Molecular Genetics of Populations of Intracellular Bacteria: The Spotted Fever Group Rickettsiae

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The obligate intracellular nature of the members of the genus *Rickettsia* is an evolutionary factor which sets them apart from the free-living bacteria, such as *Escherichia coli* or *Bacillus*, for which our knowledge of population genetics and population structure is most complete.[1,2] Details of the genetic structure of a bacterial population are needed to evaluate the evolution of a bacterial species. These details must include information on relative levels of genetic variation within and between localities. Analysis of the patterns of genetic diversity can give us insights into the effective population size, migration rate between subpopulations, and genetic parameters of mutation and recombination. In intracellular bacteria such as the rickettsiae, the situation is further complicated by the fact that the bacterium is dependent for its continued survival upon factors affecting the host. For example, it has long been known that the maintenance of *Rickettsia rickettsii* in both tick and animal reservoirs is possible.[3] What is not clear is the relative importance of these alternative reservoirs on the genetic factors affecting long-term evolution of the bacteria; nor do we understand the possible importance of movement between alternative reservoirs.

For organisms such as *E. coli*, it has generally been held that a "species" is made up of a number of distinct clones,[4] each of which may occur widely over the geographical range of the species. Here we use the term "clone" in the sense used by Milkman and Stoltzfus:[5] a group of organisms descended without recombination from a single ancestor. Genetic differences between clones arise through the interaction of the processes of selection and recombination. Changes in the frequency of different clones in the species occur both through interclonal processes[6] and through the intraclonal process of periodic selection.[7] It has recently become clear that one of the most important aspects of the clonal evolution of *E. coli* occurs not at the level of the organism (i.e., at the level of the entire genome), but rather takes place at the level of smaller segments of the chromosome.[5,8] As a result, the evolutionary histories of different segments of the *E. coli* genome often are not the same, resulting in different phylogenetic inferences for the various chromosomal segments.[9-11]

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Relatively little is known about the evolutionary forces which shape the population structure of obligate intracellular bacteria such as the rickettsiae. Consequently, we have been interested in using molecular methods to begin the study of the population structure of species in the spotted fever group (SFG) of *Rickettsia* in order to compare them with free-living bacteria. The initial goals of our studies have been, first, to estimate the levels of genetic variation which are present in local and in geographically distant populations of the tick-borne species of *Rickettsia;* second, to partition this variation within and between populations in order to establish the extent of clonality of a rickettsial species; third, to get a first approximation of the degree of mosaic clonality in the rickettsial genome; and, finally to estimate the relative importance of some of the factors which shape the existing genetic variability, such as mutation, recombination, and population size.

LEVELS OF GENETIC VARIATION IN LOCAL POPULATIONS

The approach taken by our laboratory involves estimation of levels of genetic variation, using molecular methods which can detect nucleotide changes in the genome. This is accomplished by comparison of restriction fragment length polymorphisms (RFLPs) in DNA, observed by means of Southern blot analysis of genomic DNA from different rickettsial strains. Homologous genetic regions in different bacterial strains were compared using a variety of anonymous, random DNA probes. By "anonymous" we mean that the probes were chosen because of their size (3–10 kilobase pairs), and because they represent unique sequences in the rickettsial genome, but not because of the coding potential of any genes which they might include. A set of such anonymous probes allows an unbiased estimate of the underlying genetic variability within populations, when used with statistical methods based on the proportion of shared restriction fragments between pairs of strains.[12] By using a group of random probes, we can obtain an estimate which will not be greatly affected if selective factors influence a particular gene or segment of the genome.

