Table 2. Occurrence of dry cerumen among American Indians, and estimated recessive gene frequencies (q); estimates based on two phenotypes [after C. C. Li, *Human Genetics* (McGraw-Hill, New York, 1961)]. Matsunaga (*1*) found q to range between .978 and .790 for Northern Chinese, Koreans, Tunguse, Mongols, Japanese, Southern Chinese, and Ruyuku Islanders; to be .176 and .069 for Caucasians and Negroes, respectively. "All other tribes" include Walapai, Pauite, Omaha, Winnebago, Chumibec, Tlingit, Apache, Chicasaw, Quinault, Chippewa, Cowicham, Arapaho, Mescalero, Flathead, Aleut, Yurak, Cheyenne, Tiowa, Comanche, Chuckchanci, Mono, Washo, Nez-Perce, Creek, Mohave, Delaware, Choctaw, Shoshone, Papago, Blackfoot, and Yakima. Numbers of subjects examined appear in parentheses; standard errors, in square brackets.

Peoples	Dry cerumen		
	No.	Percentage	q
Navaho (all) (183)	116	63.3	.796 [.022]
Sioux (all) (147)	54	36.7	.606 [.031]
All other tribes (153)	78	50.9	.714 [.028]

groups that migrated to the New World from Asia about 3000 years ago. The present-day Navaho arose from subsequent southerly migrations of Athabascans (as "Apacheans") to the Southwest about 400 to 500 years ago. It is known that the Navaho have maintained their scattered encampments and nomadic culture unchanged, and that historically they experienced no contact with large numbers of Caucasians until relatively recently. There has been little mixture of the Navaho with Spanish and Anglo-American settlers (*8, 9*).

The Siouan tribes are believed to be descended from early paleolithic mongoloids who first settled in the Upper Mississippi Valley before migrating to the Dakotas, Nebraska, Wyoming, and Montana (*10, 11*). In contrast to the Navaho and other Southwestern tribes, the Dakota (Sioux) and other Plains Indians experienced extensive and sustained contact with French trappers and traders from as early as 1700, and, over the last 150 years, with American traders, soldiers, and settlers. Extensive intermarriage is recorded between the Dakota and Caucasians (*11*). Undoubtedly, flow of White genes into these tribes was further accentuated by the extremely severe reductions in populations that resulted from epidemics of smallpox and cholera, famines, and military campaigns (*9, 12*) during this period.

Table 3. Occurrence of dry cerumen among American Indians of full and mixed blood, and their recessive gene frequencies (q). "All Southwest Indians" include all full-blooded Navaho, Walapai, Paiute, Apache, Papago, and Mohave. Numbers of subjects examined appear in parentheses; standard errors, in square brackets.

Peoples	Dry cerumen		
	No.	Percentage	q
Navaho, full blooded (162)	113	69.7	.835 [.020]
All Southwest Indians (251)	168	66.9	.818 [.017]
Sioux, full blooded (113)	53	46.9	.685 [.033]
Indians with white blood (78)	7	8.9	.299 [.053]
White Americans (45)	2	4.4	.210 [.072]

Certain blood-group findings may reflect these historical influences on the Navaho and Sioux also. It is acknowledged that the presence in American Indians of blood groups A_2 and B indicates non-Indian admixture (*13*). Several reports show the complete absence of blood groups A_2 and B among the Navaho (*14*), whereas up to 4.2 percent of blood group B has been found in Sioux Indians and been attributed to mixture of blood with Caucasians (*15*).

The findings in Chiapas Mayan Indians are unexpectedly at variance with what would be anticipated from reported present-day cerumen frequencies in mongoloids of Asia, and from our findings in Indians of the United States. Since the Chiapas Mayans are stated to have remained almost completely isolated from the Spaniards throughout the centuries (*16*), it is conceivable that the high frequency of sticky cerumen in these people is the result of early genetic drift, or possibly of mixture with prehistoric non-mongoloid immigrants (*17*). It seems prudent to suspend judgment pending further information.

