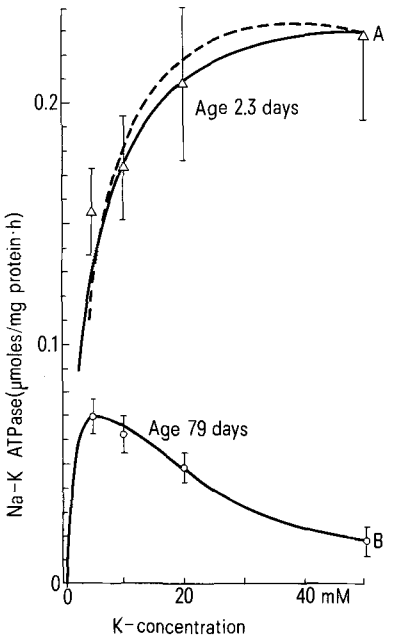
obtained, with the constants given in the legend, using the equation[4,10]

$$\text{observed } v = \frac{v_{max}}{\left(1+\frac{K^a_K}{[K]}\right)^2\left[1+\frac{K^a_{Na}}{[Na]}\left(1+\frac{[K]}{K^i_K}\right)\right]^3} \quad (1)$$

('observed v' is the rate at saturating ATP concentration, K^a_K is the dissociation constant for K at the activating (external) site at 150 mM Na and K^i_K that at the inhibitory (internal) site, K^a_{Na} the dissociation constant for Na at the activating (internal) site and v_{max} the maximal rate).

Using equation (1) to fit the points in curve B seems justified because there is good evidence that the stoichiometry in ruminant red cells is 3 Na:2 K[4,9,10]. For the sake of consistent treatment it was also applied to the data at birth (curve A) although these could be fitted equally well or even better by a rectangular hyperbola. The stoichiometry has not been assessed in young ruminants. K^a_K for curve B was taken = 1 mM in the presence of 150 mM Na, as measured by Ellory and Carleton[9] in adult cows and K^a_{Na} = 20 mM, similar to what was found in goat red cells[4,10]. There is no unique way of transforming curve A into curve B by changing the constants in equation (1). This is exemplified by the 2 approximations shown, one obtained by increasing K^i_K and the other by decreasing K^a_{Na}. However, two points seem clear: a) The ration K^a_{Na}/K^i_K must be > 1 in B[7] and < 1 in A. b) More than one of the 3 dissociation constants must be altered and v_{max} must be decreased when the behavior seen in curve A yields to the behavior seen in curve B.

The qualitative result thus is that during the first weeks in the life of cattle the affinity for K relative to that of Na at the internal metal binding site of the red cell Na-K pump increases, v_{max} decreases moderately (be it by way of a decrease in the number of pumps per cell or by a reduction in the turnover rate) and the affinity for K at the external metal binding site may increase slightly. The rise in K^a_{Na}/K^i_K seems to be more important than the fall in v_{max} for the drop in cellular K concentration observed during maturation of the animal.



Ouabain sensitive ATPase (Na-K pump ATPase) activity of disrupted membranes from red cells of the same 7 cattle at 2.3 days (curve A) and 78.7 days of age (curve B) at variable K concentration. Points: mean of 7 animals ± 1 SE. Medium (mM): NaCl 150, tris-Cl 10 (pH 7.7 at 37 °C), $MgCl_2$ 1.25, EGTA 0.5, ATP 1.25, (K + choline-Cl) 100, with or without ouabain 0.17[8]. Membrane density corresponding to 0.25 haematocrit of original cells. Sample volume 1.5 ml. Incubation 1 h at 37 °C. Start by adding Na_2ATP (Boehringer), stop by adding 0.5 ml trichloroacetic acid 20%. Phosphate liberated was determined according to Martin et al.[14], protein according to Lowry et al.[15]. Curves calculated according to equation (1). A, Solid line: v_{max} = 0.37 μmole/1 cells · h, K^a_{Na} = 20 mM, K^a_K = 2 mM, K^i_K = 1000 mM; broken line: v_{max} = 0.37 μmole/1 cells · h, K^a_{Na} = 2 mM, K^a_K = 4 mM, K^i_K = 6.5 mM. B: v_{max} = 0.19 μmole/1 cells · h, K^a_{Na} = 20 mM, K^a_K = 1 mM, K^i_K = 6.5 mM.

