trast, bolus treatment with dAVP and AVP produced a more prolonged increase in blood pressure and APir release. In addition, continuous infusion of phenylephrine (10 μg/min) produced a sustained 15-mmHg elevation in mean arterial pressure and a three- to fourfold elevation in plasma APir. Both of these effects were reversible upon termination of the infusion. The dependence of APir release on changes in arterial pressure is consistent with the observation that neither the nonpressor analog dDAVP nor AVP in the presence of its antipressor antagonist caused APir release.

Changes in systemic pressure may not be the only factor involved in the release of APir. There were significant differences in the initial amount of APir released in response to matched increases in mean arterial pressure. These differences may be related to regional changes in pressure or blood flow, especially those affecting atrial volume and wall tension. Alternatively, dAVP and AVP could be acting through a parallel but pressure-independent mechanism to augment APir release.

Average urine output was relatively stable in the control animals during the experiment (Table 1). Treatment with dAVP and AVP produced significant diuresis, while phenylephrine and angiotensin II did not (Table 1), suggesting that a sustained release of APir may be necessary to effect an increase in diuresis. Natriuresis has been shown to occur after administration of vasopressin (*14, 15*); however, the mechanism underlying this effect is not known. A possible explanation is that a sustained elevation in blood pressure causes the diuresis. Depression of proximal tubular reabsorption of sodium (*14*) and activity of a subtype of vasopressin receptor specific for the natriuretic activity have also been postulated (*16*). The observed dAVP-induced release of APir in vivo provides a further explanation of the natriuretic effects of vasopressin and related peptides.

In conclusion, we have shown that the release of endogenous APir in intact animals can be induced by a naturally occurring peptide hormone, arginine-vasopressin. In a recent study it was demonstrated that infusion of APIII lowers elevated arginine-vasopressin in dehydrated or hemorrhaged animals (*17*). This suggests the existence of a negative-feedback endocrine loop whereby arginine-vasopressin stimulates AP release, which in turn suppresses arginine-vasopressin release. These opposing hormones may interact in the regulation of fluid and electrolyte homeostasis.

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18. Radioimmunoassay of AP was performed with a rabbit polyclonal antiserum generated against a cyanogen bromide fragment of high molecular weight AP [C. B. Saper *et al.*, *Science* **227**, 1047 (1985)]. Synthetic APIII was iodinated by the chloramine-T method and purified by high-performance liquid chromatography (HPLC), yielding a specific activity of 200 μCi/μg. This antiserum was 100 percent cross-reactive with API and APII, 10 percent with the high molecular weight precursor atriopeptigen (containing 92 carboxyl terminal amino acids), and did not cross-react with biologically inactive synthetic peptides: residues 13 to 24 of APIII, APIII containing an inversion of Arg-Ile at positions 7 and 8, or arginine-vasopressin. Schwartz *et al.* (*8*) showed a correlation between the plasma immunoreactivity, HPLC migration, biological activity, and sequences of the AP's.

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Ser-Leu-Arg-Arg–Atriopeptin III: The Major Circulating Form of Atrial Peptide

Abstract. *Vasopressin induces a concentration-dependent increase in atriopeptin immunoreactivity in plasma. Rat plasma, rat atrial extract, and synthetic atriopeptin III (APIII) produced parallel displacement curves of iodine-125–labeled APIII binding to specific antiserum. Fractionation of plasma atriopeptin immunoreactivity by reverse-phase high-performance liquid chromatography showed that the major portion consists of two species of low molecular weight peptides in a ratio of 10 to 1. Both peaks exhibited potent vasorelaxant activity, suggesting the presence of the carboxyl terminal Phe-Arg sequence of atriopeptin in each species. Sequence determination of the purified peptides indicated that the major peptide is Ser-Leu-Arg-Arg–APIII and the minor peptide APIII. It appears that the former is the major species of atrial peptide in the rat circulation and that it is the product of selective cleavage of the high molecular weight precursor.*

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Several related low molecular weight peptides with potent natriuretic, diuretic, and vascular smooth muscle relaxant activity have been isolated from mammalian atrial myocytes and their sequences determined (*1–3*). All these atriopeptins (AP's) contain the same core sequence of 17 amino acids within a cystine disulfide bridge, but differ in the lengths of their amino and carboxyl termini. The vasorelaxant property of the AP's requires the presence of the Phe-Arg sequence in the carboxyl terminus (*1, 4, 5*). Variations in the peptide sequence probably result from proteolysis during extraction or processing within the atrial granules.

