saccharide moieties bound to the collagen.

The specimens for neutron diffraction were much larger than those for x-ray diffraction. Porcine intervertebral disks were used once more and sections of about 0.5 mm were cut so that the plane of the section was parallel to the vertebral column axis and the section itself was from a fixed radius. Sections were thus segments of thin cylinders, which were then flattened out and held between clamps in a cell. The specimens were immersed in a D_2O solution of 0.15*M* NaCl and the cell had quartz windows to allow the passage of neutrons through the specimen. Neutron diffraction was carried out on the D17 camera at the Institut Laue-Langevin. The diffraction pattern was recorded on multidetector of 128 × 128 elements, each element being in a 0.5 by 0.5 cm area. A contoured diffraction pattern is shown in Fig. 3, where the direction of the vertebral column is vertical. In Fig. 3 the first and the third through the sixth orders of the 670 Å collagen period may be clearly seen and are labeled by numbers. The second order is too weak to appear on the contour map. The direction of the line of reflections indicates that the collagen fibrils are oriented at about 70° to the direction of the vertebral column. A second, less distinct line of reflections in Fig. 3 reveals another population of collagen fibrils oriented again at 70° to the vertebral column but tilted in the opposite direction to the first set. Since the reflections in the first set are of similar intensity on both sides of the origin of the diffraction pattern, the collagen fibrils from which they originate must lie in the plane of the specimen. Since the specimen was cut at a fixed radius, these two sets of reflections originate from the radial lamellae of collagen fibers seen by optical polarization microscopy. Adjacent lamellae were seen by that method to be alternatively tilted in opposite senses from the vertebral column direction (*3*). The neutron diffraction pattern confirms that the collagen fibrils follow the direction of the optically visible fibers. The intensities of the reflections in Fig. 3 are strikingly different from those in the neutron diffraction pattern from rat tail tendon in a D_2O solution of 0.15*M* NaCl (*9*), particularly in the fifth order, which is considerably stronger in the pattern from annulus fibrosus.

These preliminary neutron diffraction results are consistent with the conclusion based on the x-ray diffraction pattern, namely that the differences between the diffraction patterns from tendon and annulus fibrosus either reflect a difference in molecular length or indicate that noncollagenous materials in the fibrocartilaginous tissue are regularly attached to the collagen. The latter possibility is being tested by the contrast variation method for neutron diffraction data [for example, see (*10*)], and we are also testing whether the dissected fibers used as specimens for x-ray diffraction are markedly different in chemistry and structure from the matrix in which they are embedded and whether differences are observable between fibers at different radial positions in the annulus fibrosus.

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Coexistence of Clones in a Heterogeneous Environment

Abstract. *Two genetically distinct clones of the asexual triploid fish* Poeciliopsis 2 monacha-lucida *inhabit the Rio del Fuerte of northwestern Mexico. Their coexistence apparently depends on feeding specializations that result in partitioning of the limited food resources in the desert streams. The findings suggest that these asexual organisms have sufficient clonal diversity to occupy a broad, heterogeneous, adaptive zone.*

The absence of genetic recombination in asexual organisms is generally thought to result in a slow rate of evolution and a high rate of extinction (*1*). Presumably, the genetic variability contained in sexual populations allows them to respond to diverse environmental conditions, to expand their adaptive zone, and thereby to decrease their likelihood of extinction (*2*). Recent genetic studies have revealed that naturally occurring asexual populations of weevils (*3*), fishes (*4–6*), amphibians (*7*), and lizards (*8*) contain considerable variation in the form of multiple sympatric clones. Clonal diversity in an asexual population reflects the balance between those forces that generate new clones from other asexual or sexual ancestors and those factors that cause clonal extinction (*5*). In this report I describe two clones of all-female fish in the genus *Poeciliopsis* (Poeciliidae) and provide evidence that coexistence of clones within an asexual population is associated with partitioning of the natural resources.

The triploid "species," *P. 2 monacha-lucida*, arose by hybridization between the sexual species, *P. monacha* and *P. lucida*; its name reflects the fact that it contains two sets of *monacha* chromosomes and one set from *lucida* (*9*). The all-female populations reproduce gynogenetically; sperm from males of *P. monacha* are required to stimulate development of the triploid eggs, but the sperm contribute nothing to the genotypes or phenotypes of the progeny (*10*). Cytogenetic, immunogenetic, and electrophoretic studies revealed that the all-female progeny are identical to their mothers (*6, 11*). Their lineage constitutes a true clone. Three distinct clones were identified by electrophoretic studies in which the products of 23 gene loci were used (*12*). Clones 1 and 2 coexist in the Rio del Fuerte, Sonora, Mexico, and clone 3 occurs alone in the Rio Mayo, a river system north of the Rio del Fuerte. Tissue-grafting studies confirmed the genetic distinctiveness of clones 1 and 2; clone 3 remains to be tested (*13*).

