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Allozymes and the hybrid origin of the parthenogenetic lizard *Cnemidophorus exsanguis*[1]

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Summary. Allozyme electrophoresis was used to determine the hybrid parentage of the triploid parthenogenetic species *Cnemidophorus exsanguis*. 19 of 33 loci were polymorphic in *C. exsanguis* and/or the 7 potential bisexual progenitors. It was determined from allele distributions that *C. exsanguis* containes genomes form 3 different species, probably *C. costatus*, *C. inornatus* and *C. septemvittatus*.

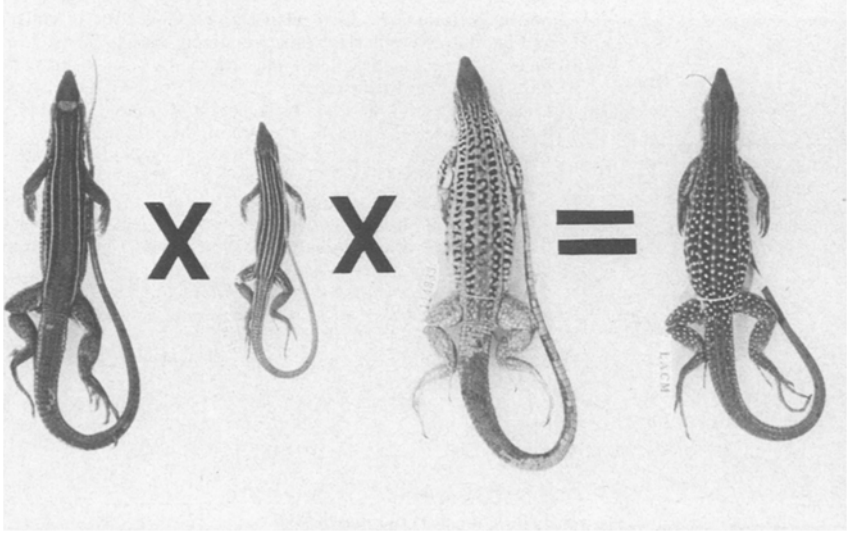
Key words. Lizard, parthenogenetic; *Cnemidophorus exsanguis;* allozymes; hybrid parentage.

15 of the approximately 45 species of the New World teiid genus *Cnemidophorus* are parthenogenetic[2]. Within the broad geographic range of the genus, the parthenogenetic species have 3 primary areas of distribution: the Amazon Basin, the Yucatan Peninsula and the inland southwest of North America[2,3]. It has been determined by chromosome analyses that these species are either diploid or triploid[2,4] and in one case both[5]. Some of the species are clearly alloploid as they contain chromosome complements characteristic of different species groups in the genus[5–7]. Most parthenogenetic species; however, have morphologically homologous chromosomes that are characteristic of a single species group (*sexlineatus* group). All but one of these are triploid. Thus, with one exception[8,9], chromosome analyses have contributed little toward the understanding of the origin of most of these parthenogenetic species. Neaves[10] and Parker and Selander[11] subjected 2 of the alloploid parthenogenetic species (*C. neomexicanus* and *C. tesselatus*) to allozyme analyses. They found that these species had fixed heterozygosity at many loci and that the alleles present could be assigned to putative parental species. These were the same putative parental species established on the basis of chromosomes and morphology[5,6] and more recently by restriction endonuclease analyses of mitochondrial DNA[3].

This study is focused on determining the origin of *C. exsanguis*, one of the parthenogenetic species with 3 complements of *sexlineatus* group chromosomes. Samples of this species and of all bisexual species in the *sexlineatus* group were subjected to allozyme analyses involving 33 gene loci. Data are presented here for 7 populations of the 5 species that are probable potential progenitors of *C. exsanguis*. We use these data to address 2 primary questions: 1) Is *C. exsanguis* of hybrid origin? and 2) Can the bisexual progenitor(s) of *C. exsanguis* be determined?