Our findings indicate that determination of the type of cerumen can provide a useful and simple genetic marker for population studies of the American Indian. More extensive anthropologic studies will be needed to establish the hypothesis that the presence of high frequencies of the sticky allele in American Indian tribes results from Caucasian admixture.

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Lymphocytic Choriomeningitis: Production of Antibody by "Tolerant" Infected Mice

Abstract. *Newborn mice infected with lymphocytic choriomeningitis virus are not immunologically tolerant to the agent but, rather, appear to make antibody to the virus. This antibody was detectable only in the kidneys, where presumably it had been deposited in the glomeruli in the form of complexes of antibody, virus, and complement.*

Traub, more than 30 years ago, reported the occurrence in mice of a lifelong, symptomless, lymphocytic choriomeningitis (LCM) viral-carrier state induced by *in utero* or neonatal infection (see *1*). This state was characterized by persistently high titers of virus in blood and organs throughout

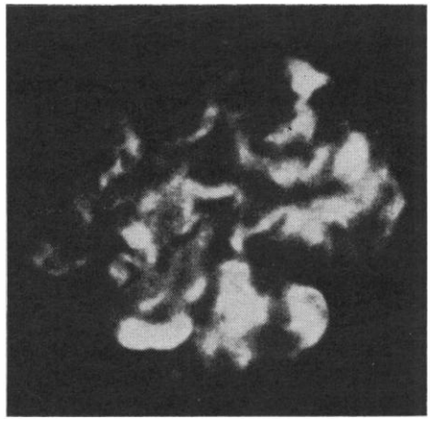

Fig. 1. Renal glomerulus from a 3-month-old B10D2 (old line) mouse infected at birth with LCM virus. The preparation was stained with fluorescein-conjugated rabbit antiserum to mouse 7*S* γ-globulin. Comparable results were obtained with fluorescein-conjugated rabbit antiserum to mouse β_{1c}-globulin (the third component of complement).

life and by absence of detectable antibody. Burnet and Fenner suggested that congenital LCM infection involved the development of immunological tolerance to the foreign microorganism during early life (*2*). Thus the animal in later life was "incapable of responding with antibody production to injection or infection with the same microorganism." Subsequent work in several laboratories (*3*) has been in basic agreement with this concept in which the parameters of the "immunologically tolerant" LCM model are defined as: (i) resistance of normal newborn mice to a viral dose lethal for mature adults; (ii) presence of high titers of virus in organs and blood of neonatally infected adults; (iii) resistance of neonatally infected adults to ordinarily lethal LCM viral challenge; and (iv) absence of detectable complement-fixing or neutralizing antibody to LCM in neonatally infected adults.

Table 1. Micro-complement-fixation assay (*12*) for antibody to LCM virus in five 3-month-old "tolerant" B10D2 (old line) mice infected at birth with LCM virus. (Similar results have been observed in B10D2 (new line), NZB, SWR/J, and C3H mouse strains.) Hemolysis was measured by optical density at 413 mμ; 1.2 $C'H_{50}$ units were used in the assay; kidney dilutions (1/2 to 1/200) were made from citrate eluates from saline-washed kidney homogenates; and the antigen was specific LCM complement-fixing antigen (*11*). The control values for antigen alone and complement alone were 0.54 and 0.55, respectively. Any reading below 0.45 indicates significant complement fixation.

1/2	1/10	1/50	1/100	1/200
0.11	0.37	0.46	0.50	0.54
0.22	0.41	0.47	0.56	0.55
0.18	0.30	0.37	0.49	0.58
0.12	0.36	0.49	0.55	0.53
0.28	0.36	0.52	0.58	0.57

Our work indicates that, contrary to previous experience, antibody to LCM virus is formed by so-called "tolerant" LCM carrier mice. This antibody is found concentrated in the renal glomeruli.

