

Rapid Screening of DNA Diversity Using Dot-Blot Technology and Allele-Specific Oligonucleotides: Maternity of Hybrids and Unisexual Clones of Hybrid Origin (Lizards, *Cnemidophorus*)

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Allele-specific oligonucleotide probes, together with dot-blot methods, can provide rapid and inexpensive screening of DNA types in large samples of organisms. Here we demonstrate their use in: (1) determining types of mitochondrial DNA in hundreds of lizards from a dynamic hybrid zone; (2) discovering intraspecific geographic variation in genes; and (3) determining and verifying the maternal ancestry of unisexual, parthenogenetic lizards in clones of hybrid origin. These methods are broadly applicable in research involving rapid screening of DNA types in large samples of specimens for any gene with sequence data from which to design specific probes. © 1996 Academic Press, Inc.

INTRODUCTION

Biologists often could benefit from rapid and efficient methods for screening DNA types of large samples of organisms. Allele-specific oligonucleotides (ASOs) are valuable reagents for such applications. They are now used extensively in the diagnosis and detection of carriers of monogenetic disease (Conner *et al.*, 1983; Kazazian, 1989) and in solving a variety of forensic problems (von Beroldinen *et al.*, 1989). We have found that they are useful also in solving a variety of problems in population genetics and evolution of lizards.

An ASO is a single strand of nucleotides (usually 15 to 25) that incorporates unique features that distinguish one allele from another at the same locus. As a probe, an ASO hybridizes to complementary sequences on a strand of DNA. In this paper we illustrate the use of ASO probes of 12S mitochondrial DNA (mtDNA) and dot-blot of PCR amplified 12S fragments of mtDNA in (1) determining the maternal parentage of hundreds of

lizards collected in a dynamic hybrid zone involving *Cnemidophorus tigris gracilis* and *C. t. marmoratus*; (2) rapidly screening large series of specimens for intraspecific polymorphisms and geographic variants; and (3) determining and/or verifying the maternal ancestry of unisexual, parthenogenetic lizards in clones of hybrid origin (*C. neomexicanus* and *C. tessellatus*). In addition, we discuss the specificity of ASOs relative to the number of sequence differences within the region of mtDNAs being compared.

MATERIALS AND METHODS

Specimens of *C. t. marmoratus* (MAR), *C. t. gracilis* (GRA and GRT), *C. inornatus* (INO), *C. septemvittatus* (SEP), *C. sexlineatus* (SEX), and *C. neomexicanus* (NEO), and six pattern types of *C. tessellatus* (TES: types A, C, D, E, F, and G) were collected in the southwestern United States (see Appendix for details on specimens examined). The specimens of *C. tessellatus* include individuals that other investigators have referred to as *C. dixonii* (Types F and G, with G referring to *C. dixonii* A from Texas; Scudday, 1973) and *C. grahamii* (Types C–E, and sometimes F), but we use *tessellatus* here for all of these forms (Zweifel, 1965) and need not digress with the nomenclatorial problems of clonal vertebrates (e.g., Frost and Wright, 1988; Cole, 1990). Tissues were removed and stored according to standard procedures (Dessauer *et al.*, 1990).

DNA was isolated from red blood cells, skeletal muscle, and/or liver using standard phenol/chloroform extractions. DNA concentrations and quality were confirmed by UV absorption of aqueous solutions and ethidium bromide fluorescence of polymerase chain reaction (PCR) products in agarose gels (Maniatis *et al.*, 1982).

Fragments of the 12S mitochondrial ribosomal RNA gene for the populations under study were amplified by PCR in a DNA thermal cycler (Perkin-Elmer Co., Norwalk, CT) using universal primers H1557 and L1091 (Knight and Mindell, 1993). PCR directed synthesis, with 0.1 to 1 µg of DNA as template, was carried out in 30-µl volumes in final concentrations of 1.5 mM

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MgCl₂, 150 μM of each of the four dNTP's, 0.5 μM of each primer, and 1 unit *Taq* DNA polymerase. Thermocycling parameters were denaturation at 94°C for 1 min, annealing at 50°C for 30 s, and elongation at 72°C for 1 min, repeated for 30 cycles. All PCR products were visualized on 2% agarose gels containing ethidium bromide.