Materials and methods. Tissue samples were secured from specimens of *Cnemidophorus* and stored in liquid nitrogen or in ultra-cold freezers (−76 °C). Voucher specimens were then prepared and deposited in the Natural History Museum of Los Angeles County (LACM). The following specimens were used in this analysis (more complete collection data are available on request): *C. exsanguis:* Arizona (10; 131759, 131761, 131764, 131770, 131772, 134797–801), New Mexico (4; 126981, 134290, 134294–95), Texas (1; 134309) and Chihuahua (4; 116150–51, 116228, 116330); *C. burti burti*: Sonora (4; 121421–23, 121426); *C. burti stictogrammus:* Arizona (4; 114743–44, 114746, 114748), Sonora (22; 114731–42, 121331–39, 121341); *C. burti xanthanotus:* Arizona (2; 135450–51); *C. costatus:* Sonora (11; 121365–71, 130368, 135469–71); *C. gularis:* Nuevo Leon (4; 116207–08, 116210, 116212); *C. inornatus:* Arizona (11; 114790–93, 114795–801), New Mexico (2; 116293, 134336); *C. septemvittatus:* Chihuahua (4; 121458–59, 135486–87). The specimens photographed in the figure are as follows: *C. costatus* (Sonora, 121366), *C. inornatus* (New Mexico, 116296), *C. septemvittatus* (Chihuahua, 114833) and *C. exsanguis* (New Mexico, 114823).

Prior to the analysis the tissues (usually heart and liver) were thawed, combined, homogenated with equal parts of tissue and deionized water and centrifuged at 15,000 rpm for 30 min. The resulting supernatant was subjected to starch-gel electrophoresis. The procedures including staining protocols were essentially those described by Selander et al.[12] with minor modifications. The gel types and the proteins (loci) scored in each are as follows: *Tris Citrate II (pH 8.0):* isocitrate dehydrogenase (ICD, 2 loci), glutamate oxalate transaminase (GOT, 2 loci), phosphoglucomutase (PGM, 2 loci), mannose phosphate isomerase (MPI, 2 loci), α-glycerophosphate dehydrogenase (α-



The probable hybridization sequence in the origin of the allotriploid parthenogenetic species *Cnemidophorus exsanguis* as determined by the analysis of alleles at 19 polymorphic allozyme loci. The species represented are (from left to right) *C. costatus*, *C. inornatus*, *C. septemvittatus* and *C. exsanguis*.

gpd, 2 loci). *Tris Citrate III (pH 7.0)* with NADP: adenosine deaminase (ADA), malate dehydrogenase (MDH, 2 loci), malic enzyme (ME), hydroxyacid dehydrogenase (HADH), glucosephosphate isomerase (GPI), leucine aminopeptidase (LAP). *LIOH (pH 8.2):* l-leucyl-l-alanine (LA), l-leucylglycyl-glycine (LGG), l-phenylalanyl-l-proline (PAP), amido black (AB, general protein stain), superoxide dismutase (SOD), esterase-D (EST-D). *Poulik (pH 8.7):* lactate dehydrogenase (LDH, 2 loci), creatine kinase (CK), β-N-acetylglucosaminidase (HEX), glutamate dehydrogenase (GLUD). *Tris EDTA Borate II (DH) (pH 9.0)* with NADP: alcohol dehydrogenase (ADH), aconitase (ACON), phosphogluconate dehydrogenase (6-PGD), pyruvate kinase (PK), glucose-6-phosphate dehydrogenase (G-6-PDH).

Results and discussion. Of the 33 gene loci (69 alleles) resolved, 14 were monomorphic in all taxa. 3 allele complements were visible at most loci in *C. exsanguis* either as separate alleles or through dosage effects. Furthermore, *C. exsanguis* had identically fixed heterozygosity in all individuals at 14 of the 19 polymorphic loci in the study samples, a level of heterozygosity (42.4%) far higher than that present in any of the bisexual species (circa 3.1%). At 2 of these loci, 3 alleles were present (ADA and ACON). The high level of fixed heterozygosity and 3 allelic components at each locus would be expected in a parthenogenetic triploid species that resulted from hybridization[10,11]. Moreover, the presence of 3 alleles at 2 loci suggests that hybridization involved the contribution of genomes from 3 species sources as in the case of triploid populations of *C. tesselatus*[5,10,11].