To establish the endocrine nature of AP's, both stimulated release and structural identification of the circulating form must be demonstrated. Circulating AP's have been detected by radioimmunoassay, suggesting that they have an endocrine role in fluid and electrolyte homeostasis (*6–9*). These studies have shown that (i) atrial extracts, plasma,

and synthetic AP's cause parallel displacement of radioligand binding to specific polyclonal antisera (*6*); (ii) the natriuretic activity of atrial extracts corresponds to the amount of AP immunoreactivity (APir) (*6*); and (iii) the plasma-derived immunoreactivity and the low molecular weight AP's have similar mobility on reversed-phase high-performance liquid chromatography (HPLC) (*6–9*). In contrast to extracts from cardiac atria, which have both high and low molecular weight peptides, the coronary venous effluent from isolated, perfused hearts contains a peptide that migrates in the vicinity of but not coincident with the 24-amino-acid APIII on reversed-phase HPLC (*10*). Atrial distension of isolated rat hearts enhances the release of bioassayable AP's (*11*). Recently, Lang *et al.* (*8*) confirmed both the presence of a low molecular weight AP in the coronary venous effluent of isolated, perfused hearts and the observation that atrial stretching facilitates release of the peptide. They also found that hypervolemia in rats results in the release of material into the blood that is immunologically related to the AP's. HPLC analysis of the coronary effluent from the isolated rat heart and of rat plasma after atrial stretching indicated that the immunoreactive substance was a low molecular weight species; however, the lack of comigration with AP standards precluded its identification. Finally, inadequate material was available for characterization of biological activity and sequence determination (*8*).

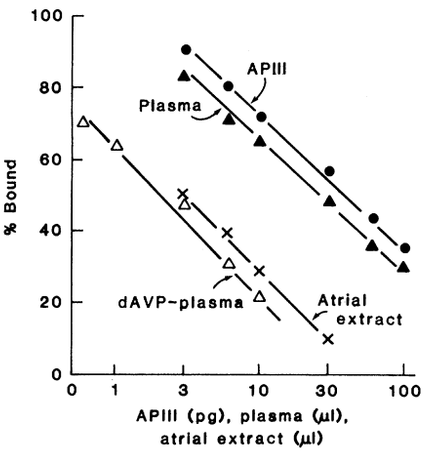

Fig. 1. Results of the AP radioimmunoassay. Atriopeptin immunoreactivity of synthetic APIII, rat atrial tissue extracts, and plasma was measured directly with polyclonal rabbit antiserum (ATRP 11; final dilution, 1:6000) raised against a cyanogen bromide fragment (92 amino acids) of the high molecular weight atriopeptigen precursor molecule (*15*). This antiserum was 100 percent cross-reactive with API and APII and did not cross-react with dAVP (Peninsula). Synthetic APIII, prepared by solid-phase synthesis in our laboratories or obtained commercially (Peninsula), was iodinated by the chloramine-T method and purified by HPLC, yielding a specific activity of 200 μCi/μg. Antibody-bound [^{125}I]APIII (10,000 count/min per assay tube) was precipitated overnight at 4°C with a 1:750 dilution of goat antibody to rabbit immunoglobulin (Linco) in a final volume of 300 μl of 100 m*M* sodium phosphate buffer (*p*H 7.0), 3 percent polyethylene glycol, and 0.25 percent bovine serum albumin (BSA). The precipitate was washed twice with 0.25 percent BSA and 10 m*M* NaN_3 and radioactivity was counted in a Micromedic Apex automatic gamma counter. Male Sprague-Dawley rats (250 to 350 g) were anesthetized (0.5 ml of 7 percent chloral hydrate per 100 g of body weight) and treated with dAVP (10 μg, intravenously). Blood was collected 5 minutes after injection in a one-tenth volume of 4.4 percent disodium EDTA (*p*H 7.0) and centrifuged (7700*g* for 10 minutes). The plasma was immediately frozen and stored at −70°C. Normal plasma was obtained from untreated rats. Rat atrial extracts were prepared by boiling finely minced tissue in 1.0*M* acetic acid for 10 minutes followed by centrifugation at 30,000*g* for 20 minutes. The supernatant was stored at −70°C and diluted 1000-fold before being assayed.