Allele-specific-oligonucleotides (Table 1) were designed and synthesized based upon unique sequences within positions 202 through 219 in the 12S mtDNA of MAR, GRA, GRT, INO, SEX, and SEP (Reeder *et al.*, unpublished data). For use as probes, the ASOs were end-labeled with [γ -³²P]adenosine-triphosphate and T4 polynucleotide kinase (Maniatis *et al.*, 1982).

The 12S mtDNA PCR products were subjected to dot-blot analysis on a ZetaProbe GT membrane in a Dot-Blot apparatus (Bio-Rad, Richmond, CA). Saiki *et al.*, (1986) and Kazazian (1989) provide detailed descriptions of the method; an outline follows. Ten microliters of each PCR product was diluted with 100 μl of a solution of 0.4 M NaOH and 25 mM Na₂EDTA, incubated at 94°C for 10 min, and cooled in an ice bath. One hundred microliters of each sample was added to sample wells of the dot-blot apparatus, and a vacuum was applied until the liquid was evacuated from the wells. Each well was rinsed with 200 μl of 20× SSPE (3.6 M NaCl, 20 mM phosphate buffer, pH 7.4, 20 mM EDTA). The wells were evacuated again and the vacuum was applied for a couple of additional minutes. After the apparatus was disassembled the membrane was air dried, its DNA side was exposed to 250 nm UV radiation for 15 s, and it was then baked briefly at 65 to 80°C. Positive controls, including samples that had been sequenced, were included in the majority of analyses.

The membrane was placed in a hybridization bottle and incubated for 15 min in 5 ml of prehybridizing buffer [5× SSPE, 0.5% sodium dodecyl sulfate (SDS),

5× Denhardt's solution]. The radioactive ASO probe was added to the prehybridization buffer, and hybridization was allowed to proceed overnight in a hybridization oven (Hybaid; National Labnet, Woodbridge, NJ), commonly at a temperature of 2°C below the T_m of the ASO, but in some experiments as much as 10°C above their T_m (Table 1). The wet membrane was blotted, covered with Saran Wrap, overlain with X-ray film (X-OMAT, RP XRP-S, Eastman Kodak, Rochester, NY) and placed in an ultracold freezer for 2 to 6 h. To reuse a membrane, it was stripped of the ASO by soaking it twice in 125 ml of a solution of 0.1× SSC (20× SSC = 175 g NaCl, 88 g sodium citrate adjusted to pH 7) and 0.5% SDS, at 95°C.

RESULTS AND DISCUSSION

Maternal Parentage of Lizards in a Dynamic Hybrid Zone

Two forms of the western whiptail lizard, *C. t. gracilis* and *C. t. marmoratus*, hybridize freely in southwestern New Mexico (Fig. 1). Analyses of 10 population samples transecting the hybrid zone revealed sharp, concordant, and superimposed clines in allele frequencies for several protein loci and for external color patterns (Dessauer and Cole, 1991), in a contact zone that may be moving in space and time.

The paper cited is a preliminary report of a comprehensive study of population genetics involving several contact zones. In addition to acquiring knowledge of morphological, karyotypic, and protein frequencies, mating patterns and hybrid viability are basic issues. Does the male of one form of lizard favor the females of the same type, or are hybrids of unequal viability depending upon which form is the maternal parent? In order to address such questions, we needed to rapidly assess the type of mtDNA present in each of a total of approximately 600 lizards.

ASOs of *C. t. gracilis* and *C. t. marmoratus* from the vicinity of the contact zones were used to determine the type of mtDNA (either *gracilis* or *marmoratus*) found in each lizard, testing up to 96 specimens per blot. Reciprocal probes were used, so each specimen was expected to produce a positive reaction one way or the other, rather than assume that no reaction meant only one alternative was possible. Thus, no reaction with either ASO would require further investigation to determine if a third allele were involved.