The sperm-dependent, all-female clones and their sexual host, *P. monacha*, inhabit the unpredictable headwater streams that drain into the Sonoran desert. During the annual dry season, streams often dry up and local extinctions occur (*14*). Surviving populations are densely crowded into small

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pools surrounding isolated natural springs, where competition for food presumably is severe. Samples of *P. monacha* and the triploid clones, collected during the dry season of March 1976 (*15*), were examined for their stomach contents (Table 1). A major part of *P. monacha*'s diet was insects and snails, an observation that is consistent with its predacious behavior in the laboratory (*16*). The two triploid clones were narrower in their food diversity, having eaten mostly algae and some small invertebrates. Significant dietary differences between the two clones were not evident in this crude examination of the percentage occurrence of food categories. A detailed study of the relative volumes of food items is more likely to reveal significant differences, however, since clones 1 and 2 forage differently in the laboratory. When commercially prepared fish foods are withheld, clone 1 individuals primarily engage in scraping algae from rocks. They often defend an area around a rock from intruders of the same or a different clone. Clone 2 individuals browse within the floating filamentous algae and in the detritus that accumulates in aquaria.

The differences in feeding behaviors are clearly reflected in the patterns of dentition in fish caught in the wild (Fig. 1). The dentary bone in clone 1, these fish being scrapers, is densely covered with numerous tricuspid inner teeth. Scanning electron microscopy revealed that the cusps of these minute teeth are often worn or broken. Clone 2, being browsers, tend to have about half as many of these teeth (arranged in four evenly spaced rows and not as worn down) as do the scrapers. A strong correlation between dental morphologies and feeding behaviors has been demonstrated for other fish (*17*).