Mice of the following strains were used: B10D2 old line, B10D2 new line, SWR/J, NZB, and C3H. A viral carrier state was induced by intracerebral inoculation of normal newborns with LCM virus. The LCM strain was derived from mouse-brain passage of the NIH 7022 strain (*4*). In all cases, newborns were inoculated intracerebrally during the first 15 hours of life with 0.03 ml of $1000 \times LD_{50}$ (minimum lethal dose, 50 percent effective for adult mice) of virus prepared from infected brains of isogenic adult mice. Within the first 2 to 3 weeks after inoculation, 10 to 20 percent of the young mice died. The remainder were, from then on, virus carriers. Other C3H mice born of infected mothers were also used as "tolerant" carriers. At weaning, the blood and organs of these mice showed high titers of virus (*5*), but there was no detectable neutralizing antibody to LCM virus in undiluted serums. When 5 $C'H_{50}$ (complement required for 50 percent lysis) units were used in a standard complement-fixation assay (*6*), we did not detect any complement-fixing antibody to LCM virus in the serums of the mice.

Immunofluorescent study of the tissues of these LCM-"tolerant" carriers indicated that mouse γ-globulin and β_{1C}-globulin (the third component of complement) were concentrated in both mesangia and capillary walls of the renal glomeruli, but that they were not present in other tissue sites (Fig. 1). Mouse albumin and mouse fibrinogen were not found in the glomeruli. These observations were made by direct immunofluorescent technique with the use of specific fluorescein-conjugated rabbit antibodies to each of the mouse proteins (*7*). The positive reaction of fluorescence could be eliminated by specific absorption of the respective conjugated rabbit antiserums with mouse γ- or β_{1C}-globulins. Kidneys of adult mice of these strains inoculated at birth with brains of normal isogenic mice, or of mice infected with LCM virus as adults, showed no such accumulation of γ- or β_{1C}-globulins in the glomeruli. Study of the kidneys of tolerant LCM carriers with fluorescein-labeled guinea pig antibody to LCM virus showed viral antigen in tubular cells as well as in glomeruli, in contrast to the exclusively glomerular localization of γ- and β_{1C}-globulins.

The mouse γ-globulin located in the renal glomeruli apparently was, at least in part, antibody to LCM virus. γ-Globulin was eluted from kidneys of 3-month-old "tolerant" virus-carrier mice by treatment of saline-washed kidney homogenates with .02*M* citrate buffer, *p*H 3.2, for 80 minutes at 37°C. The eluted material was dialyzed against phosphate-buffered saline and concentrated. When tested in a micro-complement-fixation assay (*8*) this eluate fixed complement in the presence of LCM antigen (Table 1). Identically prepared undiluted eluates from homogenates of the spleen and brain of "tolerant" animals, as well as from kidneys of mice inoculated at birth with extracts of normal isogenic brain, failed to fix complement in the presence of specific LCM antigen. Complement-fixing or neutralizing antibodies were not detected in the blood of any of these animals.

The concentration of antibodies in the glomeruli in the absence of detectable circulating antibody is not without precedence. In NZB/W mice making antibodies to nuclear DNA, the specific antibody activity of γ-globulin eluted from kidneys may be 50 to 100 times greater than that of serum γ-globulin (*9*). Not infrequently the eluates of kidney homogenate of the NZB/W mice contain considerable antibody to nuclear DNA in the absence of detectable serum antibody.

The mechanism by which the antibody to LCM virus accumulates in the glomeruli may be by trapping of complexes of circulating viral antigen–antibody complement in the glomerular filter. This process is well recognized in laboratory models in which soluble protein antigens, antibody, and complement combine to form circulating complexes which lodge in the glomeruli (*10*). If the rate of elimination or degradation of antibody in the glomeruli were slower than its deposition, as is the case in chronic serum sickness, a gradual glomerular accumulation of antibody might be expected. The deposition of virus, antibody, and complement in the glomeruli may be one of the causes of the

delayed pathologic changes observed in the kidneys of mice of certain strains infected at birth with LCM virus (*5*). These observations suggest that glomeruli may be a promising source of antibodies in other chronic infectious diseases eliciting low antibody responses.