The blots revealed (Fig. 2) that each hybrid from the contact zones has either the local *gracilis* or the *marmoratus* type of mtDNA. This finding applied to the hundreds of specimens examined from the vicinity of three independent contact zones. Thus, successful hybrid lizards are formed with either combination of male and female parents. The full details for all specimens examined will be presented in a comprehensive analysis of these contact zones (Dessauer *et al.*, in preparation).

TABLE 1

Sequences of the ASO Probes Used^a

MAR ^b	CCA ATA GTC CAC CAA CTA	$T_m^c = 52^\circ\text{C}$
MAR2	CCA ATA GTC CAC CAA	$T_m = 44^\circ\text{C}$
GRA	CTAATA GTT TCT CAA CTA	$T_m = 46^\circ\text{C}$
GRT	ATA GTT CTT CAA CTA	$T_m = 38^\circ\text{C}$
INO	CCA ACA GTC TAC CAA CTA	$T_m = 52^\circ\text{C}$
SEX	CCA ATA GTC TAC CAA CTA	$T_m = 50^\circ\text{C}$
SEP	CCA ATA GTT AAT TAA CTA	$T_m = 44^\circ\text{C}$

^a Allele-specific oligonucleotide probe designs are based upon positions 202 through 219 from the 5'-phosphate end of sequences of PCR product of the 12S mtDNA of various species of *Cnemidophorus* (Reeder *et al.*, unpublished data).

^b MAR, *Cnemidophorus tigris marmoratus* (MAR2 is simply shorter); GRA, *C. t. gracilis* from the hybrid zone area; GRT, *C. t. gracilis* from the Tucson, Arizona, area (Fig. 1, G_t); INO, *C. inornatus*; SEX, *C. sexlineatus*; SEP, *C. septemvittatus* (see Appendix for specimens examined).

^c Estimate of the melting temperature of the ASO: $T_m = 4 (\#G + \#C) + 2 (\#A + \#T)$.