If it is assumed that *C. exsanguis* is an interspecific hybrid and the parental species are extant, then it should be possible to identify those involved by comparing alleles present or absent in *C. exsanguis* with those in potential parental populations. For example, among these populations (table) alleles at 8 loci (ICD-1, PGM-1, ADA, MDH-1, HADH, LA, LGG and G-6-PDH) in *C. exsanguis* are otherwise present only in *C. inornatus*. Furthermore, at least 1 allele at all remaining loci is present in both species. The allele distributions of 7 loci (PGM-1, ADA, MDH-1, HADH, LA, SOD and ACON) indicate that only 1 genome from *C. inornatus* could be present in *C. exsanguis*. Thus, it is reasonable to conclude that *C. inornatus* was 1 and only 1 of the parental species for *C. exsanguis*. If alleles for 1 genome are assigned to *C. inornatus*, the remaining diploid set of alleles can then be compared with those of the other species. Alleles at 4 loci (GOT-1, α-GPD-2, ADH and ACON) suggest that either *C. gularis* or *C. septemvittatus* was one of the other parents. The problable presence in *C. exsanguis* of the less common allele (a) at the LDH-1 locus of

Allele distributions at 19 polymorphic loci in *Cnemidophorus exsanguis* and its potential bisexual progenitors. Gene frequencies for alleles at polymorphic loci in the bisexual species are in parentheses. Lizard samples are e, *exsanguis;* c, *costatus;* i, *inornatus;* b, *b. burti;* st, *b. stictogrammus;* x, *b. xanthanotus;* se, *septemvittatus;* and g, *gularis.*

Locus	Population samples e	c	i	b	st	x	se	g
ICD-1	aab	a	a (0.23) b (0.73) c (0.04)	a	a	a	a	a
GOT-1	aab	b	a	b	b	b	a	a
PGM-1	aab	a	b (0.96) c (0.04)	a	a	a	a	a
MPI-1	bbb	b	b	a	a (0.02) b (0.98)	b	b	b
α-GDP-2	aab	a	a	a	a	a	b	b
ADA	bcd	b (0.05) c (0.95)	d	c	b (0.02) c (0.98)	c	a (0.25) b (0.75)	a (0.25) b (0.25) c (0.50)
MDH-1	bbc	b	c	b	a (0.02) b (0.98)	b	b	b
HADH	aab	a	b	a	a	a	a	a
GPI	bbb	b	b (0.96) c (0.04)	b	b	a	b	b
LA	cce	a (0.05) b (0.09) c (0.86)	e	c	b (0.02) c (0.76) d (0.22)	c	c	c
LGG	xxx[a]	e	b	e	a (0.02) d (0.04) e (0.94)	e	c	c
PAP	bbb	b (0.95) c (0.05)	b	b	a (0.04) b (0.94) c (0.02)	b	b	b
SOD	abb	b	a	b	b	b	b	a
EST-D	bbb	b (0.95) c (0.05)	a (0.04) b (0.96)	b	a (0.02) b (0.90) c (0.08)	b	b	b
LDH-1	xxx[a]	b	b	b	b	b	a (0.13) b (0.87)	
ADH	abb	a	b	a (0.75) b (0.25)	a	a (0.50) b (0.50)	b	b
ACON	acd	c	a (0.08) c (0.92)	b (0.25) c (0.75)	a (0.02) c (0.92) d (0.06)	b (0.75) c (0.25)	d	d
6-PGD	bbb	a (0.82) b (0.18)	b	a (0.38) b (0.62)	a (0.46) b (0.54)	b	b	b
G-6-PDH	xxx[a]	b	a	b	b (0.96) c (0.04)	b (0.25) c (0.75)	b	b

[a] These loci in *C. exsanguis* are heterozygous, but the alleles involved could not be assigned with full confidence.

