Genetica **102/103:** 61–69, 1998.

Deleterious mutations in animal mitochondrial DNA

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Key words: neutral theory, slightly deleterious, evolution, mutations, mitochondrial DNA

Abstract

A simple neutral model predicts that the ratio of non-synonymous to synonymous fixed differences between species will be the same as the ratio of non-synonymous to synonymous polymorphisms within species. This prediction is tested with existing mitochondrial datasets from 25 animal species. In slightly over half of the studies, the ratio of replacement to silent polymorphisms within species is significantly greater than the ratio of replacement to silent fixed differences between species. These observations are best explained by a substantial number of mildly deleterious amino acid mutations that contribute to heterozygosity but rarely become fixed.

Introduction

Understanding the distribution of selection coefficients for newly arising mutations is a major challenge for population genetics. Ultimately, we would like to know what fraction of new mutations are advantageous, strictly neutral, or deleterious. We would also like to know what fraction of new mutations can be classified as borderline (i.e., $s = 1/N_e$, approximately) and whether most of those are deleterious (Ohta, 1972) or whether half are deleterious and half are advantageous (Fisher, 1958).

In its simplest form, the neutral theory asserts that there is a large class of deleterious mutations, a large class of strictly neutral mutations, and few advantageous or borderline mutations (Kimura, 1968). In 1971, Ohta and Kimura stressed the importance of borderline mutations and suggested that there is a large class of mildly deleterious amino acid mutations as a way of accounting for the observation that rates of protein evolution are roughly constant per year rather than per generation. Models of slightly deleterious mutations may explain a number of important observations in molecular evolution (Gillespie, 1995; Ohta & Gillespie, 1996); however, current models include roughly equal numbers of deleterious and advantageous substitutions and depend on a very narrow range of values of $N_e s$ (Gillespie, 1994a, b, 1995). Several models have also been proposed that incorporate a large number of advantageous mutations, where natural selection rather than drift is the main force in molecular evolution (e.g., Gillespie, 1991).

One attractive feature of a simple neutral model lies in its heuristic value. It makes several clear predictions that have been formalized into statistical tests based on the distribution of genetic variation within species (e.g., Watterson, 1978; Sawyer, Dykhuizen & Hartl, 1987; Tajima, 1989; Fu & Li, 1993; Kelly, 1997) or the distribution of variation within and between closely related species (e.g., Hudson, Kreitman & Aguade, 1987; McDonald & Kreitman, 1991; McDonald, 1996). While use of these tests on individual genes may not address the general applicability of the neutral model, single locus studies do provide insight into the forces governing variation at particular genes. Moreover, as data begin to accumulate from many genes, a general picture of molecular evolution may emerge.

These tests have mainly been applied to nuclear gene sequences in *Drosophila* (for reviews see Brookfield & Sharp, 1994; Kreitman & Akashi, 1995), although recent work has also included studies of plants (Gaut & Clegg, 1993a, b), bacteria (Guttman & Dykhuizen, 1994), mice (Nachman & Aquadro, 1994),

and humans (Hammer, 1995; Hey, 1997). Statistical tests of neutrality have also been applied to mitochondrial DNA sequences in *Drosophila* (Ballard & Kreitman, 1994; Rand, Dorfsman & Kann, 1994; Rand & Kann, 1996; Rand & Kann, 1997), mice (Nachman, Boyer & Aquadro, 1994), and humans (Nachman et al., 1996; Templeton, 1996). Studies of the neutrality of mtDNA are of particular interest because mtDNA is widely used as a marker in population and evolutionary research. In addition to statistical tests of mtDNA sequences, several groups have conducted experimental tests for fitness effects of mitochondrial variants (e.g., MacRae & Anderson, 1988; Hutter & Rand, 1995; Kilpatrick & Rand, 1995).

Both statistical analyses of DNA sequences and functional tests for fitness have revealed a variety of non-neutral patterns in animal mitochondrial DNA. One consistent result to emerge from studies of DNA sequences is the observation of a greater ratio of replacement to silent polymorphism within species than of replacement to silent fixed differences between species. This pattern has been documented in mice (Nachman, Boyer & Aquadro, 1994), flies (Ballard & Kreitman, 1994), and humans (Nachman et al., 1996; Templeton, 1996). This group of taxa includes only humans and species commensal with humans, raising the possibility that some of the observed fitness effects are the result of the particular ecology of humans and their commensals. Here I investigate the generality of these results by analyzing patterns of mtDNA sequence variation within and between species in 25 published datasets. A significantly greater ratio of replacement to silent polymorphisms is seen within species than expected under a simple neutral model in about half the species examined. These results indicate that many amino acid mutations in animal mtDNA may be weakly deleterious.