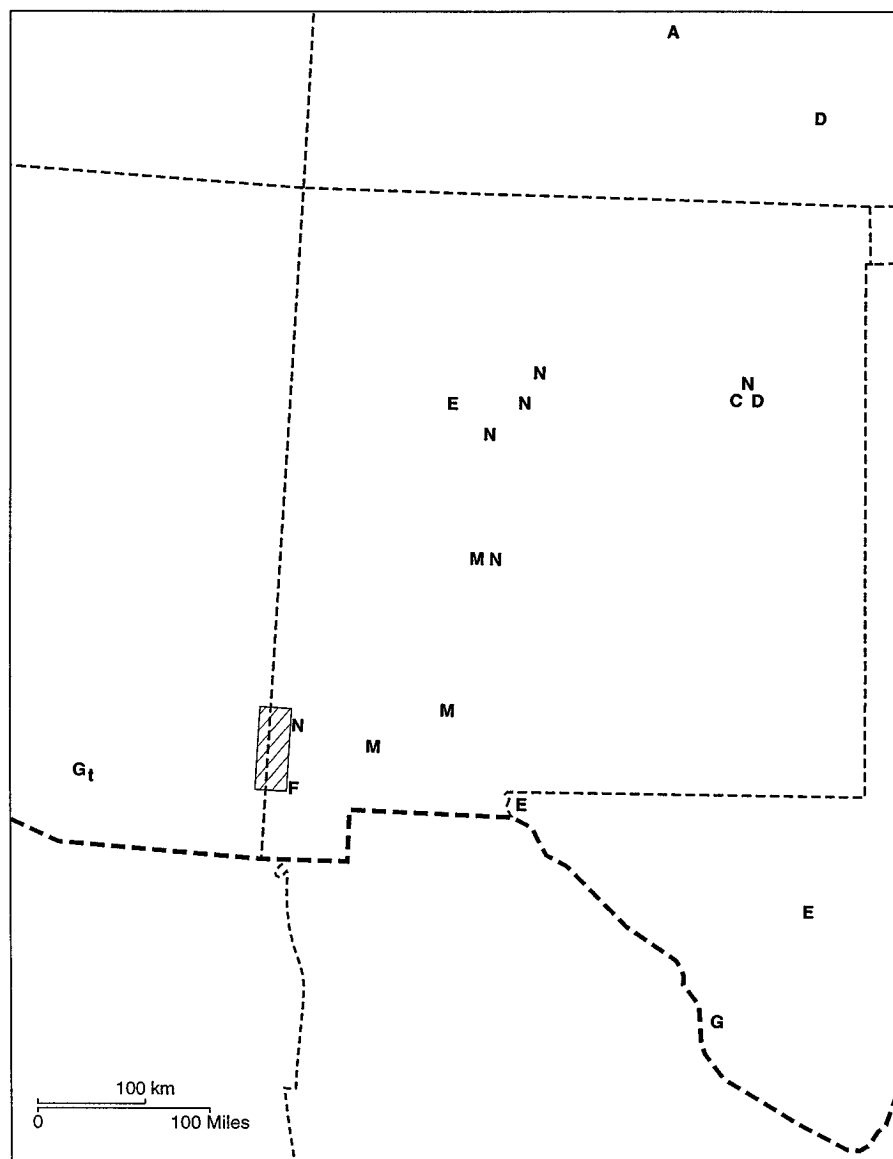


FIG. 1. Location of sites in southwestern United States (New Mexico and adjacent states) at which specimens were collected. Hatched area along the Arizona/New Mexico border is the area of the contact zones between populations of *Cnemidophorus tigris gracilis* and *C. t. marmoratus*; G_t and M are sites of *gracilis* and *marmoratus* morphs away from the contact zone. N designates sites where *C. neomexicanus* were collected. A, C, D, E, F, and G designate sites where the different *C. tessellatus* types were collected.

Rapid Screening for Intraspecific Variation

In addition to screening specimens of *C. t. gracilis* and *C. t. marmoratus* from the vicinity of the contact zones, we also screened specimens from additional population samples distant from the hybrid zones. The ASO for *marmoratus* hybridized to DNA samples of specimens collected across much of the range of *marmoratus* in New Mexico, including lizards obtained approximately 220 km from the contact zone (Fig. 1, M; Fig. 2, A7 and A8). In fact, none of our specimens of *marmoratus* failed to bind strongly with the *marmoratus* ASO.

The ASO for *gracilis* hybridized to DNA samples of specimens collected in the San Simon valley, Arizona,

at the eastern edge of its range, and in the contact zones. It failed to hybridize with DNA of *gracilis* from the vicinity of Tucson, Arizona, about 180 km to the west (Fig. 1, G_t; Fig. 2, GRA-ASO, B7 and B8). To evaluate this finding, we sequenced the 12S mtDNA from one of the latter lizards and found a two-base difference between it and that of the other *gracilis* in the 18-residue region being used (Table 1, GRT vs GRA). A new ASO based on the GRT sequence reacted positively with DNA of other western whiptail lizards from the Tucson area, but not with those from the vicinity of the contact zones, reflecting intraspecific geographic variation in mtDNA within *C. t. gracilis*

