in the number of stimulated cells when compared to the response of leukocyte cultures from the same individuals with PPD alone or with transfer factor alone ($P < .001$). There was no significant increase in the number of stimulated cells when PPD was added to cultures from PPD-negative individuals to which had been added the low-molecular-weight fraction prepared from leukocytes of a PPD-negative donor. The addition of PPD to leukocyte cultures from PPD-positive individuals to which transfer factor had been added 1 hour earlier increased the number of stimulated cells beyond that observed under the same conditions in the absence of transfer factor.

The data suggest that the response of leukocytes from PPD-negative and PPD-positive individuals to PPD was significantly increased by the addition of a low-molecular-weight dialyzate of leukocytes from PPD-positive donors which contained transfer factor. Whether this in vitro response of leukocytes is analogous to the in vivo passive transfer of delayed hypersensitivity to tuberculin with transfer factor remains to be seen.

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Interpopulation Variations in Calcium Metabolism in the Stream Limpet, Ferrissia rivularis (Say)

Abstract. *Significant differences between populations occur in calcium uptake during growth within one species of freshwater limpet. These are not related to environmental differences and may involve genetically determined physiological races. Such variation is significant in relation to aspects of evolution in freshwater animals and is important in assessing radionuclide contamination.*

Infraspecific physiological variation between populations of freshwater mollusks in aspects of growth, fecundity, and respiration has been reported (*1, 2*), and its significance discussed (*1, 3*). We now report on apparently extensive interpopulation variations in calcium uptake which occur during growth in the freshwater limpet *Ferrissia rivularis* (Say). The investigation was made in upstate New York with natural populations in freshwater creeks, whose mineral contents are very different.

If one considers only those fresh waters which can support mollusks with calcareous shells, the dissolved calcium content varies more than 100-fold. Within this range the distribution of many molluscan species is affected by calcium concentration. In temperate regions of the world, extremely soft waters (calcium concentrations < 3 mg/liter) can support only about 5 percent of the molluscan species of the region, moderately soft waters (Ca < 10 mg/liter) can support about 40 percent, intermediate waters (10 to 25 mg/liter) can support up to 55 percent, with hard waters (Ca > 25 mg/liter) being required for the rest (*3–5*). However, most of those species tolerant of low calcium could survive in, and are found in, harder waters (*3*).

The simplest possible case concerns those mollusks able to live in waters with a wide range of hardness and in whose shells and tissues the amount of calcium carbonate accumulated during growth varies directly with dissolved calcium content of the waters. Sampling of natural populations has shown that this is true in the extremely euryoecic snail *Lymnaea peregra* (*6*) where the thickness (and mass) of the shell varies according to the amount of calcium available. There are some earlier experimental data confirming this relation for some molluscan species (references in *4, 7*), and culture experiments on *Biomphalaria pfeifferi* have demonstrated that there is an optimum concentration of environmental calcium for both fecundity and growth in this species (*8*).

The case of *Ferrissia rivularis* is apparently different. In upstate New York, this species lives in creeks containing from 10.4 mg to 67.6 mg of calcium per liter. In a survey of interpopulation variations in organic growth pattern, with carbon being assessed by a colorimetric method after "wet-oxidation" of the sample (*9*), it became clear that the calcium content of the shell of *Ferrissia* did not vary in a simple direct relation with the amount of environmental calcium available. Seven natural populations, in six creeks and on a section of the shore of Oneida Lake, were studied; all of these waters form part of the Oneida division of the Seneca-Clyde-Oneida system which flows by way of the Oswego River into Lake Ontario. The environmental concentrations of dissolved calcium and magnesium were analyzed by an EDTA (ethylenediaminetetraacetate) titration (Table 1). The total hardness was also determined chemically at the same time, and the average total hardness was determined from conductivity measurements of samples made on every visit throughout the year. Regular sampling of 50 to 100 *Ferrissia* at these localities on about 15 to 30 occasions over 18 months to 2 years has allowed growth cycles to be determined. For a range of shell lengths (ages), the total organic carbon content, weights of living specimens, weights of dried specimens, "ash-free" dry weights, and shell calcium carbonate were determined. If consideration of growth changes in the ratio of calcium to carbon is postponed, the mean figures (Table 2) for shell calcium (with the confidence limits of the mean at the 95 percent probability level, $\bar{X} \pm t_{0.05}\, s_{\bar{X}}$) for each population are from 82 ± 1.3 mg of calcium per gram live wet weight (Fish Creek) to 121 ± 1.0 mg of calcium per gram (Big Bay Creek). These data are plotted against environmental calcium (Fig. 1), and it is clear that there is no direct relation. Data from studies with radioactive tracers and microanalyses (*10, 11*) show that there is direct active uptake of calcium from the medium by freshwater snails, and further that 80 percent of the calcium gained by grow-

Table 1. Pertinent chemical characteristics for localities with *Ferrissia* populations.

