MITOCHONDRIAL DNA TRANSMISSION GENETICS IN CRICKETS

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ABSTRACT

This paper presents the results of a single generation study of the transmission genetics of mitochondrial DNA in the field cricket *Gryllus firmus*. In this species, individuals heteroplasmic for at least two different-sized mitochondrial genomes can be collected easily from natural populations. The frequencies of mtDNA size variants in heteroplasmic females and samples of their offspring were estimated by densitometry of autoradiographs. The variance in mitochondrial genotype frequencies among the offspring of heteroplasmic females indicates that, through genetic drift, fixation would take several hundred animal generations. Differences between the observations and data on mtDNA transmission in yeast and cows are discussed in light of the differences in organelle sampling regime and early developmental events in these species. Our data also show shifts in genotype frequencies in the transmission from mother to offspring that suggest a bias in favor of smaller genomes. The nature of mtDNA size variation in natural populations of crickets is discussed in reference to a mutation-selection balance.

OVER the past several years, mitochondrial DNA (mtDNA) has been used increasingly as a marker for genetic variation within and among populations and for phylogenetic studies of closely related species (AVISE *et al.* 1979; BROWN and SIMPSON 1981; BROWN *et al.* 1982; FERRIS *et al.* 1983a,b; LANSMAN *et al.* 1983). Although mtDNA is becoming a standard population genetic tool, surprisingly little is known about the nature of its inheritance in higher organisms. To properly interpret the patterns of mtDNA variation in natural populations we must understand the transmission rules of this molecule in a diversity of taxa.

Surveys of mtDNA variation in arthropods and vertebrates have revealed considerable intraspecific heterogeneity in mtDNA sequences (AVISE *et al.* 1979; LANSMAN *et al.* 1983; HALE and SINGH 1985; SAUNDERS, KESSLER and AVISE 1986). Until recently, however, there has been little evidence for heteroplasmy, or the presence of more than one mtDNA type in the cells of individual animals (BROWN and DESROSIERS 1983; SOLIGNAC, MONNEROT and MOUNOLOU 1983; HAUSWIRTH *et al.* 1984; MONNEROT, MOUNOLOU and SOLIG-

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NAC 1984; BERMINGHAM, LAMB and AVISE 1985; DENSMORE, WRIGHT and BROWN 1985; HARRISON, RAND and WHEELER 1985). In metazoan animals, mtDNA is maternally inherited (DAWID and BLACKLER 1972; HUTCHINSON *et al.* 1974; FAURON and WOLSTENHOLME 1980a,b; LANSMAN, AVISE and HUETTEL 1983). Thus, for a novel mitochondrial lineage to become established in a population, a heteroplasmic state must intervene at least briefly, following a mutation of a mtDNA molecule in a female germline cell. The early observations of considerable variation among individuals but little or no variation within individuals were taken as support for the idea that the fixation of novel mitochondrial lineages is a relatively rapid process (UPHOLT and DAWID 1977; TAKAHATA and MARUYAMA 1981; HAUSWIRTH and LAIPIS 1982; TAKAHATA and PALUMBI 1985). The discovery of easily detectable levels of heteroplasmy in a variety of organisms now provides the necessary markers with which to study the transmission genetics of a mixed population of organelles in animals.

In this paper we present the results of a single generation study of the transmission genetics of mtDNA in the field cricket *Gryllus firmus*. This species and a closely related cricket, *G. pennsylvanicus*, show variation in the size of their mitochondrial genomes (HARRISON, RAND and WHEELER 1985). Moreover, approximately 15% of several hundred field-collected individuals are heteroplasmic for mtDNA size variants. Southern blot analysis and densitometry were carried out on samples of individual offspring of seven different heteroplasmic females to determine the relative frequencies of the two (or three) mtDNA size variants within each individual. The data provide estimates of the variance among offspring in the relative frequencies of mitochondrial genotypes. From our data we estimate that fixation or loss of mtDNA variants from an initial heteroplasmic condition would require several hundred animal generations. Differences between the results presented here and those from studies in other organisms (*cf.* BIRKY 1983; HAUSWIRTH and LAIPIS 1982) are discussed in reference to species-specific differences in the organelle sampling regime and in the early developmental events leading to the determination of the primary germ cells.

The data further reveal systematic shifts in genotype frequencies in the transmission from mother to offspring, suggesting a bias in favor of smaller genomes. Given that at least six mtDNA size variants are detectable in natural cricket populations, the presence of larger genomes is discussed in light of a possible mutation-selection balance.

MATERIALS AND METHODS

Establishment of heteroplasmic lines: Heteroplasmic lines were obtained by collecting a large number of field-inseminated female crickets from southern Connecticut and central Virginia. Single females were allowed to lay eggs in oviposition dishes in the laboratory. Eggs from each female were placed in chill to allow for synchronous post-diapause hatching. Total DNA was isolated from the individual females. Heteroplasmic females were identified by *Eco*RI digests (see HARRISON, RAND and WHEELER 1985), and the eggs from these females were later removed from chill and allowed to hatch. Total DNA was then isolated from individual nymphs at the third or fourth quarter.