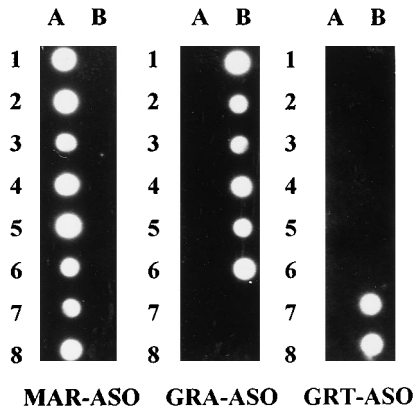


FIG. 2. Dot-blots representative of 12S mtDNA types of lizards from the contact zones between *Cnemidophorus tigris marmoratus* and *C. t. gracilis* in southwestern New Mexico (see Fig. 1, hatched area) and from 4 populations far from the contact zone (see Fig. 1, G_t and M; Appendix). Blots are contact prints, reverse images of the X-ray film. DNA samples were applied in two columns. Samples 1 through 6 in columns A and B are from the contact zone: A 1, 2, 5 (sample sequenced), and 6 are from site 48 with only *marmoratus* morphs; B 1, 2, 5, and 6 are from site 36 with only *gracilis* morphs; A 3 and 4 and B 3 and 4 are from site 26 in the middle of the central hybrid zone. Samples 7 and 8 in columns A and B are from populations distant from the hybrid zones: A 7 and 8 are from site 54 near San Antonio, NM with only *marmoratus* morphs; B 7 and 8 are from site 49 near Tucson, Arizona, with only *gracilis* morphs. The blot was hybridized with MAR-ASO, GRA-ASO, and the GRT-ASO (Table 1). Note that *gracilis* morphs from the Tucson area hybridized to the GRT-ASO but not to the GRA-ASO.

(Fig. 1, G_t vs hatched area; Fig. 2, B 7 and B 8). Thus, the ASO dot-blot method efficiently revealed allelic variation within the 12S mtDNA locus, which previously had been unexpected for these particular populations.

Maternal Ancestry of Parthenogenetic Clones

The New Mexican Whiptail Lizard (C. neomexicanus). This unisexual species occurs primarily in the Rio Grande Valley in New Mexico, with outlier populations in southwestern New Mexico (vicinity of Lordsburg) and in northeastern New Mexico (vicinity of Conchas Lake), the latter of which may be a result of artificial introduction (for reviews, see Parker and Selander, 1984; Cole *et al.*, 1988). The species originated as a result of hybridization between two diploid, bisexual species, *C. t. marmoratus* and *C. inornatus*, and one or more of the F_1 hybrid females perpetuated a lineage through parthenogenetic cloning (Dessauer and Cole, 1986, 1989).

Specimens of *C. neomexicanus* from across the vast majority of its geographic range tested strongly positive with the *marmoratus* ASO, but not with the *inornatus* ASO (Fig. 3). This confirms earlier conclusions, based on mtDNA restriction fragment analyses, that *marmoratus* was the maternal parent in the original hybridiza-

tion event(s) (Brown and Wright, 1979; Densmore *et al.*, 1989).

Brown and Wright (1979) and Densmore *et al.* (1989) examined mtDNA of a total of 10 specimens of *C. neomexicanus* from four localities in the southern central portion of its range. Here we add 14 more specimens from six additional localities covering the majority of the range (Fig. 1, N), including the two outlier populations to the SW and NE.

The following three electrophoretically distinguishable clones of *C. neomexicanus* are known: (1) the widespread common genotype (reviewed by Dessauer and Cole, 1989); (2) a variant malate dehydrogenase clone (Parker and Selander, 1984); and (3) a variant transferrin clone (Cole *et al.*, 1988). Each of these clones is represented in Fig. 3, and all have the mtDNA of *C. t. marmoratus*. Therefore, either all clones of *C. neomexicanus* stem from a single original F_1 hybrid lizard or *C. t. marmoratus* was the maternal parent of each hybrid zygote that produced separate clones of *C. neomexicanus*.

The checkered whiptail lizard (C. tessellatus). This is a complex of at least two unisexual species that occur from northern Mexico and western Texas northward, largely in the Rio Grande and Pecos river valleys, through New Mexico, and into southern Colorado. Because of the uncertainty and inconsistency with which nomenclature has been applied to these lizards, we use the name *C. tessellatus* for now and refer also to

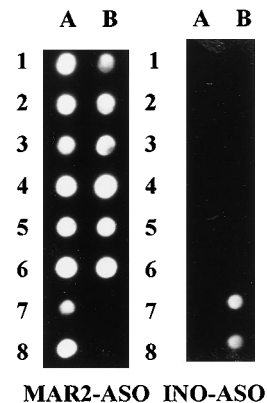


FIG. 3. Dot-blots of 12S mtDNA types for specimens of unisexual *Cnemidophorus neomexicanus* sampled throughout much of its range (Fig. 1, N; Appendix). Blots are contact prints, reverse images of the X-ray film. DNA samples were applied in two columns. All samples in column A and samples 1 through 4 in column B are from *C. neomexicanus*: A 1 through 5 are from two sites in Sandoval Co., New Mexico; A 6 and 7 are from Socorro Co., New Mexico; A 8 is from Hidalgo Co., New Mexico; B 1, 2, and 3 are from Valencia Co., New Mexico; and B 4 is from San Miguel Co., New Mexico. DNA samples B 5 and 6 are from *C. t. marmoratus*; B 7 and 8 are from *C. inornatus*. These were included to check the female parentage involved in the hybrid origin of these *neomexicanus*, representing most of its geographic range. The blot was hybridized with the MAR2-ASO and with the INO-ASO at their $T_m + 5^\circ$ (Table 1).