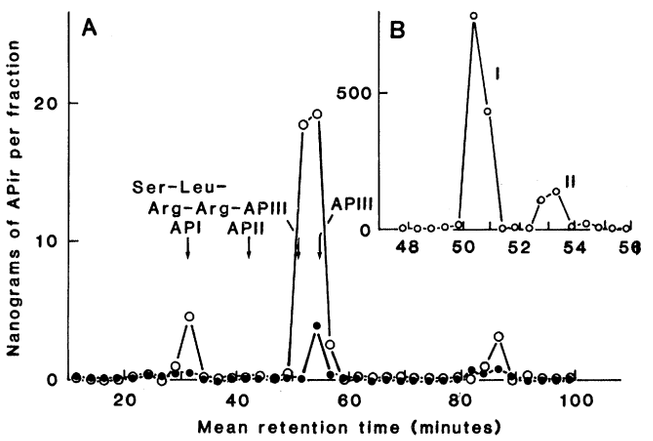

Fig. 2. Purification of immunoreactive plasma peptides by HPLC. Normal or dAVP-stimulated rat plasma was obtained as described in the legend to Fig. 1. Plasma was partially purified on octadecylsilane (Baker 7020-6) columns. Each column was washed with 3 ml of 0.1 percent trifluoroacetic acid (TFA). Plasma was applied at a rate of 0.8 ml/min, eluted with 90 percent acetonitrile and 0.1 percent TFA, and lyophilized. The eluate was reconstituted in 10 percent acetonitrile and 0.1 percent TFA and applied to a Vydak C18 (218TP54) column at a flow rate of 1.0 ml/min (A = 0.05 percent TFA and B = 80 percent acetonitrile and 0.05 percent TFA). The following linear gradients in B were applied: at 5 minutes to 0 to 22.5 percent in 5 minutes; at 30 minutes, 22.5 to 32.5 percent in 40 minutes; at 70 minutes, 32.5 to 70 percent in 20 minutes; and at 90 minutes, 70 to 100 percent in 5 minutes. (A) Immunoreactivity of 2.5-minute fractions from an HPLC run of 4 ml of plasma from a control rat (●) and a dAVP-treated rat (○). Locations of synthetic standards are indicated (arrows). (B) Immunoreactivity of 0.5-minute fractions from an HPLC run of dAVP-stimulated plasma from 17 rats.

Arginine-vasopressin and other vasoconstrictor agents induce a profound release of AP into the circulation (*9*). We report here that Ser-Leu-Arg-Arg–APIII (with smaller amounts of APIII) appears to be the major circulating form of the AP's released after stimulation.

The antiserum used is characterized in the legend to Fig. 1. Synthetic APIII displaced [^{125}I]APIII binding to polyclonal rabbit antiserum in a concentration-dependent manner, with 40 pg of APIII producing 50 percent displacement. Crude rat atrial extract, normal rat plasma, and plasma from a rat stimulated with 1-deamino-Arg^8-vasopressin (dAVP) caused displacement of radioligand binding parallel to that produced by synthetic APIII (Fig. 1). Crude atrial extract contained approximately 10 μg of APir per heart. Normal rat plasma contained 800 pg of APir per milliliter. The concentration increased approximately ten times after treatment with 10 μg of dAVP. The APir value we obtained for normal rats is similar to that of two previous studies (*6, 7*) but is much higher than that of a third study (*8*). The difference might reflect the different anesthetics used or the preparation of the plasma sample for immunoassay. Neither biologically inactive analogs of AP (residues 13 to 24 of APIII, and APIII with an inversion of Arg and Ile to positions 7 and 8), vasopressin, nor dAVP caused displacement of radiolabeled ligand binding.