Methods

All sequences were taken from published sources. Over 4,000 mitochondrial cytochrome b sequences and over 2,000 mitochondrial cytochrome oxidase sequences are listed in Genbank. These were screened for datasets that included multiple individuals from single species and sufficient intraspecific variation so that statistical analyses could be performed. An effort was made to choose the studies with the largest sample sizes. Most of the mtDNA sequences analyzed here were originally generated with the aim of reconstructing either intraspecific or interspecific phylogenies. Consequently, there are relatively few datasets with large numbers of individuals from single populations. Published sequence alignments were used when available; otherwise sequences were aligned using Pileup in GCG. The number of non-synonymous and synonymous polymorphisms and fixed differences were counted. Differences between sequences were not corrected for multiple hits so that statistical tests were based on independent observations. For most comparisons, divergence was low and uncorrected values accurately reflect the substitutions that occurred. For a few comparisons, uncorrected values underestimate the silent substitutions that occurred, and this may bias the outcome of tests (e.g., *Pomatostomus* in Table 1; see Discussion). The approach of McDonald and Kreitman (1991) was used to test the neutral prediction that the ratio of replacement to silent polymorphisms within one species is the same as the ratio of replacement to silent fixed differences between species. Contingency tables were evaluated using Fisher's Exact Test (Sokal & Rohlf, 1995). For each comparison, the neutrality index (N.I.; Rand & Kann, 1996) was calculated as:

$$\text{N.I.} = \frac{\frac{\text{(no. of non-synonymous polymorphisms)/}}{\text{(no. of non-synonymous fixed differences)/}}}{\frac{\text{(no. of synonymous polymorphisms)}}{\text{(no. of synonymous fixed differences)}}}$$

This ratio of ratios has an expectation of one under neutrality; N.I. > 1 when the ratio of replacement to silent variation is greater within species than between species, and $0 <$ N.I. < 1 when the ratio of replacement to silent variation is greater between species than within species.

The sampling formulae of Sawyer and Hartl (1992) were used to estimate $\gamma = N_e s$, where N_e is the effective population size and *s* is the average selection coefficient on non-synonymous mutations. This approach assumes that selection is acting on non-synonymous mutations only and that synonymous mutations are effectively neutral. Under this assumption, the ratio of polymorphism to divergence (r_{pd}) at silent sites can be used to estimate t_{div}, the time since divergence scaled to N_e generations:

$$t_{\text{div}} = [L(m)/r_{\text{pd(silent)}}] - 1/m \,,$$
$$\text{where} L(m) = \textstyle\sum_{i=1}^{m-1} 1/i$$

and *m* is the number of alleles sampled from a population. The divergence time estimated from silent sites can then be used to calculate $\gamma = N_e s$ based on the ratio of polymorphism to divergence (r_{pd}) at replacement sites:

Table 1. Contingency tables for nonsynonymous and synonymous polymorphisms and fixed differences in animal mitochondrial gene sequences from Genbank