the different color pattern classes designated as type A through type G (Zweifel, 1965; Scudday, 1973, type G referring to *C. dixonii* type A from Texas). The diploid parthenogens (pattern types C through G) originated as a result of hybridization between two diploid, bisexual species, *C. tigris marmoratus* and *C. septemvittatus* (for reviews, see Parker and Selander, 1976; Densmore *et al.*, 1989; Dessauer and Cole, 1989), and one or more of the F₁ hybrid females perpetuated a lineage through parthenogenetic cloning (Dessauer and Cole, 1986). The triploid parthenogens (pattern types A and B) originated as a result of hybridization between diploid *tesselatus* and the bisexual *C. sexlineatus* (for reviews, see Parker and Selander, 1976; Densmore *et al.*, 1989; Dessauer and Cole, 1989).

Specimens of *C. tesselatus* from across much of its range in the United States (Fig. 1, A–G) and including nearly all pattern types (except TES B and *dixonii* B, which were not tested) reacted strongly positive with the *marmoratus* ASO, but not with either the *septemvittatus* or *sexlineatus* ASO (Fig. 4). This confirms earlier conclusions, based on mtDNA restriction fragment analyses, that *C. t. marmoratus* specifically was the maternal parent in the original hybridization event(s) (Brown and Wright, 1979; Densmore *et al.*, 1989).

Brown and Wright (1979) and Densmore *et al.* (1989) examined mtDNA of a total of 73 specimens of *C.*

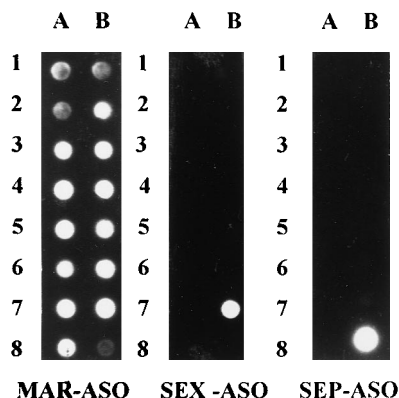


FIG. 4. Dot-blots of 12S mtDNA types for specimens of unisexual *Cnemidophorus tesselatus* (Fig. 1, A–G; Appendix). Blots are contact prints, reverse images of the X-ray film. DNA samples were applied in two columns. All samples in column A and samples 1 through 5 in column B are from *C. tesselatus*: A 1 and 2 are type A from Fremont Co., Colorado; A 3 and 4 are type C from San Miguel Co., New Mexico; A 5 and 6 are type D from San Miguel Co., New Mexico; A 7 is type E from Cibola Co., New Mexico; A 8 is type E from Reeves Co., Texas; B 1 is type E from El Paso Co., Texas; B 2 and 3 are type F from Hidalgo Co., NM; and B 4 and 5 are type G from Presidio Co., Texas. DNA sample B 6 is from *C. t. marmoratus*, B 7 is from *C. sexlineatus*, and B 8 is from *C. septemvittatus* (Appendix). These were included to check the female parentage involved in the hybrid origin of these different *tesselatus* types. The blot was hybridized with the MAR-ASO, SEX-ASO, and SEP-ASO at their respective T_m 's (Table 1). Note that the *sexlineatus* blot at B 7 hybridized with the MAR-ASO at its T_m . Raising the temperature 10° above the T_m of the MAR-ASO prevented its hybridization to the *sexlineatus* DNA.

tesselatus from various localities throughout the range, representing pattern types A through F. Here we add 19 more specimens representing types A and C through G (Fig. 1; Appendix). Thus, even though there is considerable clonal diversity in the *tesselatus* complex (Parker and Selander, 1976; Dessauer and Cole, 1986, 1989; Densmore *et al.*, 1989), *C. t. marmoratus* is the maternal parent of all known clones, both diploid and triploid, from throughout the range.