The availability of this specific radioimmunoassay along with the ability to greatly increase circulating levels enabled the circulating immunoreactive species of AP to be identified. Plasma from untreated rats that was fractionated by HPLC displayed small amounts of APir in three zones of the column eluate migrating in the vicinity of standards API, APIII, and Ser-Leu-Arg-Arg–APIII and a high molecular weight peptide (Fig. 2A). The trace amount (less than 5 percent) of immunoreactive high molecular weight peptide was inadequate for further characterization. This material may represent the amino terminal fragment of the precursor detected by the

polyclonal antibody but lacking the carboxyl terminal AP. However, plasma from dAVP-treated rats displayed an especially marked increase in APir present in the APIII and Ser-Leu-Arg-Arg–APIII zone. Figure 2B shows the pattern of immunoreactivity obtained across this zone when separated into smaller fractions in a scaled-up HPLC run. Two distinct peaks of immunoreactivity, in a 10 to 1 ratio, were obtained which comigrated on the HPLC with Ser-Leu-Arg-Arg–APIII and APIII standards, respectively. The APir peptides in peaks I and II were then subjected to further purification by second HPLC column (Brownlee RP 300) runs. Each peak was then used in a vascular smooth muscle bioassay and subjected to protein sequence analysis.

Strips of isolated rabbit thoracic aorta were used to assess the spasmolytic activity of the immunoreactive peaks (I and II) obtained by reversed-phase HPLC of plasma from dAVP-treated animals. The blood vessel preparation readily discriminates between modifications in the length of the carboxyl terminus of AP's; thus, loss of the Phe-Arg or Arg residues markedly decreases the vasorelaxant activity (*12*). Peaks I and II, which comigrated on HPLC with synthetic Ser-Leu-Arg-Arg–APIII and APIII, respectively, were biologically active, producing a response comparable to that of APIII standards (Fig. 3). The activity of the fractions on the rabbit aorta suggests the presence of a Phe-Arg on the carboxyl-terminal sequence in each of the AP-like substances.

The smooth muscle experiments and the chromatographic comigration data provide only suggestive evidence of the structure of the APir. Sequence analyses of the purified peptides derived from peaks I and II demonstrate that the more abundant form (approximately 90 percent of the APir and mass obtained from the repetitive yield calculated in the sequence analysis) of APir present in peak I is Ser-Leu-Arg-Arg–APIII, whereas that of peak II is APIII (Fig. 4). The combined data from the immunoreactivity, chromatographic migration, vasorelaxant biological activity, and sequence analysis establish that the major component of the hormone-stimulated peptides in the plasma is Ser-Leu-Arg-Arg–APIII [the 28-amino-acid peptide, previously termed cardionatrin (*2*)], with smaller amounts of APIII (the 24-amino-acid peptide). The higher abundance of the former peptide (peak I) suggests that APIII (peak II) may be a plasma hydrolysis product of the larger peptide. Indeed, the APir released by marked hypervolemia in rats is converted to a material that comigrates with APIII when proteolytic inhibitors are not added to blood samples (*8*). The data suggest that the 28-amino-acid peptide is the major circulating form in the rat after hormone stimulation.

We recently demonstrated that while Ser-Leu-Arg-Arg–APIII and APIII are equipotent in reducing renal resistance in intact dogs, the former is considerably more potent as a natriuretic and diuretic (*5*). Thus, conversion of Ser-Leu-Arg-Arg–APIII to AP-III could be an important factor in modification of the effect of the atrial peptides on fluid and electrolyte excretion in dogs.

Extracts of mammalian atria contain a mixture of high and low molecular weight peptides. It is unclear whether the precursor atriopeptigen is the granular storage form and the low molecular weight peptides are generated during purification or whether processing proceeds within the granule and is regulated

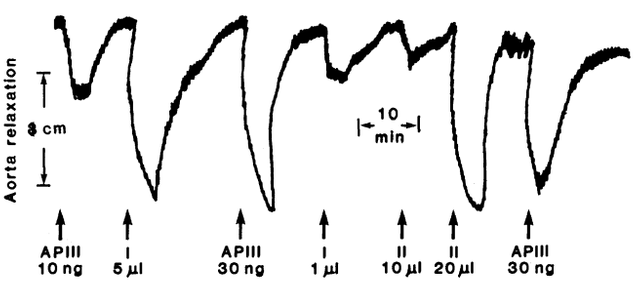

Fig. 3. Biological activity of the two purified low-molecular-weight peptides obtained by HPLC fractionation of plasma from dAVP-treated rats. Approximately 10 percent of the AP-immunoreactive peptides purified from peaks I and II was dissolved in 100 and 160 µl of 22 percent acetonitrile and 0.1 percent TFA, respectively, for biological assay. Aliquots of these samples were compared to APIII standards for vasorelaxant activity on rabbit aorta strips contracted with norepinephrine ($2 \times 10^{-8} M$) (*1, 4*). Application of 30 µl of the solvent alone did not elicit a respone or alter the sensitivity of the tissues.

