These cells are designated PTK_2C567 and PTK_2C568.

Stock populations of PTK_2 cells are maintained in standard culture flasks, in minimal essential Eagle's medium (MEM) with Hanks balanced salts, penicillin and streptomycin, 10 percent fetal calf serum, and buffered with $NaHCO_3$ (0.85 g/liter). The stock cultures are determined free of mycoplasma by standard cell plating on mycoplasma agar (Gibco No. 804 AP). Twenty-four to 48 hours prior to microbeam irradiation, cells are trypsinized from stock flasks, resuspended in culture medium, and lightly seeded into Rose multipurpose culture chambers. Cells in early to middle anaphase are selected for irradiation. The argon laser microbeam is identical to the one described in numerous publications (*4*, *5*). A videotape time-lapse system attached to the microscope provides continual documentation. Cell PTK_2C567 was irradiated on the long arm of one of its larger chromosomes, and cell PTK_2C568 was irradiated on one of its smaller chromosomes. In both of these experiments we did not attempt to follow the cell from preirradiation prophase until the time of irradiation; therefore, positive identification of the irradiated chromosome was not possible. Both irradiations resulted in typical phase "paling" reactions of the irradiated region.

Subsequent isolation and cloning procedures are identical for both cells. Immediately after irradiation and photography a small circle is drawn around the irradiated cell on the outer cover slip of the Rose chamber. The chamber is removed from the microbeam system and placed on a Nikon inverted microscope that is mounted inside a sterile Edgeguard laminar flow hood. All subsequent procedures are performed with aseptic technique. The top plate of the Rose chamber is carefully removed, thus exposing the culture medium with underlying cells and cover slip. The irradiated cell is relocated through the inverted microscope and final identification is made by comparison with the videotape playback image.

After relocation of the irradiated cell, all the adjacent cells are dissected away by using a De Fon Brune micromanipulator mounted on the stage of the inverted microscope. With a finely pulled sterile glass needle (1 to 2 μm outer tip diameter), the cells in contact with the irradiated cells, and within about a 1- to 3-mm radius of them, are gently pulled way. Next, a clean sterile cover slip is placed over the gasket, and the Rose chamber is reassembled. Additional culture medium supplemented with fetal calf serum (20 percent), nonessential amino acids (0.1 m*M*), and glutamine (2 m*M*) is injected into the chamber, which is placed in an incubator at 37°C. The irradiated cell is carefully followed and photographed over a period of several days. It is usually necessary to open the chamber once or twice during this period to dissect away cells that have migrated into the vicinity of the irradiated cell population. When a population of at least 300 cells is attained, the clone is transferred to a T30 plastic flask. This is accomplished by opening the Rose chamber, removing most of the culture medium with a sterile syringe, and carefully lowering a sterile metal cylinder over the cells. The bottom of the cylinder is lightly coated with sterile stopcock grease so that it forms a good seal with the bottom cover glass of the culture chamber. One half to 1.0 ml of 0.125 percent trypsin is placed within the cylinder over the cells. The cells are continuously viewed through the inverted scope, and when free of the glass surface, they are sucked into a sterile syringe and placed in a sterile culture flask. A larger (4 ml) volume of normal MEM with *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES) buffer (2.38 g/liter) is placed in the flask. Since the cells are not kept in a CO_2 incubator, HEPES buffer is needed to maintain a stable *p*H of the medium surrounding the small population of cells.

Table 1. Number of irradiated cells at various times postirradiation. After 32 days the clones were trypsinized and transferred to T30 culture flasks. At 64 days clones were growing in the flasks.

Cell identification No.	Days postirradiation				
	2	4	8	16	32
PTK_2C567	1	3	4	28	> 300
PTK_2C568	1	1	6	33	> 300

Table 1 summarizes the data for the two cells and the derived clones. It is clear from these data that cells can be cloned and established as viable populations after the deletion of DNA from a selected chromosome segment. The procedure opens the way for the directed mapping of genes to specific chromosomes and chromosome regions, and establishment of new genetic markers in somatic cell cultures.

Future work must firmly establish the parameters of irradiation and cell culture so that this rather intricate procedure will be easily repeatable and as routine as possible. In addition, careful analysis must be performed to determine the subsequent fate of the deleted chromosome region in the clonal population. Further work is needed to establish clonal populations of cells made deficient in various proportions of ribosomal (nucleolar) genes.