DNA isolation and Southern blot analysis: The procedures for isolation of total DNA and pure mtDNA for use as a nick-translated probe have been described previ-

ously (HARRISON, RAND and WHEELER 1985, 1986) and do not differ from those used in this study.

Samples of approximately 1 μg of total cricket DNA were digested to completion with *Eco*RI (New England Biolabs), run on horizontal 0.7% agarose gels and transferred to nitrocellulose (MANIATIS, FRITSCH and SAMBROOK 1982).

A minimum of two separate digests and gel runs was done for each set of mother and offspring. The Southern blots obtained from these replicate gels were hybridized with independent nick translations of the same whole mtDNA probe. Moreover, to estimate the effects of duration of exposure on the relative intensity of bands on the X-ray films, at least two different exposures of each hybridized Southern blot were made. Thus, differences in the values of the relative frequencies of the two (or three) mitochondrial genotypes obtained for the same individuals from different Southern blots provide an estimate of experimental error (see below).

Densitometry: The autoradiographs obtained, as described above, were scanned on a Bio-Rad laser densitometer interfaced with a printer that recorded peaks of absorbance. The densitometer was calibrated with each scanning of an autoradiograph, such that the darkest band was set as top-of-scale and the lightest interband area was set as background. Some autoradiographs were overexposed and could not be properly zeroed, or they produced square peaks on the chart recorder. Such autoradiographs were not included in the data set.

A digital planimeter was used to determine the area of the peaks corresponding to the bands on an autoradiograph. Each peak was traced three times, and the mean value was taken as the area (the error due to tracing was approximately 0.1%). The relative areas under the peaks were taken as the estimates of relative frequencies of the mitochondrial genotypes in each individual. Due to differences in the intensity of bands on some autoradiographs, it was not possible to score all sampled offspring of each female on all autoradiographs. Since each autoradiograph of the same set of individuals produced slightly different estimates of relative frequencies, the final values assigned to an individual were means of the values for that individual obtained from each of the replicate autoradiographs. The average standard error of the frequency estimates of all individuals was 0.024 (standard deviation of this mean was 0.016).

The quantification of absolute differences in nucleic acid concentrations between lanes of a gel by densitometry must be done in reference to a proper control. Heteroplasmic individuals provide an internal control since all comparisons between lanes (*i.e.*, between individuals) are of relative frequencies within lanes. This internal control could be jeopardized if the relative intensities of bands within a lane are greatly influenced by absolute differences in the intensity of signals between lanes. Figure 1 shows seven different densitometer tracings of the same triplasmic individual (female 2) from five different autoradiographs, one of which was exposed for three different lengths of time. Under each peak is the area of that peak relative to the area under all three peaks. This value is equated with the relative frequency of that mitochondrial size variant in the heteroplasmic individual. In the lower right is the mean frequency for each mitochondrial genotype and the standard error of the seven tracings. The results indicate that absolute differences in intensity have a small and nondirectional effect on relative intensities of bands. These comparisons suggest that the experimental variation is a very small proportion of the true variation in relative frequencies among individuals.

RESULTS

Size variation and heteroplasmy: In the sample of heteroplasmic females analyzed in this study, mitochondrial genomes of four different sizes were evident. Each genome differed from genomes of adjacent size classes by approximately 220 base pairs (bp); "*L*" = 16.80 kb, "*M*" = 16.48 kb, "*S*" = 16.26 kb, and "*VS*" = 16.04 kb. Two additional, larger mtDNA size classes have been identified and also appear to differ in size by approximately 220 bp.