We have used a combination of between nine and twelve different probes and three to five different restriction enzymes in our various population surveys. The probes hybridize with unique (non-repeated) portions of the genomes of each species. In total, these regions represent about 50–70 kilobase pairs of the rickettsial genome (estimated to represent 3–5% of the genome). TABLE 1 shows that this approach has resulted in data equivalent to direct comparisons of 1000 or more nucleotide sites from each strain. Anonymous probes have been utilized previously to compare strains in other bacteria, although a direct estimate of genetic variability using this method exists for only a few species, including *E. coli*.[13] Previous studies of RFLP variation in the typhus group rickettsiae[14] did not directly estimate nucleotide variability within populations.

We have estimated intraspecific genetic variation in a set of approximately 85 strains isolated from nature, as summarized in TABLE 1. These isolates represent five species of tick-borne Rickettsiae.[15,16] The first two data sets represent "local" populations, while the remaining intraspecific data were obtained from geographically dispersed populations of four species. Finally, the last entry in TABLE 1 represents the interspecific genetic differences seen in a comparison of six of the SFG species. Genetic variability is expressed in terms of nucleotide diversity, estimated following Nei and Li,[12] and is equivalent to the average pairwise nucleotide difference between strains. This statistic allows us to make a valid comparison of our RFLP estimates of genetic diversity from *Rickettsia* with data collected

on direct nucleotide sequence comparisons or on RFLP comparisons in other organisms. The validity of the RFLP approach in estimating the level of underlying nucleotide diversity in the rickettsial genome has been confirmed in our laboratory by comparison with estimates obtained by sequencing homologous genetic regions from different strains of *R. bellii* and from five North American species of the SFG.

The data in TABLE 1 illustrate several observations which we have made concerning intraspecific genetic variability in the SFG and tick-borne rickettsiae. First, average levels of nucleotide diversity are extremely low. In the largest single sample of strains reviewed in TABLE 1, the 53 strains of *R. bellii* represent strains isolated from *Dermacentor variabilis* ticks collected in Ohio over the time period 1978–1986, from various localities separated by over 200 miles.[15] The population is made up essentially of two haplotypes, which differ by a single nucleotide difference. The two major haplotypes represent, respectively, 62% and 38% of the strains. Four subhaplotypes, which occur as unique strains and which each differ from one of the major haplotypes by an additional single nucleotide site difference, complete the variation which exists in the population. The esti-

TABLE 1. Number of Strains Analyzed in Each Intraspecies Study and Underlying Variability

Species	No. of Strains	$\hat{n}$[a]	Nucleotide Diversity[b]
R. bellii (Ohio)	53	1004	0.00061
R. montana	4	1090	0.0
R. bellii (U.S.)	5	1284	0.0029
R. rickettsii	14	1128	0.0001
R. rhipicephali	4	1021	0.0049
R. siberica	2	566	0.0023
SFG interspecies[c]	6	1978	0.011–0.049

[a] $\hat{n}$, average number of nucleotides explicitly included in restriction sites.
[b] Nucleotide diversity estimated following Nei and Li.[12]
[c] SFG comparisons included pairwise comparisons between *R. rickettsii*, *R. montana*, *R. rhipicephali*, *R. conori*, *R. siberica*, and *R. parkeri*.

mate of nucleotide diversity within this population (0.0006) is about 100-fold smaller than that observed in *E. coli*,[13] and 100–1000-fold less than that seen in a similar study of the plant pathogen *Pseudomonas syringae* pv syringae.[17] The small collection of local strains of *R. montana* listed in TABLE 1 possibly represents an even more extreme level of intrapopulational homogeneity than that found in local populations of *R. bellii*.

The remaining four sets of intraspecific comparisons summarized in TABLE 1 represent groups of geographically dispersed strains which were chosen to yield a representative estimate of the level of interlocality genetic variability to be found within a rickettsial species. Nevertheless, the levels of interstrain nucleotide variability uncovered in even the most variable of these species is still 10-fold lower than that observed between different isolates of *E. coli* which can be collected at a single locality.