C. septemvittatus and the absence of a second 'a' allele at the SOD locus that is fixed in both *C. gularis* and *C. inornatus* implicate *C. septemvittatus* as a second parental species. Thus, alleles at 6 loci favor the involvement of *C. septemvittatus* and there are no alleles in conflict with this hypothesis.

The array of alleles in the unassigned haploid genome is similar to those of the 4 remaining taxa (table). The absence of alleles in *C. exsanguis* that are fixed in *C. b. burti* (MPI-1) and *C. b. xanthanotus* (GPI) remove these taxa from primary contention. All alleles favor equally the involvement of *C. b. sticto-grammus* and *C. costatus* and provide no means of allowing a choice between the two. In an effort to resolve this question, we subjected tissue samples of the 2 taxa to a battery of electrophoretic techniques (modified buffer systems, acrylamide gels and isoelectric focusing) with no success and we are unable at this time to distinguish between the 2 populations on the basis of allozymes. Fortunately, preliminary data from restriction endonuclease analyses of mitochondrial DNA of these species are available[13]. These data indicate that *C. b. sticto-grammus* and *C. costatus* have quite different mtDNA and that *C. exsanguis* has the *C. costatus* mtDNA genome. Thus, it is reasonable to conclude that not only was *C. costatus* involved in the hybridization but that it was the maternal parent involved in the original hybridization with either *C. inornatus* or *C. septemvittatus*. The resulting diploid form is not known. Although it may never be determined, we suspect that *C. inornatus* was the species involved in the first hybridization (fig). There are other parthenogenetic populations that undoubtedly contain a *C. costatus* allozyme genome but have a *C. inornatus* mtDNA genome[13], which may represent reciprocal crosses to this one. *Cnemidophorus inornatus* and *C. costatus* do not now occur sympatrically[14], but they undoubtedly did in the not-too-distant past. It is known, for example, that the geographical range of *C. inornatus* has contracted considerably in just the last 100 years[15] and that, even in historical times, favorable habitat for *C. inornatus* was present in northeastern Sonora[16]. It may also be that the presence of 6 parthenogenetic species in the immediate area may have modified the distribution of these 2 bisexual species. Regardless, the resulting diploid parthenoform was present long enough to have hybridized with another species, *C. septemvittatus*. To have done this, it may have had an extensive geographical range.

In conclusion, the analysis of alleles at 19 polymorphic loci in *C. exsanguis* and 7 potential parental progenitors indicates that *C. exsanguis* is an allotriploid resulting from hybridization involving 3 species. These 3 species are *C. costatus* (♀), *C. inornatus* (♂) and *C. septemvittatus* (♂), respectively.

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Experiments on sexual isolation between Chilean and European strains of *Drosophila subobscura*

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Summary. Males and females of Chilean strains of *Drosophila subobscura* exhibit a pronounced tendency toward homogamic mating. This tendency shows a clear relation to the distance between the geographic localities from which the respective strains came. Nevertheless, when the Chilean flies are confronted with European strains, the ethological isolation is observed in some cases but not in others, depending on the geographic origin of the strains.
Key words. Drosophila subobscura; mating isolation; sexual isolation; behavior genetics.

Drosophila subobscura is a very recent colonizing species in Chile[2,3]. It was detected for the first time in 1978. Its distributional area now extends continuously from La Serena (Lat. 29° 54′ S), located in the semi-desert and temperate northern zone of Chile, to the extremely cold and windy conditions existing near Punta Arenas on the Strait of Magellan (Lat. 53° 40′ S). Recent experiments by the present authors have revealed rudiments of sexual isolation between different Chilean populations of *D. subobscura*. It is well known that mating behavior in the *Drosophila* genera are under the control of genetically determined factors, mainly polygenes, and it could be modified by selective pressures[4-6]. The objective of the present paper is to discuss the results of these studies, and to report the results of experiments on ethological isolation between Chilean and European strains of *D. subobscura*.

Materials and methods. We used 4 Chilean strains of *D. subobscura* which originated from a large number of flies collected about 1 year before the initiation of the study in the localities