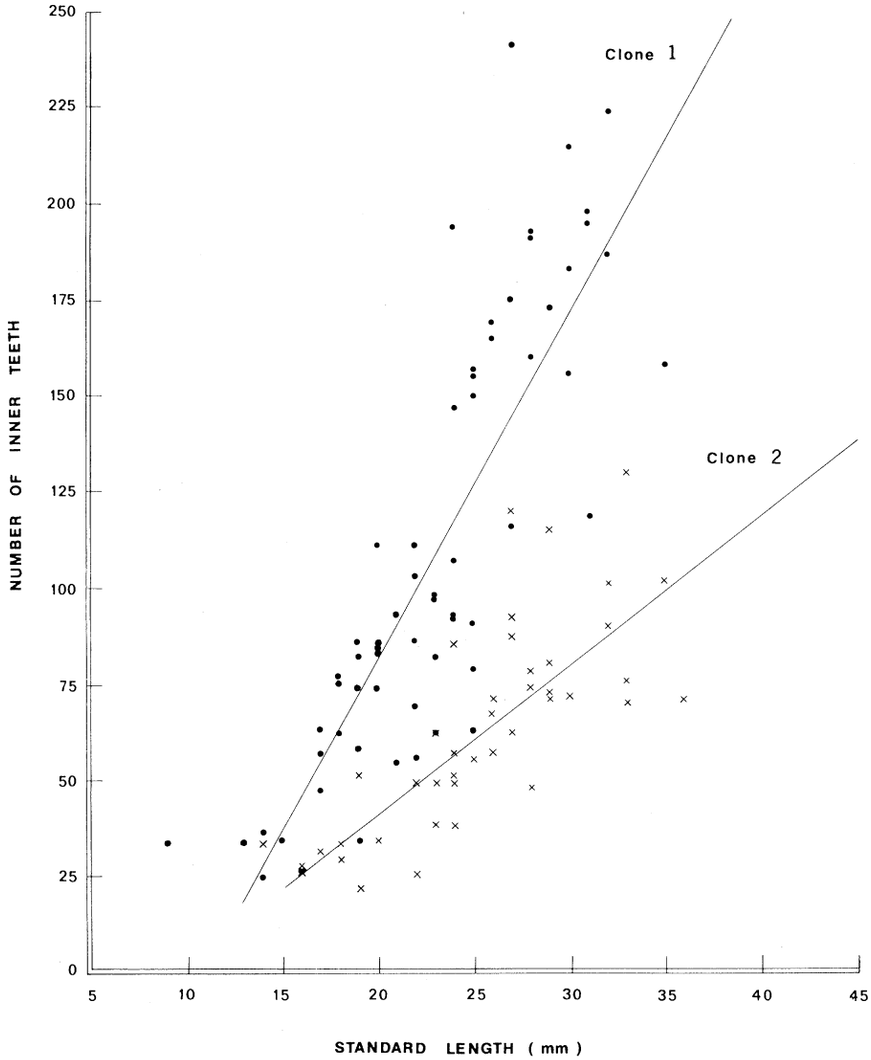

Fig. 1. Regression lines for the number of inner teeth versus standard length in clones 1 and 2. The slopes are significantly different (Student's t-test = 4.68; d.f. = 98; $P < .01$). Individual fish were identified electrophoretically for their clone and their teeth were counted. Samples used were combined from several localities shown in Fig. 2.

The relative frequencies of *P. monacha* females (*18*) as opposed to clones 1 and 2 correlated closely with the abundance of food resources in the environment (Fig. 2). Clone 2, the browsers, were most frequent in productive stream habitats. Sites *CA*, *TA*, and *AC* (Fig. 2) are broad, sun-drenched streams with sand and gravel bottoms. These habitats contained considerable floating algae and detritus. Much of the productivity is due to enrichment of the stream by manure from livestock which graze nearby and drink from the streams. The frequencies of clone 2 diminish greatly in the rocky arroyos (*PL*, *NA*, and *AT*). These shaded bedrock pools contain little floating vegetation. Leaf litter from the surrounding trees and shrubs provides the major input of nutrients. As detritus accumulates downstream in the deeper pools of the Arroyo de los Platanos (*PL*), the frequency of clone 2 increases whereas the frequency of *monacha* females decreases. The predominance of *monacha* females in upstream portions of these austere arroyos corresponds well with their demonstrated ability to withstand starvation (*19*).

The feeding specialty of clone 1 might explain its relatively constant frequencies in both austere and enriched habitats. Its numbers may be limited by the surface area available for scraping. This factor is a function of pool size, which correspondingly might limit the relative numbers of clone 2 and *monacha*. Unfortunately, the frequencies measured in this study provide no reliable information on population densities in austere as opposed to enriched habitats. Studies that follow the rise and fall of these populations throughout the wet-dry seasonal cycle are needed since other factors, such as differential rates of increase and mortality, also may be significant in clonal coexistence.

Could *P. 2 monacha-lucida* have had a single hybrid origin followed by splitting and divergence of clones? It is possible that the additive genetic component of complex traits, such as dentition and feeding behavior in a triploid hybrid fish, provides enough material for mutations and gene rearrangements to promote rapid clonal divergence without conventional genetic recombination. Although immunogenetic studies demonstrated that recombination does not occur in clones 1 and 2, they cannot exclude the possibility that recombinations occurred prior to the origins of the now stabilized genotypes (*6*). On the other hand, it is also possible that clones 1 and 2 arose independently, and have undergone no adaptive genetic change since their ori-

gins. Their dentitional and behavioral differences would then reflect the capture of distinct genomes from a variable diploid gene pool. Thus, clones 1 and 2 may represent a small, but highly selected, subset of clones that arose independently. The electrophoretic identification of these genotypes does not enable us to discriminate between these two hypotheses (*12*).

Although the annual wet-dry cycle of northwestern Mexico is a regular event, the severity and duration of droughts are unpredictable. Nevertheless, the gynogenetic species *P. 2 monacha-lucida* apparently is well adapted to this environment. Its numbers often equal and sometimes exceed those of the sexual species

Table 1. Analysis of stomach contents in *P. monacha* females and clones 1 and 2 of *P. 2 monacha-lucida* from the Arroyo Tarahumara (*TA*). Because these fish are very small as adults (25 to 44 mm), no attempt was made to quantify the contents of each stomach. The percentages represent the proportion of individuals containing each kind of food. The percentages sum to greater than 100 percent because individuals usually contained several kinds of food. A significant heterogeneity in the kinds of food taken (χ^2 = 53.2; d.f. = 8, owing to grouping of fish, snail, and seed categories; $P < .01$) was primarily due to the difference between *monacha* and the two triploid clones, which were not significantly different from one another (χ^2 = 8.8; d.f. = 4; $P > .05$). Statistical tests were performed with raw numbers of observations rather than the percentages as listed.

Species or clone	Number of specimens	Percentage of fish containing each kind of food						
		Algae*		Insect	Fish†	Snail	Seed	Small invertebrates‡
		Filamentous	Other					
P. monacha	16	88	31	63	13	31	25	19
Clone 1	18	83	100	0	0	0	0	55
Clone 2	22	100	73	9	0	0	0	9

*The algae are divided into filamentous and nonfilamentous forms including diatoms, *Pandorina*, and *Merismopedia*. †Identified by the presence of scales in the gut. ‡Invertebrate microfauna including nematodes and ostracods, for example.