The choice of strains, especially in *R. bellii*, enables us to estimate the proportion of the observed nucleotide diversity which exists within populations compared to that which exists between different populations. To do this, we use the gene diversity statistics of Nei,[18] which can be related directly to the F_{st} statistics

originally developed by Sewall Wright.[19] Analysis of the data from local and geographically disjunct populations of *R. bellii* reveals that 76% of the nucleotide variation observed in the entire species exists in the form of between-population differences.[15] In contrast, for instance, only 4% of the variation observed in *E. coli* exists as between-population variation.[20] Among other bacterial populations studied by protein electrophoresis, only *Legionella*[21] has a value of between-population differentiation as high as 30%. It is very clear that, even though *R. bellii* has much lower overall variation than that found in bacteria such as the enterics, most of the variation which does occur in *R. bellii* is not geographically homogeneous, but rather exists as differences distinguishing differentiated subpopulations.

Phylogenetic studies of the SFG using RFLP variation gives some further insight into the low levels of differentiation which are observed in these intracellular bacteria. The last row of entries in TABLE 1 shows the estimate of average nucleotide diversity between different species of the SFG.[22] The most differentiated of the SFG species are estimated to have only about 4.9% nucleotide differences. This is very minor differentiation. As mentioned above, 3–4% nucleotide divergence is equivalent to that observed between different isolates of *E. coli* collected at the same locality. A determination of the ratio of the genetic variability found within a species of the SFG with the amount of genetic differentiation between SFG species shows that 93% of the nucleotide variability exists in the form of between-species variation. Although the SFG species have low levels of genetic differentiation when compared to free-living bacteria, the genetic variation that does occur exists in large part as genetic differences between species. Studies of both the intraspecific and interspecific data strongly suggest that very little genetic transfer occurs between rickettsial populations.[15,22] They show that rickettsial populations are extremely well differentiated, with each population representing an independently evolving lineage.

Finally, the pattern of genetic variability which we find in the SFG rickettsiae differs remarkably from that seen in *E. coli* in one other respect. There is very little variation in *Rickettsia* caused by insertion/deletion events or by repeated elements. In fact, our evidence suggests that within rickettsial populations such variation is essentially non-existent.[15] A few such events have occurred and can be detected in interspecific comparison.[22] Such insertion/deletion events are frequent in the enteric population.[9] Whether the lack of insertion/deletion changes in *Rickettsia* is related to the restricted size of the genome or to other selective factors is not currently understood.

The findings which we have made concerning variation within and between rickettsial populations are consistent with recent observations made on the SFG and typhus group rickettsiae using RFLP analysis of PCR-amplified gene sequences.[23] There is, however, evidence that the scrub typhus group rickettsiae may show higher levels of intraspecific genetic variability,[24] and the possibility certainly exists that *R. tsutsugamuchi* may have a population structure substantially different from that which we have inferred for the North American SFG rickettsiae.

FORCES WHICH AFFECT GENETIC VARIATION IN THE SFG

Some of the factors which affect levels of genetic variability in populations are historical, while others are purely biological. For example, one possible reason for the low levels of genetic variability might be a recent origin of the North

American rickettsiae. Were the origin recent enough, it would explain why none of the taxa are well differentiated. We have studied the differentiation of the genes for the 16S rRNA in several SFG species and in *R. bellii*.[25] All values of nucleotide difference between 16S rDNA sequences from tick-borne rickettsiae showed less than 0.7% sequence differentiation. That level of differentiation suggests that the SFG is less than 30 million years old, and differentiation among the members is possibly much more recent. This estimate is based on the calibration of rRNA gene evolution by Ochman and Wilson,[26] which holds that a 1% divergence between 16S rRNA gene sequences would occur during 50 million years of divergence from a common ancestor. Additional data are required for all the North American SFG species to further resolve the possibility of recent origin.