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Habitat Selection by Chemically Differentiated Races of Lichens

Abstract. *The maritime European lichens of the aggregate species* Ramalina siliquosa *represent six chemical races. Where the races are sympatric they populate different habitats. Such intensive local ecological sorting of morphologically similar individuals accumulating different, highly specialized metabolic end products appears to be unknown in other plants.*

Some morphologically uniform lichens exhibit chemically different races that show distinct geographical distributions (*1*). Our study establishes that where the ranges of these races overlap, the races may select different ecological habitats. This differentiation into chemical races seems to be controlled genetically. Although a few instances of genetically controlled, quantitative differences with regard to physiological processes (especially photosynthesis and respiration) in ecotypes of flowering plants are known (*2*), no comparable examples in plants of habitat selection by qualitatively different races characterized by highly complex metabolic end products have been reported.

The unusual extracellular compounds that accumulate on lichen hyphae show taxonomic correlations of great magnitude. Generally, every individual of a morphological race produces the same substance or substances, but some morphologically uniform lichens exist in multiple chemical types that are interpreted as species by some authors and as "chemical strains" by others (*3*). The exact number of lichens showing chemical races is unknown, but a recent count (*1*) in 17 selected genera showed that 99 morphologic types exist together as 240 chemical variants. The biological interpretation of this variation has been impeded because few field observations on the behavior of chemical races have been made (the races usually do not occur together) and because laboratory experiments have been impractical (intact lichens are notoriously difficult to culture) (*4*). However, it has recently been shown (*5*, *6*) that *Ramalina siliquosa* (Huds.) A. L. Sm.—in the broad taxonomic sense one of the most common maritime lichens of western Europe—is made up of six chemical races. The aggregate species ranges from Portugal to arctic Norway and eastward through the Baltic to the Soviet Union, and the component chemical races, while having much more restricted distributions, are all sympatric in western Britain. We have now found that where the races occur together they select different habitats.

In western Europe all organisms inhabiting rocky shores are strikingly zoned (*7*). The fruticose *R. siliquosa* lichens form a conspicuous band just above a strip dominated by *Verrucaria maura*. The latter species is a black crustose lichen which brilliantly marks the top of the littoral zone, an ecological belt which, at least in sheltered localities, corresponds to the upper limit of the highest tides. Since the *R. siliquosa* zone in Britain consists of chemically different individuals, we tried to determine whether the distribution of the chemical types within it was correlated with any obvious environmental variables.

A small promontory with a well developed *Ramalina* vegetation was selected for study in Tre-Arddur Bay on Holy Island, North Wales. The headland presents cliff faces approaching three cardinal directions (south, west, and north), giving a total exposure of 225° (Fig. 1). The rock is quartz-chlorite-muscovite-schist of the Mona Complex (Precambrian). The ramalinas were sampled with six vertical line-transects, composed of strips of contiguous 1-foot-square (35 by 35 cm) blocks, passing through the entire *Ramalina* zone at each place sampled. The zone on the west face (Fig. 1, transect 5) is 25 feet (7.6 m) wide, the broadest of all, and some additional blocks at the bottom of it were therefore sampled to give a good representation of the plants occurring lowest down on the cliff. In every block the ten plants closest to the center were taken. The 980 individuals collected were assigned (by thin-layer chromatographic analysis) to the six known chemical types: five producing closely related depsidones elaborated in the medulla and one with no medullary lichen substances (*8*).

Even though the promontory is small (the northeast and south faces are only 45 m apart), the range of habitats presented by the faces bearing the ramalinas grossly exceeds the amplitude of tolerance of any of the component chemical races of the plants. Ninety-six percent of the plants produced either stictic acid (43 percent), hypoprotocetraric acid (30 percent), or norstictic acid (23 percent) (*9*), all with highly patterned distributions (Fig. 2). Plants that produce stictic acid were most abundant at the bottom of the west and south faces where they made up all of the ten-plant samples in most blocks (Fig. 2, right). The bottom of these transects, just above the *Verrucaria* zone and facing the sea, repre-