Species for polymorphism	N	Locus	Length (bp)	Species for divergence	Mutation	Polymorphisms	Fixed Differences	FET P-value	N.I.	Reference
Mesomys hispidus	29	Cytb	798	*M. stimulax*	Nonsynon	36	0	0.009	∞	DaSilva & Patton, 1993
					Synon	123	20			
Phyllobates lugubris	8	Cytb	292	*Dendrobates pumilio*	Nonsynon	11	0	0.060	∞	Summers et al., 1997
					Synon	59	19			
Ambystoma jeffersonianum	6	Cytb	307	*A. laterale*	Nonsynon	4	0	0.001	∞	Hedges et al., 1992
					Synon	3	27			
Grus antigone	9	Cytb	1143	*G. rubicunda*	Nonsynon	7	1	0.002	21.0	Wood & Krajewski, 1996
					Synon	10	30			
Drosophila melanogaster	4	ATPase6	674	*D. simulans*	Nonsynon	4	4	0.017	18.0	Kaneko et al., 1993
					Synon	1	18			
Microtus rossiaemeridionalis	9	Cytb	1143	*M. arvalis*	Nonsynon	11	7	$<$0.001	7.5	Baker et al., 1996
					Synon	10	48			
Melospiza melodia	11	Cytb	431	*Passerella iliaca*	Nonsynon	5	10	0.039	6.5	Zink & Blackwell, 1996
					Synon	2	26			
Emoia impar Group II	8	Cytb	779	*E. impar* Group I	Nonsynon	3	12	0.067	5.1	Bruna et al., 1996
					Synon	4	81			
Microtus arvalis	10	Cytb	1143	*M. rossiae-meridionalis*	Nonsynon	13	7	0.008	4.5	Baker et al., 1996
					Synon	20	48			
Heliconius erato	52	COI COII	819	*H. telesiphe*	Nonsynon	16	2	0.235	2.7	Brower, 1994
					Synon	70	24			
Passerella iliaca	19	Cytb	431	*Melospiza melodia*	Nonsynon	5	10	0.743	1.3	Zink & Blackwell, 1996
					Synon	10	26			
Ursus arctos	166	Cytb	1140	*Helarctos malayanus*	Nonsysnon	11	15	0.826	1.1	Talbot & Shields, 1996
					Synon	44	68			
Isothrix bistrata	10	Cytb	798	*I. pagurus*	Nonsynon	16	6	0.999	0.9	DaSilva & Patton, 1993
					Synon	108	38			
Ensatina eschscholtzii	24	Cytb	684	*Plethodon elongatus*	Nonsynon	38	21	$<$0.001	0.3	Moritz et al., 1992
					Synon	171	27			
Pomatostomus temporalis	35	Cytb	282	*P. isidori*	Nonsynon	0	8	0.014	0.0	Edwards & Wilson, 1990
					Synon	17	18			
Dendrobates pumilio	12	Cytb	292	*D. speciosus*	Nonsynon	0	4	0.234	0.0	Summers et al., 1997
					Synon	6	6			
Gadus morhua	236	Cytb	275	*Melanogrammus aeglefinus*	Nonsynon	0	5	0.147	0.0	Carr et al., 1995
					Synon	16	25			

$$r_{\text{pd(replacement)}} = F(m)/[t_{\text{div}} + G(m)]\ ,$$

where

$$F(m) = \int_0^1 [(1-x^m-(1-x)^m)(1-e^{-2\gamma x})]/[(1-x)2\gamma x]\ \ dx$$

and

$$G(m) = \int_0^1 [(1-x)^{m-1}(1-e^{-2\gamma x})]/2\gamma x \quad dx$$

This model is based on populations at equilibrium and assumes that sites are freely recombining. The first assumption may be violated for some of these datasets, and the second assumption is clearly violated for mtDNA as all sites are linked. This is likely to inflate the variance associated with estimates of $N_e s$; the values given should be interpreted only as rough estimates.

Table 2. Contingency tables for nonsynonymous and synonymous polymorphisms and fixed differences in animal mitochondrial gene sequences from previous studies testing the neutrality of mtDNA

Species for polymorphism	N	Locus	Length (bp)	Species for divergence	Mutation	Polymorphisms	Fixed Differences	FET P-value	N.I.	Reference
Mus domesticus	56	ND3	342	*M. spretus*	Nonsynon	11	2	0.003	9.73	Nachman et al., 1994
					Synon	13	23			
Drosophila mel, sim, & yak	47	Cytb	1137	*Drosophila* mel, sim, & yak	Nonsynon	6	10	0.013	4.85	Ballard & Kreitman, 1994
					Synon	12	97			
Homo, Pan, & *corilla*	21	COII	684	*Homo, Pan,* & *Gorilla*	Nonsynon	14	8	0.001	4.71	Templeton, 1996
					Synon	42	113			
Homo sapiens	61	ND3	342	*Pan troglodytes*	Nonsynon	4	4	0.079	4.43	Nachman et al., 1996
					Synon	7	31			
Holochilus brasiliensis	82	ND3	342	*H. vulpinus*	Nonsynon	8	7	0.056	3.5	Kennedy & Nachman, unpublished data
					Synon	16	49			
Homo sapiens	3	All loci	11367	*Pan troglodytes*	Nonsynon	31	179	<0.001	2.88	Nachman et al., 1996
					Synon	55	915			
Drosophila mel & sim	88	ND5	1515	*D. simulans* & *D. melanogaster*	Nonsynon	11	15	0.129	2.24	Rand & Kann, 1996
					Synon	17	52			Rand et al., 1994
Drosophila mel & sim	66	ND3	351	*D. simulans* & *D. melanogaster*	Nonsynon	1	2	0.999	1.30	Rand & Kann, 1996
					Synon	5	13			