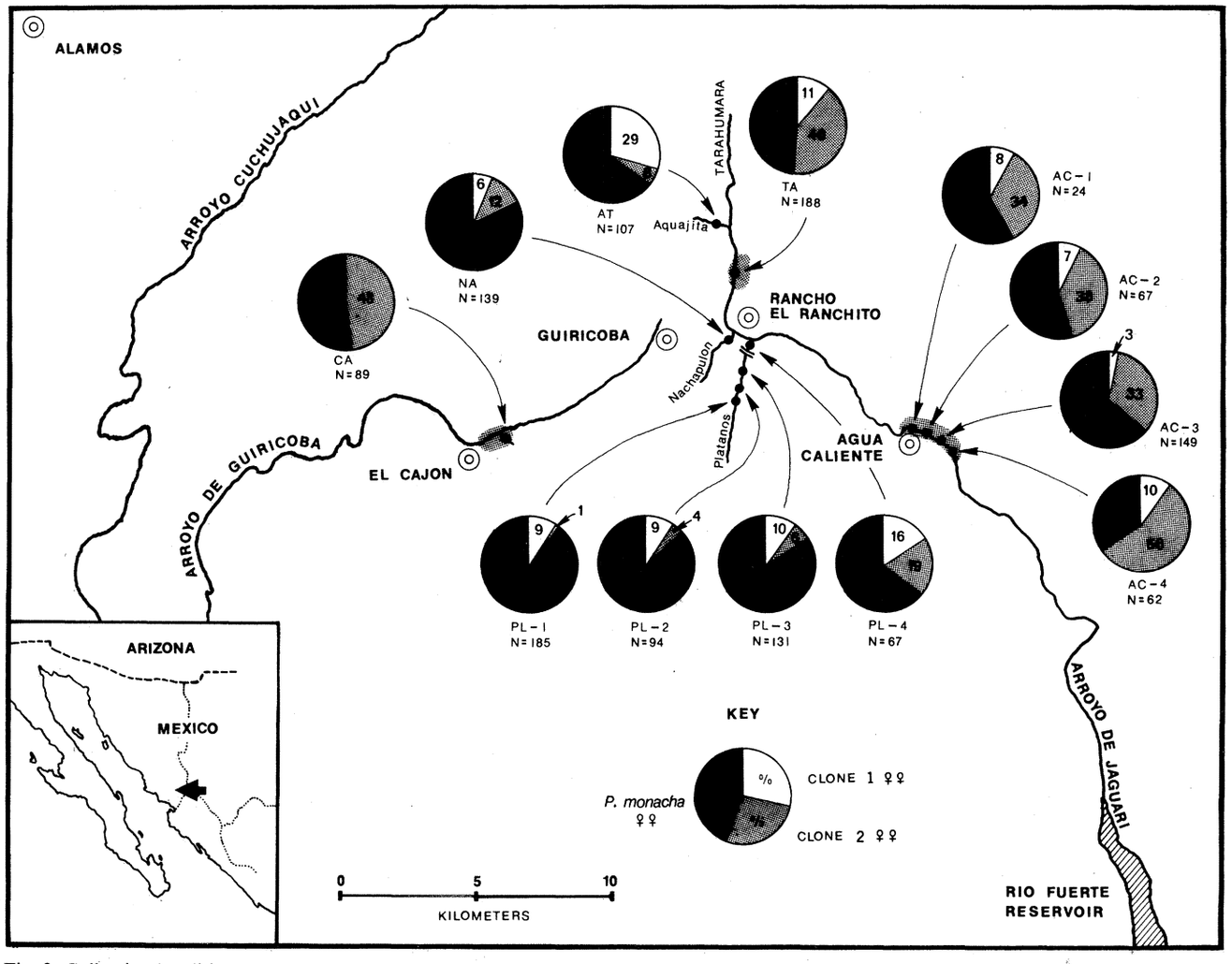

Fig. 2. Collection localities and the relative frequencies of clones 1 and 2 and *monacha* females in the Rio del Fuerte. Shaded areas on the map indicate portions of streams often enriched by manure from livestock. The numbers (*N*) below each pie diagram are the sample sizes included in estimating the frequencies of members of the *monacha* complex. The abbreviations *CA*, Cajon; *NA*, Nachapulon; *AT*, Aguajita; *TA*, Tarahumara; *PL*, Platanos; and *AC*, Agua Caliente, designate collection localities.