Location	By analysis			By conductivity: total hardness (mg $CaCo_3$/liter)
	Ca (mg/liter)	Mg (mg/liter)	Total hardness (mg $CaCo_3$/liter)	
Limestone Creek	67.60	14.58	199.0	169.46
Chittenango Creek	44.80	10.96	156.0	128.64
Oneida Lake	42.20	7.20	144.7	136.63
Big Bay Creek	14.78	4.46	59.0	64.67
Fish Creek	13.60	4.86	54.0	42.79
Slocum Creek	12.28	2.85	48.1	71.25
Black Creek	10.40	3.17	39.0	30.28

* Converted from means of seasonal conductivities as milligrams of NaCl per liter.

ing snails (*12*) comes directly from the water and only 20 percent indirectly through their food (*11*).

Thus it is legitimate to consider our results expressed as concentration ratios (Ca per mg of body weight/Ca per mg of environmental water). These ratios range from 1609 for the Limestone Creek population to 10,615 for the Black Creek limpets. This implies that during their growth, the Black Creek limpets have expended approximately 6.6 times more metabolic energy on the active transport of calcium than those of the same species in the harder water. If it were not for the significant differences among the four soft-water populations (Fig. 1), it might be possible to hypothesize that, unlike *Lymnaea peregra* and other freshwater snails, *Ferrissia* is capable of considerable regulation with respect to its calcium uptake. The content of calcium carbonate would thus be maintained at around 28 percent with perhaps some "over-compensation" when environmental calcium was less. This would correspond to conditions better known in plants, where pairs of closely-related species and subspecies occur, with one partner being "calcicolous" and the other being apparently "calcifuge", and where some forms can achieve widely different "concentration-ratios" under different environmental conditions (*13*). An example offering a close parallel to the stream limpets concerns the grass, *Festuca ovina*, where population differentiation has resulted in "races" termed edaphic ecotypes, among which response in culture to a range of calcium levels is significantly different (*14*). A survey of 25 "strains" of 6 "species" of the microorganism *Azotobacter* has revealed that some strains have specific optimum calcium requirements, whereas other strains are without specific requirements, and in one strain growth is actually inhibited by calcium (*15*). The calcium content of the cells can be presumed to be similar in most strains.

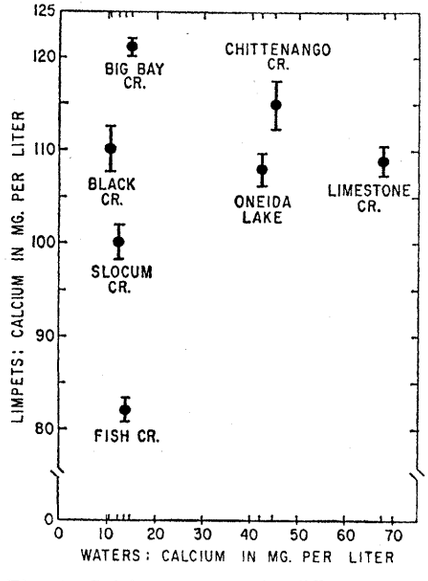

Fig. 1. Calcium content in different populations of the freshwater limpet, *Ferrissia rivularis* (mean values and confidence limits at the 95 percent probability level), in relation to the dissolved calcium content of their environments.

In *Ferrissia*, the differences among the four soft-water limpet populations would suggest genetically determined "physiological races," especially if one contrasts the Fish Creek population (82 ± 1.3 mg/g) with that of Big Bay Creek (121 ± 1.0 mg/g). The variation with age is not great. In the Big Bay Creek population, the calcium content varies from 114.0 to 123.6 mg/g, as measured on individuals from 0.7 mg live wet weight (equal to approximately 32 μg organic carbon) up to 17.5 mg live wet weight (equal to approximately 801 μg organic carbon). Although it might be expected that physicochemical or biological erosion of shells would be more significant in older limpets in localities where the water is soft, there seems to be no systematic relation between the environmental concentration and changes in the percentage of calcium content with age. In fact in Limestone and Fish Creeks, the juveniles have the highest percentages of calcium; in Black Creek and Oneida Lake the oldest adults have the highest percentages of calcium; while in Chittenango, Slocum, and Big Bay Creeks, the juveniles have percentages intermediate between different age groups of adults.

Additional data on natural populations of stream limpets could prove or disprove the existence of genetically determined "physiological races." In any case, considerable differences in energy expenditure in calcium metabolism are involved between different populations of the same species, which are almost certainly genetically isolated at present. Environmental causes cannot be invoked to explain these metabolic differences. Two points of general significance arise from these observations on interpopulation variations in calcium metabolism in the growth of *Ferrissia*. First, mollusks of this sort have been proposed as indicators of environmental radiocontamination with strontium-90 (*16*) which is accumulated indiscriminately with calcium. The work of Van Der Borght and Van Puymbroeck (*10*) clearly demonstrates active direct uptake of both calcium and strontium by freshwater snails such as *Lymnaea stagnalis, L. auricularia* and *Planorbarius corneus*. Obviously, such direct concentration is in significant contrast to the more usual transport over several trophic levels in a food-chain, and possible concentration in a series of stages, occurring in an environment contaminated with radionuclides. Thus it is important that some

Table 2. Calcium content of shell in different populations of *Ferrissia rivularis*.