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mtDNA heterogeneity in *Panulirus argus*[1]

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Summary. Restriction endonuclease analysis of mtDNA polymorphisms in *Panulirus argus* has revealed significant heterogeneity and possible species subdivision.

The techniques of both Mendelian genetics and molecular biology have been utilized to quantify intraspecific and interspecific genetic heterogeneity in a variety of organisms[3-6]. Although nucleotide sequences yield maximal information, determination of sequence heterogeneity among large numbers of individuals is not yet feasible. On the other hand, restriction endonuclease analysis permits the indexing of sequence differences, and these techniques may

Digestion phenotypes observed in samples of *P. argus* mtDNA for the 6 polymorphic restriction endonucleases

endonuclease	Hollywood Florida H	Alligator Light A1	A2	Islamorada, Florida 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Key West KW
(2) BstEII	-	2b	-	2b	-	2b	2b	2b	-	2b	-	2b	2b	2b	2a	2b	2b	2a	2b	2b	-	2b	2b	2c
(3) EcoRI	-	3c	-	-	-	-	-	-	-	-	3c	3c	-	3a	3a	3a	3c	3c	3a	3a	3a	3c	-	3c
(4) HincII	4b	4b	4b	4e	4e	4b	4b	4b	4b	4b	4c	4b	4b	4b	4b	4b	4b	4b	4b	4a	-	4a	4a	4e
(5) HindIII/HsuI	5b	5b	5b	-	5e	5b	5b	5a	5b	5c	5b	5b	-	5b	5b	5b	5b	5b	5b	5b	-	5b	5b	5b
(6) MspI	-	-	-	-	-	-	6a	6a	6a	6a	6a	6a	6a	6a	6b	6a	6a	6b	6a	6a	-	6a	6a	6a
(8) XbaI	8b	8c	8a	8a	8a	-	8b	8b	8b	8b	8b	8b	-	8b	8b	8b	8b	8c′	8b	8b	-	8b	-	8a
Sex	m	f	f	f	f	m	m	f	m	f	m	f	m	m	f	m	f	f	f	m	m	m	f	f
Carapace Length in mm	65	95	80	50	65	60	60	58	60	60	52	58	63	60	60	60	55	60	65	50	95	65	60	91

For a given restriction endonuclease, phenotypes labeled by adjacent letters (e.g., 2a, 2b, or 4b, 4c) can be interconverted by a single genetic change (base substitution); phenotypes labeled by nonadjacent letters are not readily traceable to a common evolutionary orgin, and hence the letter order is abritrary. 8c and 8c′ differ by 2 genetic changes, but by 1 each when compared with 8b. Dashes indicate the sample was not assayed. BamHI(1), SstII(7) and XhoI(9) were monomorphic.

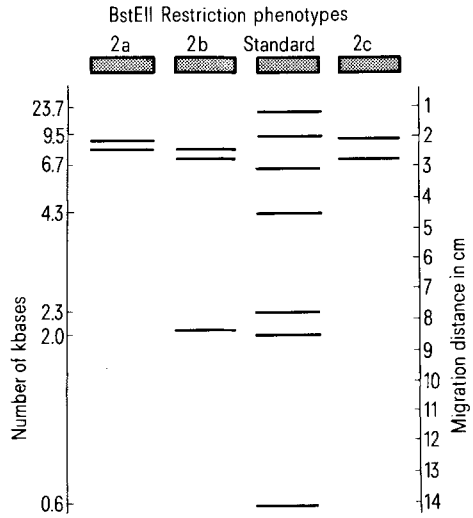
be used in large sample comparisons[7-9]. Moreover the resolving power of restriction analysis is greater than that of most other techniques[4,5,7], and mitochondrial DNA is circular and relatively small. Consequently restriction analysis of mtDNA is the method of choice where other techniques have failed to detect heterogeneity.