P. monacha, upon which it depends for sperm. Theoretically, unisexual individuals produce two female offspring for each one produced by a sexual individual, but this reproductive advantage is offset by a strong mating preference on the part of *P. monacha* males for conspecific females (*20*). Mathematical models were developed that showed how the interplay of these counteracting forces could permit a dynamic coexistence between the sexual host and its unisexual parasite (*21*). These models are unrealistic, however, because they assume that *P. 2 monacha-lucida* and *P. monacha* compete directly for limiting resources. Although females of the two forms may compete for sperm when males of *P. monacha* are rare, the unisexual and sexual females differ considerably in their utilization of food resources. Furthermore, it is erroneous to treat *P. 2 monacha-lucida* as if it were a single ecological entity. Although the exact origins of clones 1 and 2 are unknown, they are genetically and ecologically distinct. Nevertheless, from the present findings it is apparent that their continued coexistence, with one another and with *P. monacha*, depends in part on their ability to differentially exploit food resources in the heterogeneous environments of these desert streams.

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Limitation of Excessive Myelopoiesis by the Intrinsic Modulation of Macrophage-Derived Prostaglandin E

Abstract. *The clonal proliferation of the committed granulocyte-macrophage stem cell is controlled by a balance between mutually opposing factors, colony stimulating factor and prostaglandin E, both of monocyte-macrophage derivation. Increases beyond a critical concentration of colony stimulating factor within the local milieu of the mononuclear phagocyte induces the coincident elaboration of prostaglandin E, a self-regulated response which serves to limit the unopposed humoral stimulation of myelopoiesis.*

Simple cloning methods in semisolid medium which permit the selective in vitro proliferation of a particular population of committed stem cells have facilitated investigations into the cellular and molecular events controlling the proliferation and differentiation of hematopoietic cells. The bipotentially committed granulocyte-macrophage stem cell (colony forming unit-culture, CFU-C) can be detected by its ability to undergo clonal proliferation in soft agar medium when provided with stimulatory macromolecules (colony stimulating factor; CSF) (*1, 2*). Colony stimulating factor is active in vitro at extremely low concentrations (*2*) and does not stimulate the growth of other hematopoietic and nonhematopoietic cells (*3, 4*). Since CSF is detected in normal serums (*5*) and is generally increased in situations where there is increased granulopoiesis and monocyte-macrophage production (*3*), the humoral regulatory role of CSF in vivo may be analogous to that of erythropoietin. Thus, after the injection of antigens and bacterial endotoxin, during acute viral and bacterial infections, as well as preceding and during active myelopoietic regeneration following sublethal irradiation or treatment with cyclophosphamide, serum CSF concentrations are markedly increased (*6, 7*). Conversely, serum CSF levels are lower in germfree mice in which granulopoiesis is subnormal (*7, 8*). The principal CSF-producing cells, which have been identified as the blood monocyte and tissue macrophage (*9–11*), retain the ability to respond in vitro to endotoxin and markedly increase their production and release of CSF (*11*). Thus, granulopoiesis and monocyte-macrophage formation may be stimulated by a positive feedback mechanism involving CSF, which if not otherwise limited may result in accelerated myelopoiesis and further recruitment of CSF-producing cells. We reported previously (*4*) that the stimulatory actions of CSF on the committed stem cell CFU-C can be effectively limited by the synthetic E-series prostaglandins (PGE_1 and PGE_2). Conversely, increasing CSF concentrations counteract the PGE-mediated inhibition of CFU-C (*4*), indicating a dualistic modulation of committed stem cell proliferation. Here we extend these earlier, solely pharmacological observations of the CSF-PGE dualism into a self-regulating model of granulopoiesis and monocyte-macrophage production, which is controlled by the mononuclear phagocyte.

Soft agar cultures of normal human bone marrow cells were prepared as described (*4, 10*). Briefly, normal human bone marrow was separated on the basis of differential buoyant cell density by centrifugation in bovine serum albumin (density 1.070 g/cm^3) and active adherence to plastic culture dishes. The light density (< 1.070 g/cm^3) and nonadherent cells were suspended at a nucleated cell concentration of 1.5×10^5 cells per milliliter in McCoy's 5A modified medium containing 0.3 percent Bacto agar and supplemented with 15 percent fetal calf serum, essential and nonessential amino acids, vitamins, and sodium pyruvate. The bone marrow cell–agar suspensions were dispensed into tissue culture dishes (35 mm in diameter) and allowed to gel. After 10 days of incubation at 37°C in a humidified atmosphere of 10 percent CO_2 in air, the dishes were scored for the presence of colonies containing greater

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