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Rana pipiens Complex: Hemoglobin Phenotypes of Sympatric and Allopatric Populations in Arizona

Abstract. *Electrophoretic comparison of hemoglobin samples from numerous populations of* Rana pipiens *in Arizona reveals three distinct phenotypes that closely correlate with morphological differences. Hemoglobin samples from sympatric locations contain parental phenotypes with only the occasional occurrence of a hybrid. These data support the contention that the* Rana pipiens *complex consists of several species.*

There is substantial evidence suggesting that the *Rana pipiens* complex is not one highly variable species as presently treated, but actually represents a number of primarily allopatric species. Mating calls, differences in morphology, and electrophoretic patterns all change abruptly over short

geographic distances (*1*, *2*). Our research in Arizona confirms an earlier report (*2*) of two distinct forms of *R. pipiens* occurring sympatrically in the White Mountains of eastern Arizona. We also report for the first time evidence of a third form of *R. pipiens* in Arizona.

Hemoglobin samples were taken from 598 specimens from 33 collecting sites in Arizona (Table 1). Morphological data were noted, and specimens were preserved and tagged. Blood from a ventricular nick was collected in capillary tubes containing heparin and centrifuged immediately to separate red blood cells from serum. Cells were subsequently washed three times in 20 times their volume of 0.9 percent NaCl solution and then hemolyzed in twice the sample volume of distilled water. Starch gels (13 percent starch) and EBT buffer (*3*) at *p*H 8.6 were used for horizontal electrophoresis at 22° to 23°C with fan cooling for 5 hours at 16 volt/cm at 12 to 14 ma (d-c). Samples were stained in 1 percent Amido Schwarz in destaining solution (water : methanol : acetic acid, 5 : 5 : 1) for 5 minutes and then destained overnight. The resulting five phenotypes and a map of their distribution are shown in Fig. 1.

Two of the hemoglobin phenotypes are well correlated with the morphological forms designated by Mecham (*2*) as "Northern" and "Southern." Allopatric samples of both of these forms were monomorphic for the respective hemoglobin phenotypes (inset in Fig. 1). Gel data from the Northern-Southern sympatric populations revealed 31 Northern, 41 Southern, and 2 hybrid individuals (NSH type). The level of hybridization (2/73) is slightly lower than Mecham's (*2*) estimate (1/22) based on morphological features. This low level is significant, because laboratory crosses between Northern and Southern type individuals have produced high percentages of embryos that develop normally (*2*).

The third morphological form found in Arizona is widely distributed in areas of low elevation (*4*). We designate this type "Lowland." Allopatric samples from Lowland populations were also monomorphic for hemoglobin (*5*) (inset in Fig. 1). Collecting at intermediate elevations (around 1150 m) yielded three samples containing both Lowland and Southern phenotypes and included 71 Lowland, 22 Southern, and 3 hybrid individuals (LSH type).

Northern and Southern type individuals are readily identified on the basis of morphological differences reported by Mecham (*2*) which will not be repeated here. The Lowland form is morphologically very similar to the Southern type. Both have dorsolateral ridges that are displaced medially in the region of the urostyle, an incomplete lip stripe (*6*), and a variable degree of mottling in the region of the chin. Lowland type males lack vestigial oviducts, and Southern type males either lack oviducts or possess only slightly developed ones. A yellow patch on the ventral surface of the upper thigh and adjoining belly is common to both, as is the tendency toward a slightly tuberculate dorsum. Dorsal spot number is variable in both, but the Lowland type tends to have fewer spots. Dorsal coloration in the Lowland type is light gray, gray-green, or gray-brown, in contrast to the lime to olive-green color of the Southern type. Call type in the Lowland form is strikingly different from both the Northern or Southern type (*7*). Egg masses of Lowland types were observed in the field in 1970 and 1971 in late March and early April and