Panulirus argus, the Florida spiny lobster, inhabits western Atlantic coastal waters from North Carolina to Brazil[10], and planktonic phyllosoma are potentially capable of colonizing the entire species range during their more than 6 months existence[11]. Menzies[12,13] has quantified alloenzyme heterogeneity in *P. argus,* but diagnostic differences[14] among local populations have not been reported. We have employed nine restriction endonucleases (table) to estimate mtDNA heterogeneity among *P. argus* collected in the Florida Keys.

24 lobsters were collected by divers between Hollywood and Key West, Florida. Mitochondria were collected from ovaries, tail muscles and midgut glands, and mtDNA was purified on CsCl gradients[7]. mtDNA preparations were individually digested with different restriction endonucleases and the fragments were end-labeled with α^{32}P-labeled 2′-deoxyribonucleoside triphosphates[15]. Restriction fragment phenotypes were analyzed using the gel electrophoresis autoradiograms (fig.).

Restriction morphs, the patterns of fragments observed on a gel following digestion with a single restriction enzyme, are presented in the table for 6 restriction endonuclease analyses; the notation is explained in the figure. Three enzymes, BamHI, SstII and XhoI, yielded monomorphic phenotypes. The other 6 enzymes yielded polymorphic phenotypes. No mtDNA size heterogeneity was observed, and no sequence heterogeneity among tissues was observed. Seven enzyme phenotypes - 2c, 5a, 5c, 5e, 6b, 8c and 8c′ were unique. The number of base substitutions required to interconvert composite phenotypes was calculated for all restriction morphs[4]. For example, 4b5a and 4b5b differ by 1 substitution - 5a to 5b; 4b5a6a and 4b5b6b differ by 2 substitutions - 5a to 5b and 6a to 6b. Within sampling sites the mean number of interconversion substitutions was 0.7 ± 1.2 for Alligator Light and 2.0 ± 2.2 for Islamorada. Among all sampling sites the mean number was 2.8 ± 2.7; excluding Key West the mean was 1.6 ± 1.8. When Key West was compared with the other 3 sampling sites the mean number of interconversion substitutions was 5.8 ± 2.3.

These analyses suggest that mtDNA heterogeneity in this lobster is similar to that reported in *Drosophila*[18], man[19] and deer mice[4]. In addition the distinctiveness of the Key West lobster suggests that the species may be subdivided into local races. A similar study of *P. argus* has also identified the Key West population as different[20].



Diagrammatic representation of all the BstEII restriction fragments observed in 18 samples of *P. argus* mtDNA. Autoradiograms of 1.5% agarose gels were produced by 48 h exposure before developing. The standard used was bacteriophage lambda DNA digested with HindIII and the sizes of the 7 fragments are indicated on the left[17]. The 2a and 2c phenotypes have 2 fragments totalling approximately 16.2 kbase pairs, the 2b phenotype has 3 fragments totalling 16.2 kbase pairs. The largest 2a fragment 8.6 kb is equivalent to the 2 smaller 2b fragments 6.8 plus 1.8 kb; these fragments may be interconverted by a single base substitution. The 9.4 kb fragment of 2c is equivalent to the 7.6 plus 1.8 kb fragments of 2b.

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Interspecific hybrids of *Rana ridibunda* without germ line exclusion of a parental genome[1]

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Summary. Hybrids of *Rana ridibunda* × 2 unnamed Balkan taxa show no evidence, in electrophoretic comparison of soma and oocytes I for 4 enzyme loci, of germ line exclusion of their non-*ridibunda* genome. In this they differ from hybrids *R. ridibunda* × *R. lessonae,* × an unnamed Italian taxon, and × *R. perezi,* which in most cases clonally pass only their *ridibunda* genome to gametes.

The western Palearctic water frog *Rana ridibunda* Pallas 1771 (Amphibia) has been reported as a parental species of 3 different groups of natural interspecific hybrid lineages, the other parental species being *R. lessonae* Camerano 1882 in central Europe[2,3], *R. perezi* Seoane 1885 in southern France[4], and an unnamed taxon related to *R. lessonae* in peninsular Italy[5]. In all 3 cases, diploid hybrids of both sexes reproduce by 'hybridogenesis[6]': only their *ridibunda* genome is usually transmitted to progeny, the entire non-*ridibunda* genome being lost in the germ line before completion of gametogenesis (*R. ridibunda* × *R. lessonae*[7,8]; × *R. perezi*[4]; × Italian taxon[5]). Such hemiclonal[9] reproduction also occurs in fishes (interspecific *Poeciliopsis* hybrids[10]) and possibly in newts (interspecific *Triturus* hybrids[11]). The hybridogenetic genome exclusion can be detected electrophoretically in gonadal samples[4,12-17] and in individual oocytes I[14-16]: for somatically heterozygous loci, only *ridibunda* alleles are expressed in normal gametocytes of the hybrids.