Is such a date for the origin of the SFG sufficiently recent to explain why so little genetic difference between species is observed? The answer is probably yes, although the observed levels of differentiation between species would still be considered to be small. Is this date a sufficient explanation for the low levels of variation within populations? The answer is definitely no. Given 30 million years to accumulate neutral mutational variation, and with other factors being similar, we would expect that levels of genetic variation in *Rickettsia* and in the enterics would probably be equivalent.

Could the relative importance of transtadial/transovarial transfer versus horizontal transfer between hosts affect levels of genetic variability? The answer is clearly yes. Bacteria which do not come into contact with other strains may have no opportunity for genetic recombination. The relative frequency with which independent rickettsial strains interact would seem to depend strongly on the ease with which these organisms move between different hosts. Horizontal transfer of rickettsiae between hosts certainly occurs. It is the essence of bacterial infection. Does it contribute to genetic interaction between isolated strains? This question is not as easily answered. Comparisons of geographically diverse *R. bellii* strains obtained from the same or different species of tick demonstrate that genetic similarity is affected more strongly by geographic proximity than by host similarity.[15] This indicates that effective horizontal transmission occurs, although it does not indicate how frequently transfer takes place. Nevertheless, evidence for the occurrence of recombination between variable RFLP sites in the genome of any of the SFG is not compelling. Further sequence data studying the possibility of mosaic segmental evolution of the rickettsial genome are required to answer this question. Additionally, we need studies which can determine how frequently effective horizontal genetic exchange of strains between both infected and naive hosts occurs. One approach which we consider promising involves the simultaneous study of bacterial and mitochondrial DNA from rickettsial-infected female ticks. The examination of the strength of any association between particular tick mitochondrial genotypes (which must be transmitted vertically by a female tick) and particular bacterial genotypes (which may be transmitted either vertically together with the mitochondria or horizontally, independent of the mitochondria) would allow the relative importance to bacterial evolution of vertical and horizontal transmission to be determined.

Genetic factors related to the size of the genome or even difference in the mutation rate for genes in *Rickettsia* could affect levels of genetic variability. However, evidence for any major effects of genome size per se (such as the density of important genes in the smaller Rickettsial genome) are lacking. For example, the vertebrate mitochondrial genome is very densely packed with genes vital for the survival of the organism.[27] Nevertheless, the rate of divergence for genes in the mitochondrial genome is very rapid, and levels of interspecific varia-

tion are substantially larger than those measured in *Rickettsia*.[27] Nor does there seem to be any reason to invoke a lower mutation rate in *Rickettsia* to account for lower intrapopulation variation. Our data on differences between species for nucleotide sequences such as the 16S rRNA genes, which have been extensively studied in other bacteria, suggest that overall mutational changes are occurring in tick-borne rickettsiae at rates similar to those seen in other bacteria.

EFFECTIVE POPULATION SIZE

How large is the rickettsial population (in an evolutionary sense) and have population bottlenecks occurred which might account for the observed homogeneity within populations? The very low values of nucleotide diversity obtained in our studies suggest that populations of *R. bellii* are not large, compared to free-living bacteria such as the enterics. The stability of allele frequencies over time has been shown to estimate the relative effects of different population sizes on observed population structure.[28] The stability of RFLP allele frequencies has been studied in the Ohio populations of *R. bellii*.[15] The relative stability observed for these frequencies suggests that the population size of this species is not extremely small (measured relative to the parameters of mutation and selection which determine how rapidly genetic drift can occur).[19] Bacterial population sizes, in general, are expected to be much larger than those observed in multicellular eukaryotes.[4] Rickettsial populations, however, show levels of intrapopulational gene diversity which are not unlike those seen for many vertebrate species. Why might this be so? Note that the gene diversity in higher organisms is much lower than in species such as *E. coli*, reflecting in part the differences in population sizes.[29] Population sizes of higher organisms would certainly be considered small in comparison to almost any bacterial population. One possibility is that, from an evolutionary viewpoint, rickettsiae may be considered to be essentially one bacterial "individual" per host (i.e., per tick). The same seems to be true of organelle genomes in eukaryotic organisms,[30] even though there are many (sometimes hundreds) of organelles in a single cell.