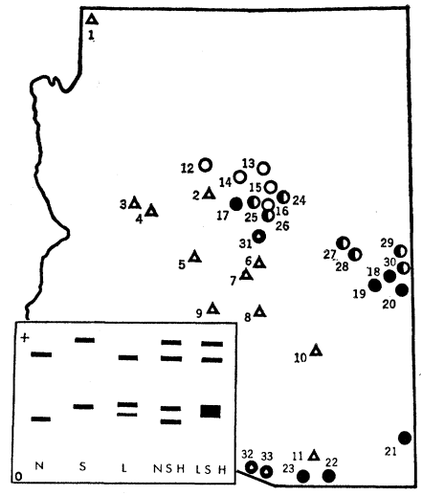

Fig. 1. Distribution map of populations sampled for hemoglobin phenotypes (inset) within the *Rana pipiens* complex in Arizona. Allopatric populations are Lowland type (open triangles), Northern type (open circles), and Southern type (solid circles). Sympatric populations between Northern and Southern types (split circles) and Lowland and Southern types (solid circles with superimposed open triangles) are also shown. The inset shows phenotypes apparent after starch-gel electrophoresis of hemoglobin of Northern type (*N*), Southern type (*S*), a Northern-Southern hybrid (*NSH*), Lowland type (*L*), and a Lowland-Southern hybrid (*LSH*) from Arizona; *O*, origin.

Table 1. Collection sites for *Rana pipiens* samples in Arizona.

No.	Locality	Latitude	Longitude	Sample size
1	Virgin River	36°54′	113°55′	2
2	Josephines Tunnel	34°45′	112°05′	30
3	Burro Creek	34°34′	111°35′	3
4	Santa Maria River	34°22′	113°11′	10
5	Tule Creek	34°00′	112°16′	45
6	Sunflower	33°52′	111°27′	19
7	Sugarloaf Mountain	33°42′	111°32′	30
8	Second Water	33°29′	111°24′	2
9	Tempe	33°26′	111°55′	5
10	Aravaipa Creek	32°55′	110°31′	3
11	Babocomari River	31°38′	110°27′	3
12	Perkins Tank	35°07′	112°04′	30
13	Ashurst Lake	35°01′	112°24′	29
14	Foxbord Lake	34°54′	111°40′	31
15	Daves Tank	34°44′	111°17′	28
16	Clint Wells	34°33′	111°19′	15
17	New Tank	34°32′	111°29′	32
18	Pacheta Creek	33°50′	109°32′	4
19	Tonto Lake	33°42′	109°36′	26
20	Highway 666	33°44′	109°12′	3
21	Herb Martyr Dam	31°52′	109°14′	32
22	Bear Canyon	31°23′	110°22′	4
23	Santa Cruz River	31°21′	110°35′	31
24	96 Crossing	34°33′	111°09′	3
25	Maxwell Tank	34°35′	111°24′	9
26	Jones Crossing	34°32′	111°17′	5
27	Lakeside	34°09′	109°59′	2
28	Bog Creek	34°05′	109°42′	3
29	Nelson Reservoir	34°03′	109°11′	24
30	San Francisco River	33°45′	109°04′	42
31	Dillers Pond	34°20′	111°21′	41
32	California Gulch	31°27′	111°15′	43
33	Sycamore Canyon	31°26′	111°11′	12

again in early October, whereas the Southern type spawns predominantly in June and July.

The evidence suggests that three distinct forms of *R. pipiens* occur in Arizona. All three bear consistently different hemoglobin phenotypes which are in turn correlated with morphological differences; in cases of geographic replacement of one form with another, the transition is discontinuous with respect to these features. Furthermore, the small number of hybrids detected in regions where two forms are sympatric indicate either that mating is preferential for the conspecific type or that, if mating is random, hybrid survival is very low.

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3. EBT buffer: 0.001*M* ethylenediaminetetraacetic acid, 0.025*M* boric acid, 0.045*M* tris(hydroxymethyl)aminomethane [S. H. Boyer, D. C. Fanier, M. A. Naughton, *Science* **140**, 1228 (1963)].
4. The Lowland type is widely distributed in Arizona at elevations between 300 and 1100 m, with one population at approximately 1600 m.
5. The hemoglobin band closest to the cathode (Fig. 1) is probably due to dimerization and is not seen in Lowland samples first treated with β-mercaptoethanol.
6. The Northern type has a white narrow stripe along the upper lip, from a point a short distance posterior to the angle of the jaw to the tip of the snout. In both the Lowland and Southern types any narrow pigmented lip stripe extends anteriorly no further than a point below the eye.
7. All three forms have been heard calling during fieldwork and can be readily differentiated by ear alone.