Specificities of ASOs

In conducting the tests described above, several false positive nucleic acid hybridizations were seen. These never occurred when the sequence of the ASO and mtDNA of the test subject differed by four or more base pairs in the 18-base region used (Table 1). However, when the ASO and mtDNA of the test subject differed by only 1 or 2 bases, as in *C. tigris marmoratus*, *C. inornatus*, and *C. sexlineatus*, some false positives were obtained (e.g., Fig. 4, B 7 with the MAR-ASO). The false positive reactions involving these ASOs in most cases could be eliminated by using one or more of the following procedures:

(1) increasing the stringency of the test by raising the temperature of the hybridization step above the T_m of the ASO;

(2) decreasing the size of the ASO to eliminate some bases shared in common with the problematical test subject (thus increasing the percentage difference between the ASO and that subject), and

(3) designing the probes so that most base-pair differences are near the center.

These false positive reactions resulted from an extension of the original problem, which was to distinguish between *C. tigris gracilis* and *C. t. marmoratus*. The 18-base region chosen for synthesizing the ASO probes was selected because these two taxa had significant variation in this region (5 bases). For the additional comparisons later added to the study, we chose the same 18-base region for all ASOs and comparisons. The message here is that for any particular investigation, one should carefully select the base region with which to design the ASO so as to maximize the differences and reduce the possibilities of false positive hybridizations. For example, to design a better ASO to distinguish *C. inornatus* from *C. sexlineatus*, we could have used an alternative region of the 12S ribosomal mtDNA, where the sequences differ by four residues (Reeder *et al.*, unpublished data).

CONCLUSIONS

The dot-blot method can be used to study any gene for which sequences are known for the populations and/or species of interest. With sequence information in hand, one can design and synthesize ASO probes. These allow rapid and inexpensive screening of allelic variation in

large numbers of specimens, which may include: (1) analysis of geographic variation; (2) determining the maternity of captive organisms of uncertain or unknown hybrid status; and (3) determining the provenance of organisms of unknown population origin.

APPENDIX

Collecting sites and voucher numbers of specimens used to obtain the dot blots of Figs. 2, 3, and 4 and to design the allele-specific oligonucleotides are described below.³

Cnemidophorus tigris gracilis

Fig. 2: Site 36, 3 mi. E and 10 mi. S of San Simon, Cochise Co., Arizona, AMNH⁴ 127047-48, 127050, and 138509. Site 49, Huerfano Butte, 27 mi. SSE of Tucson, Pima Co., Arizona, AMNH 127056-57.

ASO-GRA was designed from the sequence of the 12S mtDNA of specimen AMNH 127052 from 3 mi. E and 10 mi. S of San Simon, Cochise Co., Arizona; ASO-GRT was designed from the sequence of the 12S mtDNA of AMNH 127066 from Huerfano Butte, 27 mi. SSE of Tucson, Pima Co., Arizona.

C. t. marmoratus

Fig. 2: Site 48, 0.6 mi. E and 9.6 mi. N of Animas, Hidalgo Co., New Mexico, AMNH 127069-70, 127072, 127075; Site 54, 1.4 mi. W of San Antonio, Socorro Co., New Mexico, AMNH 131072-73.

Fig. 3: Site 48, 0.6 mi. E and 9.6 mi. N of Animas, Hidalgo Co., New Mexico, AMNH 127074, 127076.

Fig. 4, Site 48, 0.6 mi. E and 9.6 mi. N of Animas, Hidalgo Co., New Mexico, AMNH 127073.

ASO-MAR (Figs. 2 and 4) and ASO-MAR2 (Fig. 3) were designed based on the sequence of the 12S mtDNA of AMNH 127072 from 0.6 mi. E and 9.6 mi. N of Animas, Hidalgo Co., New Mexico.