If we take this view, and its analogy with the intracellular organelle, two consequences may be deduced. First, the effective population size may be reduced by 10-fold or even 100-fold compared to a free-living bacteria with an equivalent number of bacterial cells in the population. Further reduction in effective population size would accompany any reduction in the importance of horizontal transmission between hosts, compared to vertical transovarial transmission. The effect in reducing effective population size will become most pronounced when only transovarial transmission occurs (as it does in the case of the vertebrate mitochondrion). The second consequence of having more vertical transmission than horizontal transmission is the reduction in the opportunity for genetic exchange between clones or subclones. Consequently, there will be an increase in the ratio of interpopulational to intrapopulational diversity.[31] Additionally, we must ask how frequently multiply infected hosts occur. Such hosts would be the equivalent of "heteroplasmic" individuals for organelle genomes. Only with multiple infection is there an important opportunity for genetic exchange between bacterial strains.

Let us return to a consideration of the possibility that population bottlenecks have affected the levels of genetic variation in *Rickettsia*. The residual effect of a population bottleneck would persist for a very long time, possibly millions of years.[32,33] If the bacterial population suffered a bottleneck, it seems reasonable

that insufficient time has elapsed in a species such as *R. rickettsii* to allow for the reintroduction of significant new variability.[32] The fact that the greatest intraspecific genetic differences in the North American SFG seem to exist between populations separated by the Rocky Mountains[15,16] suggests that founding effects and population bottlenecks may be playing an important role in the evolution of this group. The levels of genetic variation to be found in *R. conori* in the Old World offer interesting possibilities for comparison with the North American SFG.

An effect similar to that seen from a population bottleneck could occur if there are frequent cycles of periodic selection sweeping through the entire population, removing preexisting variability. In populations such as the enterics, recombination is able to mute the effects on the entire genome of cycles of periodic selection. In a species with the structure of the tick-borne rickettsiae, recombination may not be effective. Again, the relative importance of horizontal versus vertical transmission comes into play in determining how periodic selection would affect the population. If vertical transmission is much more important in the effective transmission of the bacteria from one generation of host to the next, then periodic selection must affect both bacteria and host to be important. If horizontal transmission between hosts is the primary mode of intergenerational exchange, then periodic selection might affect only the bacteria.

Finally, we are continuously struck by the analogy between the SFG rickettsiae and cellular organelles, especially mitochondria. From an evolutionary point of view, how similar might be the factors which homogenize the rickettsia within a host to those factors which make hosts homoplasmic for mitochondrial genomes? The intracellular lifestyle, per se, may be one which fosters low genetic variation. It is interesting that the intracellular bacteria *Mycobacterium leprae* has recently been reported to be essentially homogeneous.[34]

The examination of organelle genome variation in a variety of eukaryotic organisms has revealed that segregation of organelles within heteroplasmic individuals occurs at a rapid rate in most organisms. This results in the observation that most eukaryotic cells are effectively homoplasmic, despite the fact that the progenitor zygote may initially be a mixture of two organelle haplotypes.[30] As mentioned above, it is known that the factors affecting transmission of organelle genomes within a population act to reduce the effective population size of the organelle gene pool, compared to the nuclear gene pool of the same set of individuals.[31,35]

It is very likely that similar factors will be affecting the transmission and, ultimately, the levels of genetic variation for the obligate intracellular members of the rickettsiae. The one clear difference which exists between rickettsiae and organelles is, again, the possibility of horizontal transmission of the rickettsial organism. The determination of the relative importance of vertical versus horizontal transmission in natural populations of the invertebrate host would seem to be a fertile future area of research.