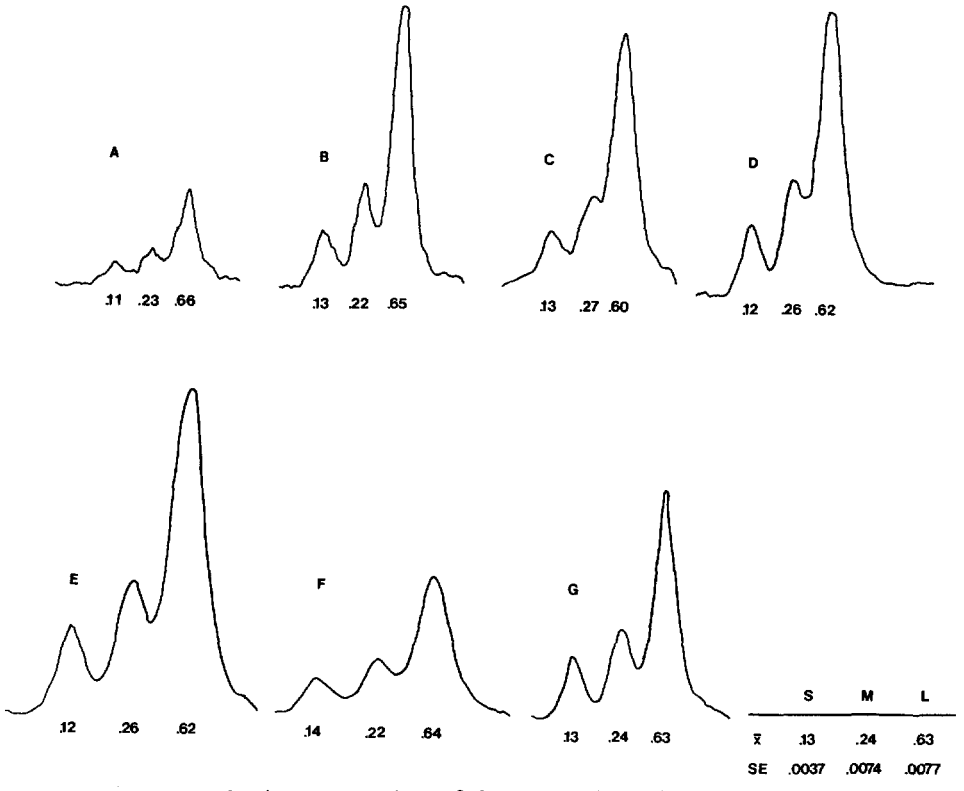

FIGURE 1.—Seven densitometer tracings of the same individual (female 2). A–E, Tracings of autoradiographs from five different Southern blots. E–G, Tracings from the same Southern blot exposed for three different lengths of time. The values under each peak are estimates of the relative frequencies of the three mitochondrial genome size variants in the same individual. The values in the lower right of the figure are the mean frequency and the standard error for each of the three mitochondrial genotypes ("*S*", "*M*" and "*L*") in all seven tracings.

Preliminary sequence data from the variable length region reveal a 222-bp tandem repeat with 14-bp terminal palindromic repeats (D. RAND, unpublished sequence data). This suggests that mtDNA size variation in Gryllus is due to variation among molecules in the number of tandem repeats. A more detailed analysis of this size variation, and its distribution in natural populations, will be described in subsequent reports. For our purposes here, the mtDNA size variants serve as markers with which to study the transmission of a mixed population of organelle genomes.

Of the seven heteroplasmic female lines studied, three were heteroplasmic for three different mtDNA size classes (*i.e.*, were "triplasmic"), and the remaining four were heteroplasmic for two mtDNA size classes (*i.e.*, were "biplasmic"). All but one of the females were heteroplasmic for mtDNA size variants that differed from one another by a single size interval. The one exception (female 27) was heteroplasmic for the "*VS*" and "*M*" mitochondrial genomes.

Single generation transmission: Figure 2 is an autoradiograph of a South-

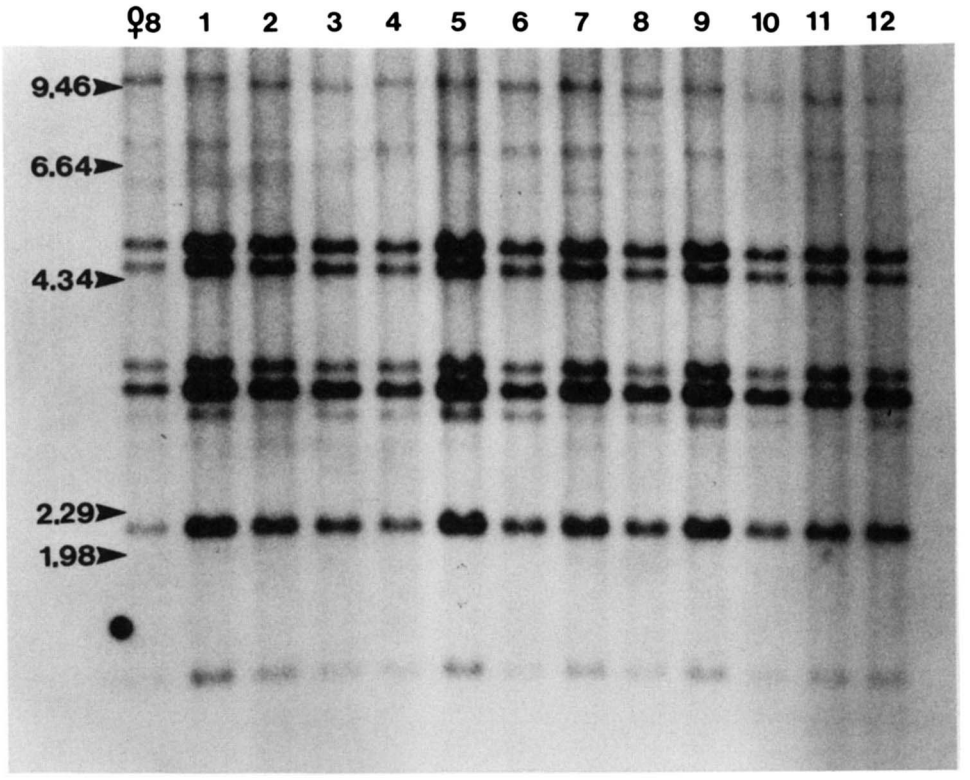

FIGURE 2.—Autoradiograph of heteroplasmic female 8 (lane 1) and 12 of her offspring (lanes 2–13). The middle three bands show variation in the relative frequencies of the three mitochondrial genotypes ("*VS*," "*S*" and "*M*") in the heteroplasmic offspring.