Results

Patterns of polymorphism and divergence for 17 species are shown in Table 1. These 17 comparisons include samples gathered as part of phylogenetic studies. Many of the samples from within single species come from different geographic localities and may not correspond to single, panmictic populations. Sample sizes range from 3 to 236. This group of taxa includes mammals (*Mesomys*, *Microtus*, *Ursus*, *Isothrix*), birds (*Grus*, *Melospiza*, *Passerella*, *Pomatostomus*), reptiles (*Emoia*), amphibians (*Phyllobates*, *Ambystoma*, *Ensatina*, *Dendrobates*), fish (*Gadus*), and insects (*Drosophila*, *Heliconius*). The 17 studies in Table 1 are arranged in order of decreasing N.I.

Eleven of the comparisons in Table 1 are significant ($P < 0.05$) or marginally significant ($0.05 < P < 0.10$). Of these, nine show a greater ratio of replacement to silent variation within species than between species, and two studies (*Ensatina* and *Pomatostomus*) show the opposite pattern. In 12 of the 17 studies, N.I. > 1.

Eight previous studies have been conducted with the specific aim of testing the neutrality of mtDNA through statistical comparison of nonsynonymous and synonymous mutations. These are summarized in Table 2. In most cases, these studies include large samples of individuals from single populations. Six of the eight studies show a significantly ($P < 0.05$) or marginally significantly ($0.05 < P < 0.10$) greater ratio of replacement to silent variation within species than between species. In all eight studies, N.I. > 1.

Estimates of $N_e s$ for each of these comparisons are given in Table 3. $N_e s$ is negative in most of these comparisons, consistent with the observation that N.I. > 1 in most cases. Estimates of $N_e s$ fall within a fairly narrow range from -3.3 to 13.6, and the majority of the estimates fall between -1 and -3. Surprisingly few estimates are in the range $|N_e s| < 1$ as expected under a simple neutral model. The average of the four positive estimates of $N_e s$ is 7.5, not very different from the value of 10 obtained by Sawyer and Hartl (1992) for the *Adh* data of McDonald and Kreitman (1991).

Discussion

Of the 25 contingency tables analyzed here, 17 deviate from the neutral expectation of equal ratios of replacement and silent variation within and between species. Most of the deviations (15 out of 17) are in the direction of a greater ratio of replacement to silent variation within species than expected based on interspecific comparisons. $N_e s$ was estimated for 21 datasets

Table 3. Divergence time and strength of selection estimated from ratios of polymorphism to divergence

Species for polymorphism	Species for divergence	Locus	m	t_{div}	γ
Mesomys hispidus	*M. stimulax*	Cytb	29	0.29	−2.3
Phyllobates lugubris	*Dendrobates pumilio*	Cytb	8	0.71	−2.1
Ambystoma jeffersonianum	*A. laterale*	Cytb	6	10.11	−3.3
Grus antigone	*G. rubicunda*	Cytb	9	3.97	−2.8
Drosophila melanogaster	*D. simulans*	ATPase6	4	16.17	−2.8
Microtus rossiaemeridionalis	*M. arvalis*	Cytb	9	6.41	−2.1
Melospiza melodia	*Passerella iliaca*	Cytb	11	18.93	−1.9
Emoia impar (Gr. II)	*E. impar* (Gr. I)	Cytb	8	18.39	−1.4
Microtus arvalis	*M. rossiaemeridionalis*	Cytb	10	3.29	−1.6
Heliconius erato	*H. telesiphe*	COI,II	52	0.76	−1.6
Passerella iliaca	*Melospiza melodia*	Cytb	19	4.49	−0.3
Ursus arctos	*Helarctos malayanus*	Cytb	166	4.40	−0.7
Isothrix bistrata	*I. pagurus*	Cytb	10	0.40	−0.1
Ensatina eschscholtzii	*Plethodon elongatus*	Cytb	24	0.25	2.6
Pomatostomus temporalis	*P. isidori*	Cytb	35	2.15	13.6
Dendrobates pumilio	*D. speciosus*	Cytb	12	1.43	7.3
Gadus morhua	*Melanogrammus aeglefinus*	Cytb	236	4.71	6.4
Mus domesticus	*M. spretus*	ND3	56	4.06	−2.4
Homo sapiens	*Pan troglodytes*	ND3	61	10.38	−1.8
Homo sapiens	*Pan troglodytes*	All loci	3	12.15	−1.3
Holochilus brasiliensis	*H. vulpinus*	ND3	82	7.61	−1.8

Species are listed in the same order as in Tables 1 and 2. Number of sequences is given by m, divergence time (t_{div}) is in units of N generations, and $\gamma = N_e s$ (see Methods). Values of γ are approximate when $m > 50$.

representing 20 different species. In a large majority of these (17 of 21), $N_e s$ was negative, and for most, -3 $< N_e s <$ -1 (Table 3), consistent with the presence of many mildly deleterious mutations (see below).