Cycle no.	1	2	3	4	5	6	7	8	9	10	11	12
Peak I	Ser	Leu	Arg	Arg	Ser	Ser	(Cys)	Phe	Gly	Gly	Arg	Ile
Peak II	Ser	Ser	(Cys)	Phe	Gly	Gly	Arg	Ile	Asp	(Arg)	Ile	(Gly)

Cycle no.	13	14	15	16	17	18	19	20	21	22	23	24
Peak I	Asp	Arg	Ile	Gly	Ala	Gln	Ser	Gly	Leu	Gly	(Cys)	Asn
Peak II	Ala	(Gln)	Ser	(Gly)	(Leu)	Gly	(Cys)	Asn	(Ser)	(Phe)	(Arg)	Tyr

Cycle no.	25	26	27	28
Peak I	Ser	Phe	(Arg)	Tyr

Fig. 4. Gas-phase sequence analysis of the two immunoreactive low molecular weight peptides obtained by fractionation of plasma from dAVP-treated rats. Peaks I and II from five Vydac HPLC runs (see Fig. 2B) involving the plasma from 84 dAVP-treated rats were combined and applied (in 10 percent acetonitrile and 0.1 percent TFA) to a Brownlee RP-300 column and separated at a flow rate of 1 ml/min with the following linear gradients in B (A = 0.1 percent TFA and B = 80 percent acetonitrile and 0.1 percent TFA): at 5 minutes, 0 to 22.5 percent in 5 minutes and at 10 minutes, 22.5 to 35 percent in 50 minutes. Single components were collected in appropriate fractions (approximately 1 minute each) following the profile of absorbance at 215 nm. HPLC of peak I produced a single immunoreactive fraction (mean retention time, 39.6 minutes). Similarly, peak II gave a single immunoreactive fraction (mean retention time, 41.5 minutes). Ninety percent of each peak was sequentially degraded with an Applied Biosystems 470A protein sequencer. The respective phenylthiohydantoin amino acids were identified by the HPLC analysis (*16*). Amino acid sequence analysis of the peptide from peak I shows an average repetitive yield of 84 percent calculated from the least-squares linear regression of individual amino acids recovered at each sequencer cycle. All values were used in the linear regression analysis except those for serine and arginine. The log picomolar yield per cycle number was plotted by linear regression analysis to determine the slope (repetitive yield) and intercept of the resulting line. The intercept yield indicated 741 pmol of starting peptide from peak I. This compares with the estimate, by radioimmunoassay, of 643 pmol of APir in peak I, and lends support to the quantitative (as well as qualitative) accuracy of the immunoassay. Peptide purified from peak I was sequenced with comparable results on two separate occasions from plasma from 45 and 84 rats, respectively. Amino acid sequence analysis of the peptide purified from peak II shows an average repetitive yield of 85 calculated (as above, with peak I) from the least-squares linear regression. The intercept yield indicated 67 pmol and compares to an estimate of 167 pmol of APir. The amino acids in parentheses gave an inadequate signal and were assigned on the basis of known sequence (*1, 2*). Peptide purified from peak II was subjected to sequence analysis on three occasions, giving partial sequences that were in agreement from batches of 45 rats. The complete sequence reported here was obtained by using plasma from 84 rats.

by secretion. Vasopressin stimulation might cause the atria to release the high molecular weight prohormone, which then undergoes proteolytic conversion in the plasma. However, Trippodo *et al.* (*13*) recently reported that the high molecular weight peptide extracted from rat atria is not converted to the low molecular weight peptide when incubated in rat plasma at 37°C. In addition, the fact that the perfusate from isolated hearts (*8, 10, 11*) and rat plasma contain only low molecular weight peptides (Fig. 2) (*6–8*) strongly suggests that proteolytic conversion occurs within the heart itself. Prohormone processing enzymes associated with membrane structure or secretory granules have been identified for a number of peptides including pro-opiocortin, somatostatin, glucagon, insulin, and adrenocorticotropic hormone (*14*).

