R-146635 [6/11/98], R-146636 (K, P, X-F) [6/12/98], R-146637 [6/12/98], R-146638 (K, P, X-I) [6/12/98], R-146639 (K, P, X-F) [6/12/98], R-146640 and R-146641 [6/12/98], R-146642–146649 [6/13/98]; Regis University (RU) 99008 [maintained in *Cnemidophorus* colony, AMNH], AMNH R-147547 [5/14/99].

Cnemidophorus tigris marmoratus: New Mexico, Chaves Cty., N side Arroyo del Macho (33°39′1″N, 104°33′17″W), 22 km N on Hwy. U.S. 285 from junction with Hwy. U.S. 70 N of Roswell, New Mexico, then 0.8 km E on Eden Valley Road. AMNH R-146650(♂) (P) [6/11/98], R-146651(♀) (P) [6/11/98], R-146652(♂) (P) [6/13/98], R-146653(♂) (P) [6/13/98].

New Mexico, Chaves Cty., 1.6 km W Pecos River, north side of Hwy. U.S. 70, approximately 16.1 km east of interchange of Hwys U.S. 285 and U.S. 70. AMNH R-146654–146680 [6/14/98].

We follow Dessauer and Cole (1991), Lemos-Espinal et al. (1994), and Dessauer et al. (2000) in applying the name *C. tigris marmoratus* to the populations of *C. tigris* in Chaves Cty., New Mexico.

CNEMIDOPHORUS TESSELATUS × *C. TIGRIS MARMORATUS* HYBRIDS: New Mexico, Chaves Cty., N side Arroyo del Macho (33°39′1″N, 104°33′17″W), 22 km N on Hwy. U.S. 285 from junction with Hwy. U.S. 70 N of Roswell, New Mexico, then 0.8 km E on Eden Valley Road. AMNH R-145145(♂) (K, P, X-I) [5/12/99], R-145146(♀) (K, P, X-F) [5/12/99], R-145147(♀) (K, P, X-I) [5/14/99], R-145148(♂) (K, P, X-I) [5/14/99], R-145149(♂) (K, P, X-I) [5/15/99], R- 146681(♀) [6/10/96], R-146682(♂) [7/20/97], R-146683(♂) [7/20/97], R-146684(♂) [7/20/97], R-146685(♂) [7/21/97], R-146686(♂) [7/21/97], R-146687(♀) [7/22/97], R-146688(♀) [7/22/97], R-146689(♀) [7/22/97], R-146690(♂) (K, P, X-I) [6/11/98], R-146691(♀) (K, P, X-I) [6/12/98], R-146692(♂) (P) [6/12/98], R-146693(♂) (K, P, X-I) [6/15/98], R-146694(♀) (K, P, X-I) [6/15/98], R-146695(♂) [5/12/99].

ADDITIONAL MATERIAL

CNEMIDOPHORUS EXSANGUIS: New Mexico, Chaves Cty., N side Arroyo del Macho (33°39′1″N, 104°33′17″W), 22 km N on Hwy. U.S. 285 from junction with Hwy. U.S. 70 N of Roswell, New Mexico, then 0.8 km E on Eden Valley Road. AMNH R-146771–146776, R-146777–146780, R-146781.

CNEMIDOPHORUS INORNATUS: New Mexico, Chaves Cty., N side Arroyo del Macho (33°39′1″N, 104°33′17″W), 22 km N on Hwy. U.S. 285 from junction with Hwy. U.S. 70 N of Roswell, New Mexico, then 0.8 km E on Eden Valley Road. AMNH R-146696, R-146697–146713.

Several new subspecies of *C. inornatus* were described by Wright and Lowe (1993), including *C. i. juniperus*, the population at Arroyo del Macho according to their range map. Couplets in the Wright and Lowe (1993) key to the subspecies of *C. inornatus* are based on proportions of samples with particular numbers and states of pale stripes. The alternative percentages provided in this key leave the population at Arroyo del Macho unidentified to subspecies. In addition, stripe character values were omitted from their table 1, without explanation, for a Chavez Cty. sample (including a specimen from Salt Creek, approximately 7 km south of the hybridization site). Therefore, use of the name *juniperus*, as presently conceived, for this population is undesirable.

CNEMIDOPHORUS GULARIS SCALARIS: México, Durango, Rio Florido near Canutillo (bridge for Méxican Hwy. 45) (elev. 1585 m). AMNH R-129176 (P).

CNEMIDOPHORUS GULARIS SEPTEMVITTATUS: Texas, Brewster Cty., 28.0 km (by Hwy. U.S. 385) S Marathon. AMNH R-126906 (P).

We follow Walker (1981) in using the name *septemvittatus* for a subspecies of *C. gularis*. Strong genetic support for the conspecificity of *C. gularis* and *C. septemvittatus* is provided by Forstner et al. (1998), although these authors considered specific rank to be appropriate for this taxon. Dessauer and Cole (1989) also presented genetic data consistent with a conspecific relationship between these taxa.

CNEMIDOPHORUS TIGRIS MARMORATUS: Texas, Brewster Cty., 17.3 km N, 1.2 km E Panther Junction (BBNP) on Phillips Ranch. LSUS 971.

CNEMIDOPHORUS TIGRIS PUNCTILINEALIS: Arizona, Pima Cty., 30.9 km W, 29.9 km S Ajo (elev. 595 m). LSUS 4135.

CNEMIDOPHORUS SEXLINEATUS: Louisiana, Caddo Parrish, LSUS campus, Shreveport. LSUS 1014, 1018, 1019.

PRESUMED *CNEMIDOPHORUS TESSELATUS* × *C. TIGRIS MARMORATUS* HYBRIDS: New Mexico, Chaves Cty., Hwy. U.S. 380, approximately 40 km E Roswell. MSB 15039; approximately 29 km E Bottomless Lake State Park, MSB 15098.

New Mexico, Sierra Cty., 0.8 km SE Caballo Reservoir dam. UCM 23377; 1.6 km SW Cutter, UCM 29197. Texas, Presidio Cty., 4.8 km E Presidio, near Ft. Leaton. TCWC 22324; 3.2 km SE Presidio, TCWC 27884, 27885; 0.4 km S Presidio, TCWC 29405; Presidio, ‘‘Jones Ala Rio’’ TCWC 30503.