ern blot of female 8 and 12 of her offspring. The relative intensities of the three middle, variable bands in the mother (lane 1) indicate that the intermediate-sized mitochondrial genome is present in a higher proportion than the other two genomes. The variation in the relative intensities of the same bands in lanes 2–13 show that the proportions of the three genomes are different in each offspring. Figure 3 shows densitometer tracings of the middle three bands of the autoradiograph in Figure 2, with the relative frequencies of the three mitochondrial genotypes indicated under each peak. Two further statistics are listed below the tracing and frequencies of the mother: (1) the mean genotype frequency among the 12 offspring ($\bar{p}$) and (2) the variance in frequency among these offspring (V). The difference between the mother's relative frequencies (*i.e.*, "input frequencies") and the means of the relative frequencies among the offspring (*i.e.*, "output frequencies"), indicate that the two smaller genomes are present in higher frequencies in the sample of offspring.

The replicate autoradiographs of each female line (see MATERIALS AND METHODS) were quantified as in Figure 3 and are summarized in Table 1. Table 1 shows the frequency of the two (or three) mitochondrial size variants in the mother, the mean frequency of those genotypes among a sample of her off-

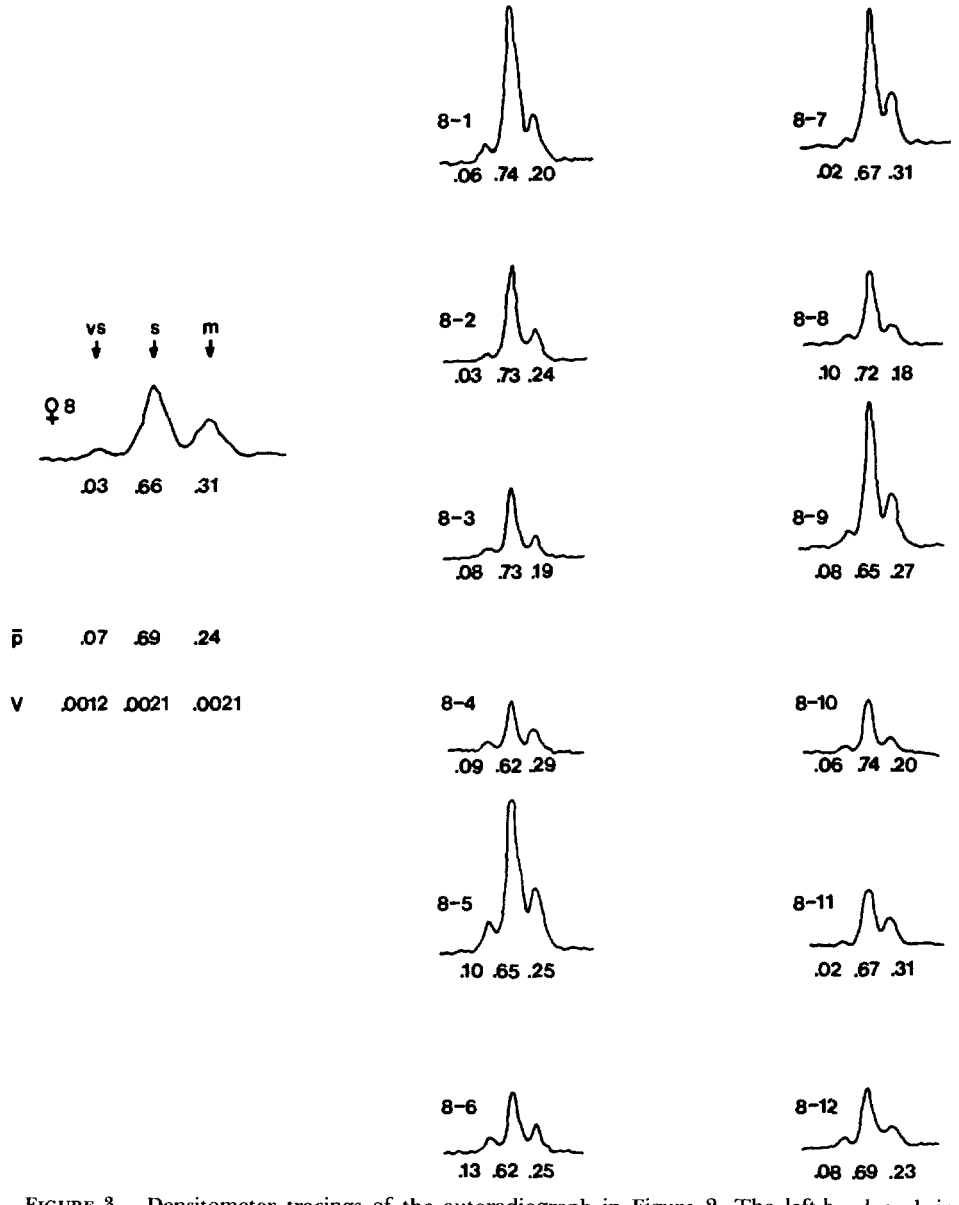

FIGURE 3.—Densitometer tracings of the autoradiograph in Figure 2. The left-hand peak in each tracing is the smallest of the three mitochondrial genotypes indicated in Figure 2. The relative frequency of each genome is indicated under each peak. $\bar{p}$ is the mean frequency of each genome among the 12 offspring; V is the variance in frequency among the offspring. The differences in frequencies between the mother (female 8) and the sample of her offspring show an increase in the frequencies of the smaller genomes.

spring, the variance in frequency of the *largest* mtDNA size class among these offspring, and the number of offspring used to obtain the mean and variance values. The female lines are listed in Table 1 in order of increasing frequency

TABLE 1

Frequencies of mtDNA size variants in mothers and samples of their offspring

Female line	Genotype frequencies				V(largest)	No. of nymphs
	f(*VS*)	*f*(*S*)	*f*(*M*)	*f*(*L*)		
Female 11		0.55	0.35	0.10		
Offspring		0.61	0.32	0.07	0.0025	12
Female 27	0.75		0.25			
Offspring	0.84		0.16		0.0137	23
Female 89		0.715	0.285			
Offspring		0.73	0.27		0.0051	9
Female 8	0.03	0.68	0.29			
Offspring	0.06	0.72	0.22		0.0052	17
Female 3		0.43	0.57			
Offspring		0.44	0.56		0.0267	11
Female 2		0.13	0.24	0.63		
Offspring		0.14	0.26	0.60	0.0165	11
Female 1		0.26	0.74			
Offspring		0.24	0.76		0.0051	11