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Conservation Genetics of Endangered Fish Populations in Arizona

Abstract. *Genetic diversity in remnant populations of the Sonoran topminnow* Poeciliopsis occidentalis *(Pisces: Poeciliidae) from Arizona, where the species is endangered, is compared with that in populations from Sonora, Mexico, where the fish is widespread and abundant. Geographically peripheral Arizona populations contain substantially lower levels of genetic variation than do Mexican populations near the center of the species' range. Allelic differences among three genetically and geographically distinct groups are responsible for 53 percent of the total genetic diversity in this species, 26 percent is due to differences among local populations within the groups, and 21 percent is due to heterozygosity within local populations. Recommendations for conservation and restocking efforts in Arizona are based on these genetic findings.*

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Alteration and destruction of natural habitats by human activities threatens many plant and animal species. Beyond the immediate possibility of extinction for geographically restricted and endemic forms, there is a more subtle threat: erosion of the genetic diversity upon which long-term persistence and adaptability depend. Habitat destruction can subdivide a species into a series of small, partially isolated demes (local populations) that may lose genetic variability through inbreeding and random genetic drift. Erosion of genetic variability may contribute to an immediate reduction in fitness and to constraints on future adaptive potential (*1*). Knowledge of the distribution of genetic variation within and between remnant populations of an endangered species is necessary if we are to design sound conservation programs (*2*).

The arid southwestern United States is a critical region for conservation of aquatic organisms because ground-water pumping, stream modifications, and widespread introduction of exotics have extensively disrupted the natural habitats of numerous endemic species (*3, 4*). For example, the Sonoran topminnow, *Poeciliopsis occidentalis*, was once the dominant fish in lowland streams, springs, and marshes of the Gila river system in Arizona (*5*). During the past 40 years, habitat destruction coupled with introduction of nonnative predaceous fishes, particularly the mosquito fish, *Gambusia affinis* (*4, 6*) have reduced formerly large and widespread populations of *P. occidentalis* to small, geographically isolated demes inhabiting tiny headwater springs and stream segments (*4*). In 1973 *P. occidentalis* was placed on the federal Endangered Species List (*7*). An effective conservation program for endangered Arizona populations requires not only familiarity with their regional ecology but also a broad knowledge of their genetic structure, including populations in Sonora, Mexico, where the species remains abundant. We report on the distribution of genetic variation in 5 Arizona and 16 Sonoran populations of *P. occidentalis*.

We surveyed the products of 25 protein-determining gene loci for electrophoretic variation, and 12 loci exhibited polymorphic phenotypes (*8*). Genetic variability is greatest near the center of the species' range, in populations inhabiting the Rió Matape and lower Río Sonora and Rió Yaqui (Table 1). Variability decreases at the northern periphery of its distribution and in upstream portions of most rivers. Numerical fluctuations in population size that lead to genetic drift and a loss of genetic variability are generally thought to be more severe in partially isolated peripheral populations (*9*). Although the immediate consequences of low heterozygosity in Arizona populations of *P. occidentalis* are not known, studies of a variety of organisms (including a related species of *Poeciliopsis*) indicate that growth rate, developmental stability, survivorship, and competitive abilities may be negatively affected (*1, 10*).

To assess relations among the 21 populations, pairwise genetic distances were computed from allelic frequencies and then clustered by the Distance-Wagner method (*11*). Three major groups were apparent in the resulting dendrogram (Fig. 1). Groups 1 and 2 correspond with morphologically defined subspecies that have previously been recognized in Arizona (*12*). Group 3, the most divergent group, inhabits partially isolated headwater tributaries of the Río Mayo above a man-made impoundment. However, differences among these groups cannot be attributed to recent disturbances. For example, the timespan necessary to explain genetic divergence between groups 2 and 3 is roughly equivalent to 2.8 million years; it is 1.7 million years for groups 1 and 2, and 4.3 million years for 1 and 3 (*13*). These estimates are consistent with the Pliocene–early Pleistocene separation of these drainage basins (*14*). Groups 1 and 2 intergrade in the Río Matape and the lower Río Sonora (locations M and N in Fig. 1), where populations are polymorphic for alleles that would elsewhere be diagnostic of the two groups. The multivariate coefficient of variation for morphological