Location	No.	Shell calcium (mg/g wet weight)	
		Mean ± S.D.	Confidence* limits of mean
Limestone Creek	70	109 ± 6.7	± 1.6
Chittenango Creek	40	115 ± 8.3	± 2.7
Oneida Lake	24	108 ± 4.2	± 1.8
Big Bay Creek	36	121 ± 2.8	± 1.0
Fish Creek	31	82 ± 3.5	± 1.3
Slocum Creek	39	100 ± 5.9	± 1.9
Black Creek	38	110 ± 7.5	± 2.5

* Confidence limits at the 95 percent probability level, $\pm t_{0.05}s_{\bar{x}}$.

freshwater mollusk species have populations which differ significantly from each other in the extent to which they *do* concentrate calcium from the environment. Secondly, this example of variation in calcium metabolism between different populations of a single species of freshwater snail can be added to the other types of physiological variation already known for freshwater mollusks, which include aspects of respiration (*2, 3, 5* and references therein), patterns of shell growth (*17*), and cycles of growth and reproduction (*1, 18* and references therein). The fundamental significance of such extensive physiological variability has been related to the temporal and spatial nature of the freshwater environment (*1, 3*). It can be claimed that the short-term, small-scale genetic isolation which occurs is not only responsible for the extensive interpopulation infraspecific diversity (with little full speciation) but also for the existence of much adaptive plasticity in several aspects of the physiology of such freshwater animals (*1–3*).

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Mutations, Chromosomal Aberrations, and Tumors in Insects Treated with Oncogenic Virus

Abstract. *An increased incidence of lethal mutations, visible mutations, chromosomal losses, chromosomal nondisjunctions, and tumors resulted when drosophila were placed in medium containing Rous-sarcoma virus. In the group treated, a few mosaics of the eye and translocations appeared as well. There is a suggestion, from preliminary data, that the size of chromosomal puffs may be reduced by this RNA virus, but the difference is not significant. Tests for the persistence of virus in progeny have been negative so far.*

The Bryan (*1*) high-titer strain of Rous-sarcoma virus containing Rous-associated virus 2 was mixed with yeast and added to the surface of medium on which 2-day-old *Drosophila melanogaster* larvae ($sc^8.Y.B^8/y^3w^1ct^6f^1$) were feeding. Two different concentrations of virus were used—0.2 ml of undiluted material and the same volume of a 50-fold dilution of virus in drosophila Ringer's solution without calcium. We checked the activity of virus by determining the production within 10 days of characteristic sarcomas in chicks after they had been subcutaneously injected. Germ cells of male flies remaining on the virus-yeasted food throughout larval, pupal, and imaginal stages of development were tested for mutations and chromosomal abnormalities by methods (*2*) adapted from Muller and Muller and Oster.

Table 1. Production of tumors in drosophila by Rous-sarcoma virus (RSV) in dilutions of 1:1 and 1:50.

Treatment	Animals with tumors (%)	Dead animals (%)	Total studied (No.)
RSV 1: 1	4.9	30.4	491
RSV 1:50	8.7	29.4	1245
Control	0.4	2.8	508

The mortality was moderately high among those larvae treated, and some of the larvae and pupae exhibited black, pigmented bodies (*3*) adjacent to the gut (Table 1). The largest were in the pupae and reached a size equal to three-quarters of the diameter of the body. None of the individuals with these tumors survived. Melanization of pericardial cells were found in progeny of flies exposed to Rous virus (*4*).

An increased rate of lethal mutations on the X chromosome was found when males were exposed to Rous virus in the medium and tested by the Muller-5 method (Table 2). Increased nondisjunction and losses of the Y chromosome, along with more numerous visible mutations, were found when tested

Table 2. Mutations, mosaics, and chromosomal abnormalities in drosophila treated with Rous-sarcoma virus (RSV) in dilutions of 1 : 1 and 1 : 50.

Aberrations	Treated with RSV 1:1		Treated with RSV 1:50		Untreated	
	Total studied (No.)	With aberration (%)	Total studied (No.)	With aberration (%)	Total studied (No.)	With aberration (%)
Loss of Y	1181	0.01	4442	0.27	711	0.0
Nondisjunction	3191	0.38	10960	0.61	2473	0.11
Mosaics (eye)	3191	0.00	10960	0.03	2743	0.0
Visible mutations	3191	0.13	10960	0.12	2743	0.0
Lethal mutations	426	3.29	1083	2.87	648	0.0
Translocations	245	0.00	794	0.63	202	0.0

Table 3. Effect of Rous-sarcoma virus (RSV) on mean size of chromosomal puffs.

Puff	Control			Treated with RSV		
	Number measured	Size (μ)	Ratio*	Number measured	Size (μ)	Ratio*
X:2-B	20	8.3	1.48	25	7.6	1.36
III L:71	16	6.4	1.43	14	5.9	1.28
III L:72	16	5.9	1.28	15	5.3	1.19
III L:74	19	7.4	1.44	19	5.9	1.23
III L:75	19	7.3	1.41	17	5.6	1.24

* Ratio of puff to adjacent band.