The four mtDNA size classes are represented as *VS* = very small, *S* = small, *M* = medium, *L* = large. *V*(largest) is the variance in frequency of the largest mtDNA size class among the sample of offspring indicated in the last column (no. of nymphs).

of the *largest* genotype (*e.g.*, 10% is the frequency of "*L*" for female 11, and 74% is the frequency of "*M*" for female 1).

Input and output frequencies: In six of the seven female lines studied, the mean frequency of the largest mitochondrial genotype among the offspring was lower than the frequency of that genotype in the mother (see Table 1). From a Wilcoxon signed-rank test, this decrease is significant ($P < 0.04$; SOKAL and ROHLF 1981). In the same six of seven lines studied there is an increase in the frequency of the smallest genotype in the generation from mother to offspring, but the increase is not significant ($P < 0.07$). The difference in the results of the significance tests is due to the presence of the triplasmic lines. With three mitochondrial size classes within a female line, a decrease in the frequency of the largest genotype is not necessarily associated with an equivalent increase in the frequency of the smallest genotype. Thus, the absolute values of the rank scores change when the test is done for either an increase in the smallest genotype or a decrease in the largest genotype.

Genetic drift model of mitochondrial transmission: In a study of mitochondrial transmission in *Drosophila mauritiana*, SOLIGNAC *et al.* (1984) modified a classic genetic drift equation used by WRIGHT (1968) to model the evolution of the distribution variances. This model can be used to predict the number of generations required for *all* descendants of a heteroplasmic female to become homoplasmic. Moreover, the fixation process can be modeled from any arbitrarily chosen (or empirically determined) initial frequencies. This approach is appropriate for studying the predicted outcome of random segrega-

TABLE 2

Estimated number of mitochondria per cell and number of generations to fixation in homoplasmic cells based on genetic drift model (see text)

Female line	V	α	N	n(fix)	n(half)
11	0.0025	0.9722	355	510	24
27	0.0137	0.9269	132	170	8
89	0.0051	0.9750	395	510	26
8	0.0052	0.9747	391	510	26
3	0.0267	0.8911	87	120	5
2	0.0165	0.9292	137	180	9
1	0.0051	0.9734	371	480	25

V = V(largest) from Table 1. α is from equation (2) (see *text*). N = the estimated number of segregating mitochondria. n(fix) = the estimated number of generations to fixation. n(half) = the estimated number of generations required to reduce by one-half the difference between the initial variance and the fixation variance.

tion processes. Although the above data suggest that mitochondrial transmission in crickets may not be entirely random, an initial assumption of random transmission is useful in understanding the process of fixation and loss.