SUMMARY

The population structure of tick-borne rickettsiae show the following characteristics: (1) the amount of genetic differentiation between strains within subpopulations is very small. (2) The evolution of the subpopulations does not fit into models based on either host or geographic similarities, suggesting the need for more information on the frequency of vertical versus horizontal transmission of

strains between hosts. (3) The species are highly clonal, with little evidence of genetic exchange between populations. (4) The dominant class of genetic change is single-nucleotide point mutation. No evidence for major rearrangements was observed. (5) Differentiation between species of the spotted fever group is equivalent to that seen between local strains of *E. coli*.

REFERENCES

1. SELANDER, R. K., D. A. CAUGANT & T. S. WHITTAM. 1987. Genetic structure and variation in natural populations of *Escherichia coli*. *In Escherichia coli and Salmonella typhimurium:* Cellular and Molecular Biology. J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter & H. E. Umbarger, Eds.: 1625–1648. American Society for Microbiology. Washington, D.C.
2. YOUNG, J. P. W. 1989. The population genetics of bacteria. *In* Genetics of Bacterial Diversity. D. A. Hopwood & K. F. Chater, Eds.: 417–438. Academic Press. New York.
3. RICKETTS, H. T. 1909. Some aspects of Rocky Mountain spotted fever as shown by recent investigations. Med. Rec. **76:** 843–855.
4. SELANDER, R. K. & B. R. LEVIN. 1980. Genetic diversity and structure in *Escherichia coli* populations. Science **210:** 545–547.
5. MILKMAN, R. & A. STOLTZFUS. 1988. Molecular evolution of the *Escherichia coli* chromosome. II. Clonal segments. Genetics **120:** 359–366.
6. MARUYAMA, T. & M. KIMURA. 1981. Genetic variability and effective population size when local extinction and recolonization of subpopulations are frequent. Proc. Natl. Acad. Sci. USA **77:** 6710–6714.
7. ATWOOD, K. C., L. K. SCHNEIDER & F. J. RYAN. 1951. Selective mechanisms in bacteria. Cold Spring Harbor Symp. Quant. Biol. **16:** 345–355.
8. HARTL, D. & D. DYKHUIZEN. 1984. The population genetics of *Escherichia coli*. Annu. Rev. Genet. **18:** 31–68.
9. STOLTZFUS, A., J. F. LESLIE & R. MILKMAN. 1988. Molecular evolution of the *Escherichia coli* chromosome. I. Analysis of structure and natural variation in a previously uncharacterized region between *trp* and *tonB*. Genetics **120:** 345–358.
10. DUBOSE, R. F., D. E. DYKHUISEN & D. L. HARTL. 1988. Genetic exchange among natural isolates of bacteria: Recombination within the *phoA* gene of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **85:** 7036–7040.
11. DYKHUISEN, D. E. & L. GREEN. 1986. DNA sequence variation, DNA phylogeny, and recombination in *E. coli*. Genetics **113:** s71.
12. NEI, M. & W-H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA **76:** 5269–5273.
13. HARSHMAN, L. & M. RILEY. 1980. Conservation and variation of nucleotide sequences in *Escherichia coli* strains isolated from nature. J. Bacteriol. **144:** 560–568.
14. REGNERY, R. L. & C. L. SPRUILL. 1984. Extent of genetic heterogeneity among human isolates of *Rickettsia prowazekii* as determined by restriction endonuclease analysis of Rickettsial DNA. *In* Microbiology, 1984. L. Lieve & D. Schlessinger, Eds.: 297–300. American Society for Microbiology. Washington D.C.
15. POETTER, K., C. PRETZMAN, P. S. PERLMAN & P. A. FUERST. 1990. Population genetics of intracellular bacteria: Low levels of genetic variation in *Rickettsia bellii*. Manuscript in preparation.
16. POETTER, K., D. RALPH, C. PRETZMAN, P. S. PERLMAN & P. A. FUERST. 1990. Population genetics of intracellular bacteria. II. Variation within species of the spotted fever group rickettsiae. Manuscript in preparation.
17. DENNY, T. P., M. N. GILMOUR & R. K. SELANDER. 1988. Genetic diversity and relationships of two pathovars of *Pseudomonas syringae*. J. Gen. Microbiol. **134:** 1949–1960.
18. NEI, M. 1987. Molecular Evolutionary Genetics. Columbia University Press. New York.