Recently, 2 related additional taxa, each specifically distinct from and sympatric with *R. ridibunda,* have been discovered in the Adriatic parts of the Balkan peninsula; one in SW Yugoslavia[18], and the other in NW Greece[18,19]. Both are electrophoretically, immunologically and morphologically distinct from all other European water frog species; they are possibly most nearly related to *R. lessonae* and the Italian non-hybrid taxon[18]. Whether the 2 new taxa are conspecific is not known. Interspecific hybrids of both sexes in low frequencies have been detected electrophoretically in 4 of 7 syntopic populations of *R. ridibunda* and either new Balkan taxon[18]. To investigate whether these hybrids also show hybridogenetic gametogenesis, we compared electrophoretic phenotypes of their soma and their individual oocytes or testis extracts.

Material and methods. The 7 hybrids *R. ridibunda* × either new Balkan taxon discussed here were collected in August and September 1980 and 1981 in Yugoslavia (5 ♀ at Virpazar, Skadarsko Jezero, Crna Gora) and in Greece (1 ♀ + 1 ♂ at 10 km NW Igoumenitsa, Epeiros). For comparison we used electrophoretically identified hybrids, *R. ridibunda* × *R. lessonae* (May and June 1980: surroundings of Zürich, Switzerland; May 1981: surroundings of Poznań, Poland; all were syntopic with *R. lessonae:* the *'lessonae-esculenta* system'[20]), × Italian taxon (September 1980: Tarsia, Calabria, Italy), and × *R. perezi* (May 1981: SW Arles, Bouches-du-Rhône, France). Small pieces of frozen skeletal or skeletal and heart muscle and of testes were crushed in equal volumes of water; individual enlarged oocytes I of average size were squashed, adding a drop of water. Samples were applied on filter paper tabs to 12–15% starch gels pH 5.7/8.0 (electrode buffer 0.22/0.69 M tris + 0.09/0.14 M citric acid, gel buffer 0.008/0.023 M tris + 0.003/0.005 M citric acid). Proteins were separated horizontally at 13 V/cm for 3–4 h (pH 5.7 gels) or at 9 V/cm for 4–6 h (pH 8.0 gels) in a refrigerator. We examined 4 enzymes that partly discriminate the 2 parental species[18] and that were scorable in gonads; they were localized on gel slices in 0.5–1% agar overlays with modified standard chromogenic methods[21,22]: glucosephosphate isomerase (GPI, EC 5.3.1.9), lactate dehydrogenase (LHD, EC 1.1.1.27), NAD-dependent malate dehydrogenase (MDH, EC 1.1.1.37) and a peptidase cleaving leucyl-tyrosine and leucy-valine (Pep, EC 3.4).

Results and discussion. The 6 ♀ and 1 ♂ hybrids *R. ridibunda* × either new Balkan taxon, all of which appear to be diploid according to erythrocyte size[18], were somatically heterozygous 11 times for GPI and LDH-1, the 2 enzyme loci examined in gonads for all of them. All individual oocytes of each ♀, with 1 exception, and testis extracts of the ♂ gave the same pattern as the soma, whether the soma was homozygous or heterozygous (table, fig. D). This is supported by the ♀ hybrid from Greece being heterozygous for MDH-1 in the soma[18] and in 18 oocytes, and by 1 ♀ hybrid from Yugoslavia being heterozygous for Pep-2 in the soma[18] and in 17 oocytes. The exception, a ♀ somatically heterozygous for GPI and LDH-1, had a heterozygous pattern of LDH-1 in all oocytes examined, but only the translation product of the *a* GPI allele, characteristic of