The approach used by SOLIGNAC *et al.* (1984) relates the variance at the nth generation, Vn, to the frequency of one mitochondrial genotype in the initial female (here the largest *Eco*RI variant), the number of segregating units taken from an infinite pool, N, and the number of germ-cell generations per animal generation, g:

$$Vn = p(1 - p)[1 - (1 - 1/N)^{gn}] \tag{1}$$

Since N and g are unknown, by setting

$$\alpha = (1 - 1/N)^g, \tag{2}$$

the equation can be modified to

$$Vn = p(1 - p)(1 - \alpha^n). \tag{3}$$

From Table 1, Vn and p can be used to find α with $n = 1$:

$$\alpha^n = \frac{p(1 - p) - Vn}{p(1 - p)}. \tag{4}$$

Equation 2 can then be rearranged to solve for N as follows:

$$N = \frac{1}{1 - \sqrt[g]{\alpha}}. \tag{5}$$

Table 2 shows for each female line the initial frequency of the largest genotype in the mother, the variance in frequency of that genotype among the offspring, the calculated values of α and N, and an estimate of the number of generations required for all descendants to be homoplasmic (*i.e.*, fixation/loss). To obtain N one must know g, the number of germ-cell generations per animal

generation. SOLIGNAC *et al.* (1984) used $g = 10$ for *D. mauritania.* For comparison we have used the same value for *G. firmus.*

The number of generations to fixation or loss, n(fix), was determined with a simple computer program that iterates (3) through n generations. Vn was calculated to six decimal places for each female line every ten generations, n(fix) being taken as the generation in which $Vn = p(1 - p)$. It is clear from (3) that Vn goes to $p(1 - p)$ as α^n goes to zero. n(fix) can thus be made arbitrarily large by calculating Vn to many decimal places. To circumvent this problem we calculated the number of generations required to reduce the difference between the initial variance and the maximum (fixation/loss) variance by one-half (see Table 2).

DISCUSSION

Our single generation data suggest that the transmission rules for mtDNA in Gryllus are similar to those in Drosophila. Estimates of the variance in genotype frequencies among offspring of heteroplasmic females are comparable and indicate that heteroplasmy would be maintained for several hundred animal generations in both crickets and flies. To determine the relative frequencies of the mitochondrial genotypes in a single fly, SOLIGNAC *et al.* (1984) prepared mtDNA from the pool of unfertilized eggs obtained from the F_2 descendants of that fly. Frequency estimates were based on densitometry of photographs of ethidium-bromide-stained gels of digested mtDNA. Variance estimates were made after three to five animal generations (minimum variance = 0.0095; maximum = 0.0296). In the present study of Gryllus we were able to estimate the relative frequencies of mitochondrial genotypes in heteroplasmic individuals directly by densitometry of autoradiographs. Because of the longer generation time, however, we quantified only a single generation of transmission.

Given these methodological differences, the variance estimates for Gryllus and Drosophila are remarkably similar. Thus, by applying the transmission variance estimates in crickets to the genetic drift model of SOLIGNAC *et al.* (1984), the estimates of N, n(fix) and n(half) should, and do, agree well with the respective values in Drosophila (400 mitochondria per cell, 500 generations to fixation and 26 generations to the "half-way" variance, assuming ten germ-cell generations per animal generation). CHAPMAN *et al.* (1982) have adapted equations of KIMURA and OHTA (1969) to predict the average number of generations to fixation of an organelle genotype in heteroplasmic cells. Their equation requires an estimate of the number of segregating units. Since this value must be obtained from (1)–(5) above, we have used the evolution of the variance approach to characterize the process of fixation/loss.

In light of the predicted number of generations to fixation/loss, it is notable that most examples of heteroplasmy involve mtDNA size variation rather than restriction site variation (see the Introduction). If these two types of mtDNA variants are neutral, they will presumably obey the same rules of transmission (however, see below for discussion of a transmission bias favoring smaller mitochondrial genomes). That restriction-site heteroplasmy is so rare may be the

result of a much lower mutation rate for base substitution than for size variation. As shown by BIRKY, MARUYAMA and FUERST (1983), it is the waiting time between mutations in relation to the number of generations to fixation/loss that determines levels of heteroplasmy.

Variation among taxa in the rules of transmission: The data from crickets and flies are in contrast to those from yeast (BIRKY 1978, 1983) and cows (HAUSWIRTH and LAIPIS 1982; OLIVO *et al.* 1983). In yeast, mitochondrial transmission can be studied by mating two haploid cells that differ in mitochondrial genotype. The resulting heteroplasmic diploid zygote reproduces by budding to produce a colony of diploid cells, or a zygote clone (BIRKY 1978, 1983). At each mitotic division there is an opportunity for random drift in the frequencies of the mitochondrial genotypes. In *Saccharomyces cerevisiae*, only about 20 generations are required for each of the cells in the zygote clone to become homoplasmic; this is presumably a result of vegetative segregation of mitochondria during mitosis (BIRKY 1983). Assuming that ten cell generations per animal generation in crickets and flies is accurate, fixation in yeast occurs about 100 times faster than in crickets or flies. This rapid fixation is most likely due to the relatively smaller number of segregating mitochondria in yeast.