19. WRIGHT, S. 1969. Evolution and Genetics of Populations. Vol. II. The Theory of Gene Frequencies. University of Chicago Press. Chicago.
20. WHITTAM, T. S., H. O. OCHMAN & R. K. SELANDER. 1983. Multilocus genetic structure in natural populations of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **80:** 1751–1755.
21. SELANDER, R. K., R. M. MCKINNEY, T. S. WHITTAM, W. F. BIBB, D. J. BRENNER, F. S. NOLTE, P. E. PATTISON. 1985. Genetic structure of populations of *Legionella pneumophila*. J. Bacteriol. **163:** 1021–1037.
22. POETTER, K., D. RALPH, C. PRETZMAN, P. S. PERLMAN & P. A. FUERST. 1990. Population genetics of intracellular bacteria. III. Interspecific differences between members of the spotted fever group rickettsiae. Manuscript in preparation.
23. REGNERY, R. 1990. Use of DNA probes for differentiation of spotted fever group and other rickettsiae. Ann. N.Y. Acad. Sci. This volume.
24. SPRUILL, C. L. & R. L. REGNERY. 1990. Analysis of *Rickettsia tsutsugamushi* amplified DNA that encodes an antigenic gene product. Ann. N.Y. Acad. Sci. This volume.
25. CLARK, J., C. PRETZMAN, P. S. PERLMAN & P. A. FUERST. 1990. Evolutionary relationships between tick-borne rickettsiae revealed by 16S rRNA gene sequences. Manuscript in preparation.
26. OCHMAN, H. & A. C. WILSON. 1987. Evolutionary history of enteric bacteria. *In Escherichia coli and Salmonella typhimurium:* Cellular and Molecular Biology. J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter & H. E. Umbarger, Eds.: 1649–1654. American Society for Microbiology. Washington, D.C.
27. BROWN, W. 1985. The mitochondrial genome of animals. p. 95–130. *In* Molecular Evolutionary Genetics. R. J. MacIntyre, Ed.: 95–130. Plenum. New York.
28. NEI, M. & F. TAJIMA. 1981. Genetic drift and estimation of effective population size. Genetics **106:** 569–574.
29. NEI, M. 1983. Genetic polymorphism and the role of mutation in evolution. *In* Evolution of Genes and Proteins. M. Nei and R. K. Koehn, Eds.: 165–190. Sinauer. Assoc. Sunderland, Mass.
30. BIRKY, C. W. 1990. Evolution and population genetics of organelle genes: Mechanisms and models. *In* Molecular Evolution. A. G. Clark, T. Whitham & R. Selander, Eds. Sinauer Assoc., Sunderland, Mass. In press.
31. BIRKY, C. W., P. FUERST & T. MARUYAMA. 1989. Organelle gene diversity under migration, mutation, and drift: Equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. Genetics **121:** 613–627.
32. MARUYAMA, T. & P. FUERST. 1985. Population bottlenecks and nonequilibrium models in population genetics: III. Genic homozygosity in populations which experience periodic bottlenecks. Genetics **111:** 691–703.
33. NEI, M., T. MARUYAMA & R. CHAKRABORTY. 1975. The bottleneck effect and genetic variability in populations. Evolution **29:** 1–10.
34. CLARK-CURTISS, J. E. & G. P. WALSH. 1989. Conservation of genomic sequences among isolates of *Mycobacterium leprae*. J. Bacteriol. **171:** 4844–4851.
35. BIRKY, C. W., T. MARUYAMA & P. FUERST. 1983. An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts and some results. Genetics **103:** 513–527.