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Electrogenic Sodium Pump and High Specific Resistance in Nerve Cell Bodies of the Squid

Abstract. *An electrogenic sodium pump contributes to the membrane potential in squid nerve cell bodies, imparting a temperature dependence to the resting potential that is abolished by strophanthidin. The existence of a potential produced by the pump in the soma but not the axon is correlated with a higher membrane resistance in the soma. Thus, membranes from different parts of a neuron may have functionally significant differences in resistance.*

The giant stellar axon of the squid arises by a fusion of the processes of several hundred small nerve cell bodies (*1*). Although the squid axon has been a classic preparation for the study of the ionic events underlying nervous electrical activity there has been little interest in the cell bodies, since the somata are not electrically excitable and do not form synaptic contacts (*2*).

There are, however, some interesting questions concerning the relative electrical properties of somata and axons that can be examined in these cells. In several molluscan nerve cell bodies an electrogenic Na^+ pump is responsible for generation of a sizable fraction of the resting membrane potential and imparts a temperature dependence to the resting membrane potential that is much greater than would be predicted from the passive electrical properties of the membrane (*3*). In the squid axon the resting membrane potential is not determined by an electrogenic pump (*4*) and is not temperature dependent to any marked extent (*5*). It has been proposed that the difference between the molluscan nerve cell bodies and squid axon is not in the character or rate of the Na^+ pump but rather is due to a greater specific membrane resistance, R_m, in the somata (*6*). Whereas R_m for squid axon is about 1000 ohm cm^2 and the membrane time constant, τ, is about 1 msec (*7*), in both *Aplysia* (*6*) and *Anisodoris* (*8*) somata R_m is higher by a factor of about 100 and τ has a value that may be as great as several hundred milliseconds.

Intracellular recordings were made from the cell bodies of neurons in the stellate ganglion of the squid, *Loligo pealii*, with glass micropipettes filled with 3*M* KCl and having resistances of 15 to 30 megohms. Most recording techniques were as in (*6*). The reference electrode consisted of a heavily and freshly chlorided silver wire. Junction and tip potentials were kept as small as possible and the temperature dependence of the d-c level when the electrode was in seawater was always monitored. Ganglia were dissected under flowing seawater with entering and exiting nerve bundles tightly tied. The ganglion was pinned to a soft resin in a Lucite chamber and perfused with oxygenated seawater. Temperature was changed by flowing the perfusate through an ice bath and was monitored through a small thermistor near the ganglion.

A total of 34 cells were studied in some detail. Many other cells were penetrated but were injured so badly that useful information was not obtained. The cell bodies studied were between 50 to 150 μm in diameter; those of the giant fiber lobe tended to be considerably smaller than many cells found in other parts of the ganglion. Resting membrane potentials ranged from 30 to 60 mv (average 43 mv). In agreement with Miledi (*2*) most cells were electrically silent and on depolarizing current pulses would show only very small all-or-nothing action potentials (2 to 15 mv). Occasionally cells were found that showed small spikes after penetration, presumably as a result of the injury. These small spikes probably result from electrotonic propagation of potentials originating in an axonal process (*2*).

Figure 1 shows results of one experiment with a recording from a cell not in the giant fiber lobe. In normal seawater the resting membrane potential reversibly increased by 10 mv when the temperature was increased from 5° to 20°C. This increase in the resting membrane potential was abolished and even reversed after exposure to strophanthidin, where now there is a 5-mv depolarization with a similar temperature change. These results suggest that strophanthidin abolishes a current which generates a potential that is the sum of the hyperpolarization seen in the control and the depolarization seen in presence of strophanthidin. In this case the total potential generated is 15 mv for a 15°C temperature change. We conclude that an electrogenic Na^+ pump contributes to the resting membrane potential in squid somata as in a variety of other molluscan nerve cell bodies (*3*). The depolarization on warming in the presence of the glycoside is also similar to what is seen in other preparations and probably reflects a greater temperature dependence of the passive Na^+ than K^+ conductance (*6*, *9*).

The greater role of the Na^+ pump in generation of the resting membrane potential in squid somata than in axons might reflect a greater pump rate, a different pump mechanism such as a larger coupling ratio of Na^+ to K^+ transported, or simply a greater mem-