Differences in the nature of mitosis in the budding yeast *S. cerevisiae* and the fission yeast *Schizosaccharomyces pombe* do suggest that the sampling regime can influence the transmission process. In Saccharomyces, a daughter cell is formed by budding from the parent cell, and far fewer than one-half of the mitochondria in the parent cytoplasm are passed into the newly formed bud. In Schizosaccharomyces, cytokinesis is more balanced, essentially dividing the mitochondrial population in half (BIRKY 1983; THRAILKILL *et al.* 1980). Fixation/loss through vegetative segregation takes longer in Schizosaccharomyces than in Saccharomyces (BIRKY 1978, 1983). Assuming the total number of mitochondria do not differ greatly between the two species, this observation is to be expected since the number of segregating units is greater in Schizosaccharomyces than in Saccharomyces.

In a lineage of Holstein cows descended from a single female, HAUSWIRTH and LAIPIS (1982) have identified a mtDNA restriction site polymorphism. Although they do not have DNA from the original female, their results suggest that this female was heteroplasmic for two different mitochondrial genotypes. Of the 16 animals scored for mitochondrial genotype in the Holstein pedigree, 11 animals were fixed for one mtDNA restriction site variant and five were fixed for another. BIRKY has calculated that the effective number of segregating units is 65–163 in Holsteins (W. BIRKY, personal communication; from data provided by W. HAUSWIRTH). Our estimates in crickets (87–395; see Table 2) indicate that fixation/loss would be reached faster in cows than in crickets, but probably not by an order of magnitude.

In a subsequent sequence analysis, OLIVO *et al.* (1983) identified four different mitochondrial genotypes in the Holstein lineage. Their data and the pedigree from HAUSWIRTH and LAIPIS (1982) suggest that three instances of complete genotypic shifts (*e.g.*, $p = 0$ to $p = 1.0$) occurred in five generations.

Assuming 50 cell generations per Holstein generation (see UPHOLT and DAWID 1977), ten cell generations per cricket generation, and an equivalent number of mitochondria per germline cell, this translates to approximately 25 cricket generations. For comparison then, would one expect to observe a comparable frequency of genotypic shifts in 25 cricket generations? If one assumes random sampling, at fixation/loss (*i.e.*, all descendants homoplasmic) the proportion of offspring fixed for the rare mtDNA will equal the frequency of that mtDNA type in the original female. With 100 mitochondria per cell and two mtDNA molecules per mitochondrion, a new mutant molecule would have an initial frequency of $p = 0.005$. Fixation/loss in crickets is estimated to be reached in well over 100 generations (see Table 2). In 25 generations, fewer than one in 200 cricket offspring would be expected to become fixed for a rare ($p = 0.005$) mtDNA type. These observations further suggest that fixation is achieved much more rapidly in cows than in insects.

Differences between taxa in early developmental events: In the early embryo of insects many nuclei are present in the egg cytoplasm. These nuclei later migrate to the periphery, where cellularization takes place to form the syncitial blastoderm. At the posterior end of the embryo, two pole cells are formed that ultimately give rise to the germline. It is during the formation of these cells that the population of mitochondria in the syncitium is sampled and germline mitochondria are sequestered. This is clearly a different sampling process than that which occurs during the holoblastic cleavage of many vertebrate zygotes. In these cases, cytoplasm is essentially divided in half at each division (with the exception of the first transverse cleavage, which results in animal pole cells considerably smaller than vegetal pole cells). Paradoxically, however, one would predict that sequestration of the pole cells from a heteroplasmic syncitium would lead to higher variance among descendant cells. This would lead to more rapid fixation/loss in insects than in organisms with holoblastic cleavage—a conclusion inconsistent with the data discussed above.

To address this question properly, comparative data on mtDNA transmission are needed from organisms with different egg cleavage patterns (*e.g.*, holoblastic, meroblastic and superficial). It seems likely that such fundamental differences in early development could influence organelle transmission considerably. With recent discoveries of size heteroplasmy in fish (BERMINGHAM, LAMB and AVISE 1985), frogs (MONNEROT, MOUNOLOU and SOLIGNAC 1984) and lizards (DENSMORE, WRIGHT and BROWN 1985), these comparisons can now be made. However, species-specific differences in cleavage patterns alone may not account for differences in transmission rules. Variation between taxa in the nature of germ cell determination could influence organelle heredity in very characteristic ways.

In insects, descendants of the pole cells become determined as germ cells by the presence of cytoplasmic inclusions known as polar granules. This determination takes place shortly after the sequestration of the pole cells. In many mammals the germ cells are also determined by the presence of polar granules, but this takes place after some tissue-layer differentiation has occurred (KARP and BERILL 1981), which may be several cell generations later than an equiv-

alent stage in insects. EDDY (1975) has compiled a taxonomic list of organisms in which substances implicated in germ-cell determination have been observed in germline tissue. In mammals such substances (polar granules and "nuage") are more frequently associated with mitochondria than in other classes of animals. This could introduce significant mitochondrial clustering, which would tend to accelerate fixation/loss. It is unclear whether differences in the stage of germline determination would affect the variance among germ cells in the frequencies of two mitochondrial genotypes. Not enough is known about the fate of mitochondria in germline cells to resolve this question.