Sampling and statistical considerations

While the McDonald Kreitman test does not require sampling from a single population (Ballard & Kreitman, 1994), it is possible that sampling from different populations may affect the sensitivity of the test to particular deviations from the neutral expectation. For example, if replacement polymorphisms are typically present at lower frequencies than silent polymorphisms (e.g., Templeton, 1996), then they may be missed if only a few individuals are sampled from each of several distinct populations. Because many of the datasets in Table 1 include only one or a few individuals from each of several different geographic regions, contingency-table tests of these data may be insensitive to differences in the ratio of replacement to silent polymorphisms among low-frequency variants.

The P values reported in Tables 1 and 2 have not been corrected for multiple tests. Of the 25 comparisons, only 6 have P values less than 0.002 and thus remain significant after correcting for multiple tests (0.002 x 25 = 0.05). This correction is quite conservative, yet it still suggests that nearly one quarter of the tests reject the null hypothesis.

The numbers of fixed differences reported in Tables 1 and 2 do not include corrections for multiple substitutions at the same site. Given the low level of divergence in these comparisons, uncorrected values will not underestimate the number of replacement substitutions but may underestimate the number of silent substitutions that have occurred. This will lead to an overestimate of the ratio of replacement to silent fixed differences. Such a bias will make the test conservative for rejecting the null hypothesis when N.I. > 1, but may lead to false rejections of the null hypothesis when N.I. < 1. For example, in the comparison involving *Emoia impar* (Table 1), 81 silent fixed differences were observed and the P-value (using uncorrected silent divergence) is P = 0.067. A Jukes-Cantor (1969) correction leads to an estimate of 118 silent

fixed differences, and the P value of a contingency table based on this corrected silent divergence is P = 0.029. Using the uncorrected value makes the test conservative in this case. On the other hand, in the comparison involving *Pomatostomus temporalis* (Table 1), 18 silent fixed differences were observed and the P value is P = 0.014. A Jukes-Cantor correction leads to an estimate of 22 silent fixed differences, and the P value based on this corrected silent divergence is P = 0.038. Thus, using the uncorrected value makes it more likely to reject the null hypothesis. Most of the rejections of the null model (15 out of 17) are in the direction of N.I. > 1; the P values associated with these results may be viewed as upper estimates.

Models of molecular evolution

The observation that 17 of 25 comparisons deviate from the null expectation and that 15 of these 17 deviate in the direction of N.I. > 1 is rather surprising. What might account for the high ratio of replacement to silent polymorphisms within species? At least three formal possibilities exist. First, a relaxation of selective constraint at mitochondrial genes may have occurred in the recent past such that replacement mutations that were previously not tolerated have accumulated as polymorphisms. If this change in selection pressure occurred recently, ratios of replacement to silent fixed differences would be unaffected. This hypothesis might explain patterns of variation seen in humans (Takahata, 1993) or human commensals, but it is unlikely that selection pressures have changed more or less simultaneously for species as different as those listed in Table 1 for which N.I. > 1.

Second, some form of positive selection may be maintaining an excess of replacement polymorphisms within species. It is difficult to evaluate the likelihood of this hypothesis without a formal theoretical model. However, there are two observations that need to be explained by any model of positive selection. One is that many sites would have to be under selection at once. For example, in the human dataset in Table 2 consisting of three complete mitochondrial genomes, an 'excess' of 20 replacement polymorphisms is observed over the neutral expectation, assuming neutrality at silent sites. The conditions for maintaining polymorphisms under selection in mitochondrial genomes are somewhat restrictive (e.g., Clark, 1984) and unlikely to give rise to multiple balanced polymorphisms. A second important observation is that many of the replacement polymorphisms in these datasets are at