³ Tests were carried out on many additional specimens. These included 594 lizards from within the contact region and 35 from four populations distant from the contact regions of the 2 subspecies of *C. tigris*; 4 specimens of *C. inornatus*; 2 specimens of *C. sexlineatus*; and 2 specimens of *C. septemvittatus*.

Two additional *C. neomexicanus* were examined: AMNH 128329 from the Bernalillo population and AMNH 125565 from 16.7 mi. NW of Lordsburg, Hidalgo Co., New Mexico. Six additional *C. tessellatus* were tested: Type C, San Miguel Co., New Mexico, Conchas Dam, UADZ 3241, 3246; Type D, Higbee, Otero Co., Colorado, UADZ 3429, 3175; Type E, Reeves Co., Texas, 2.7 mi. SW of Balmorhea, AMNH 129216; Type F, Hidalgo Co., New Mexico, 7 mi. W of Animas, FT 1934.

⁴ AMNH denotes voucher specimen in the American Museum of Natural History, New York. FT denotes frozen tissue sample number of specimens yet to be catalogued in the permanent voucher collection.

Hybrids of *C. t. gracilis* × *C. t. marmoratus*

Fig. 2: Site 26, 0.6 mi. S and 0.7 mi. W of Road Forks, Hidalgo Co., New Mexico, AMNH 138510, 138518-19, 138521.

Cnemidophorus neomexicanus

Fig. 3: Sandoval Co., New Mexico, Rio Grande crossing at Cochiti Dam, AMNH 122931, 122933, 122946; 5.3 mi. S of Bernalillo, AMNH 128330-31. Socorro Co., New Mexico, 0.6 mi. E of San Antonio along the Rio Grande, AMNH 128326, 128328. Hidalgo Co., New Mexico, 17.2 mi. NW of Lordsburg, AMNH 131067. Valencia Co., New Mexico, Rio Puerco, 15.9 mi. W of Los Lunas, AMNH 133142, 133146, 133151. San Miguel Co., New Mexico, Conchas Lake State Park, South Campground, AMNH 136881.

Cnemidophorus inornatus

Fig. 3: Gila Co., Arizona, 2 mi. N of Four Peaks, Mazatzal Mountains, AMNH 134995. Cochise Co., Arizona, 2.2 mi. SE of Willcox, AMNH 134999.

ASO-INO was designed from the sequence of 12S mtDNA of specimen AMNH 126861 from 9.3 mi. S of Gray Mt., Coconino Co., Arizona.

Cnemidophorus tessellatus

Fig. 4: Type A, Fremont Co., Colorado, 1 mi. N of Florence, AMNH 131415, 131419. Type C, San Miguel Co., New Mexico, Conchas Lake State Park, South Campground, AMNH 123029, 136877. Type D, San Miguel Co., New Mexico, Conchas Lake State Park, South Campground, AMNH 123038, 136880. Type E, Cibola Co., New Mexico, 1.5 mi. W of Canoncito, AMNH 136845; Reeves Co., Texas, 2.7 mi. SW of Balmorhea, AMNH 129217; El Paso Co., Texas, Tom Mays Memorial Park, 10 mi. N of El Paso, AMNH 127001. Type F, Hidalgo Co., NM, 7 mi. W of Animas, FT 1521,⁴ 1937. Type G, Presidio Co., Texas, San Antonio Canyon, UADZ⁵ 3557, 3561.

Cnemidophorus sexlineatus

Fig. 4: Brooks Co., Texas 7.1 mi. S of Falfurrias, AMNH 126893.

ASO-SEX was designed from the sequence of 12S mtDNA of specimen AMNH 126901 from 7.1 mi. S of Falfurrias, Brooks Co., Texas.

Cnemidophorus septemvittatus

Fig. 4: Brewster Co., Texas, 3.5 mi. S of Marathon, AMNH 126764. ASO-SEP was designed from the sequence of 12S mtDNA of specimen TNHC⁶ 53902 from Marathon, Brewster Co., Texas.

⁵ UADZ denotes voucher specimen in the University of Arkansas Department of Zoology.

⁶ TNHC denotes voucher specimen in the Texas Natural History Collection, Texas Memorial Museum, Austin.

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