Although early developmental events apparently can influence the patterns of transmission in different taxa, it is not clear that the embryological differences between Holstein cows and insects account for the observed differences in rates of fixation/loss in heteroplasmic lines. A thorough explanation of the rapid genotypic shifts of mtDNA in the Holstein lineage may require more information on the dynamics of nonrandom processes such as mitochondrial gene conversion and/or repeated replication of a small number of mtDNA templates.

BOGENHAGEN and CLAYTON (1977) have shown that mtDNA molecules are selected at random for replication throughout the cell cycle in mouse L-cells (see also CLAYTON 1982). In the cell generations from primary germ cell to mature oocyte in animals, however, the size of the mtDNA population varies considerably. A flush in the production of mitochondria could allow for nonrandom replication of mtDNA templates. If a new mutant genome was overreplicated during this flush stage, fixation could be quite rapid. Even if replication were random, differences in the extent of this flush stage in different animals would suggest that the effects of drift may not be the same in all taxa. Although any one of these processes alone may have a relatively weak effect on transmission, in some generations sampling drift, gene conversion, directional mutation and overreplication could act in concert to produce large genotypic shifts. With major differences between taxonomic groups in the biology of germline sequestration and development (see BUSS 1983), the likelihood of such "concerted drift" occurring, and its potential effect on organelle transmission, will certainly vary between groups.

Transmission bias favoring smaller mitochondrial genomes: The data from yeast indicate that mitochondrial transmission is an essentially random process (BIRKY 1978, 1983; THRAILKILL *et al.* 1980). The results presented above, however, suggest that the transmission of mtDNAs of different sizes may be nonrandom. In six of the seven female lines studied, the smallest mitochondrial genome increased in frequency in the transmission from mother to offspring. SOLIGNAC *et al.* (1984) found evidence for a selective advantage of smaller genomes over ten to 15 generations in heteroplasmic female lines of *D. mauritiana.* It may be that larger genomes are at a disadvantage simply because more time is required to complete the replication of an entire mtDNA molecule. The data from Gryllus female line 27 are suggestive in this light: the mother was biplasmic for mtDNAs that differed by two size intervals (=444 bp), and this line shows the greatest increase of all female lines in the frequency

of the smaller genome. This size effect will be reduced, however, if replication need not be completed before it is reinitiated, *i.e.*, if several replication forks can be active on a single mtDNA molecule.

In Drosophila, size variation of the mitochondrial genome, both within and between species, has been mapped to the AT-rich region lying adjacent to the small rRNA gene. This region contains the origin of replication of the mtDNA molecule (FAURON and WOLSTENHOLME 1980a,b; SOLIGNAC *et al.* 1983, 1984; WOLSTENHOLME and CLARY 1985). In Gryllus, we have evidence that size variation is due to the insertion or deletion of a tandemly repeated sequence of DNA between an AT-rich region and the small rRNA gene (D. RAND, unpublished sequence data). Larger genomes could have several copies of the origin of replication which might introduce a bias in the transmission process. If multiple start sites increased the probability of replication initiation, or if they allowed for multiple replication forks, the prediction would be inconsistent with the observed data.

Alternatively, a higher mutation rate from larger to smaller mtDNA molecules could explain the transmission bias. The effects of such events each generation would be indistinguishable from a replicative advantage of the smaller genomes when measured by densitometry of autoradiographs. Intramolecular recombination would produce smaller mtDNA molecules, whereas intermolecular recombination could allow for larger mtDNA molecules to be produced. It may be that intramolecular recombination occurs more frequently than intermolecular recombination, which would appear as an increase in the frequency of smaller mtDNAs. Additional mechanisms, such as transposition or slippage and mismatching during replication, could also provide molecular bases for the observed bias.

The existence of size variation of the mitochondrial genome in natural populations of Gryllus, and the possibility of a replicative advantage of smaller genomes in heteroplasmic individuals, suggests an interesting mutation/selection balance. If larger genomes are at a selective disadvantage, their presence indicates a high rate of mutation from smaller to larger genomes. However, there is clearly a lower limit to the size of Gryllus mtDNA, as genomes in which the origin of replication has been deleted will be lost rapidly. Suggestive of a mutation/selection balance is the observation that the two largest mitochondrial genomes in Gryllus populations have been found only in heteroplasmic individuals (D. M. RAND and R. G. HARRISON, personal observations). In the absence of a replicative advantage of the smaller genomes, a recombination dynamic between intramolecular and intermolecular recombination or a slip-mismatch dynamic rather than a mutation/selection balance may account for size variation in natural populations. Multiple generation studies of mtDNA transmission in Gryllus and a detailed sequence analysis of the variable region will further elucidate the relationship between genome size and possible selective advantage.

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