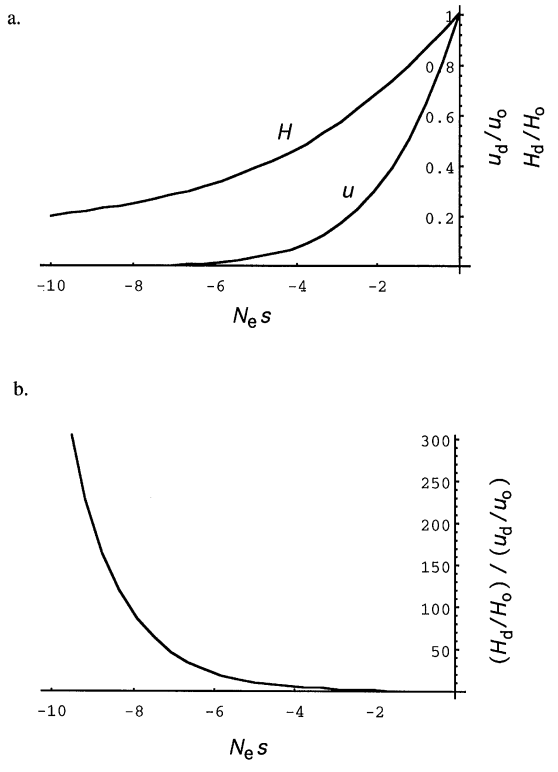

Figure 1. (a) Relative contributions of weakly selected deleterious mutations to heterozygosity (H) and substitution (u) as a function of $N_e s$ (Kimura, 1983). Heterozygosity and fixation probability are given by H_d and u_d for deleterious mutants and H_o and u_o for neutral mutants. H_d/H_o = 2(S - 1 + e^{-S})/ [S(1 - e^{-S})] and u_d/u_o = S/ (1 - e^{-S}), where $S = N_e s$. (b) Ratio of heterozygosity to substitution of weakly selected deleterious mutants relative to neutral mutants as a function of $N_e s$.

low frequencies and at lower frequencies than silent sites (Nachman et al., 1996; Templeton, 1996). Typical models with balancing components to their selection will increase the frequency of selected sites, although some selection models may lead to a negative Tajima's D (Gillespie, 1994).

A third explanation for the data is that many of the replacement polymorphisms are slightly deleterious and rarely become fixed. Figure 1a shows the contribution of weakly selected mutants to heterozygosity and to fixation, relative to strictly neutral mutants. When -10 $< N_e s <$ -1, the contribution of mutants to heterozygosity is substantially greater than their contribution to fixation (Kimura, 1983). Figure 1b shows the ratio of the proportional contributions to heterozygosity and fixation of weakly selected mutants. The Y axis is analogous to N.I., assuming replacement mutations are under selection and silent mutations are strictly

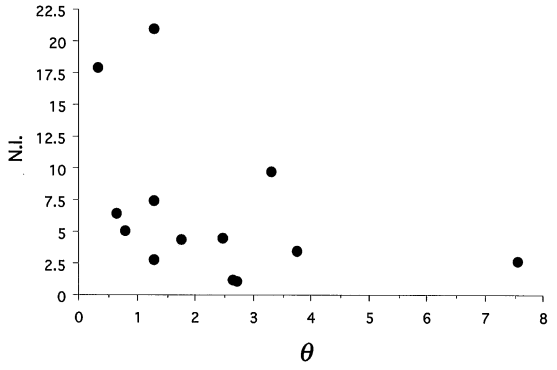

Figure 2. Scatterplot of N.I. versus θ for the 13 comparisons in Tables 1 and 2, where N.I. > 1. Three comparisons where N.I. = ∞ were excluded, as were comparisons where polymorphism data are pooled from more than one species. θ = *S*/*L* ($1/\sum_{i=1}^{m-1} 1/i$), where *S* is the number of silent polymorphic nucleotides, *L* is the number of silent sites, and *m* is the number of sequences in the sample (Watterson, 1975).

neutral. This ratio is extremely sensitive to changes in $N_e s$.

The expected relationship between N_e and N.I. under a model of deleterious mutations will depend on several factors. Figure 1b shows that for a given average selection coefficient, species with larger effective population size should have a higher value of N.I., because fixation of deleterious mutations becomes very unlikely as N_e becomes large. However, if $N_e s << -10$, (i.e., strong selection) deleterious mutations should contribute neither to heterozygosity nor to fixation. Figure 2 is a scatterplot of $\theta = N_e \mu$ versus N.I. for the 13 species with N.I. > 1 (excluding the 3 species with N.I. = ∞). There is no evidence of a positive correlation as might be expected if differences in θ accurately reflect differences in N_e and if $-10 < N_e s < -1$. However, it is unlikely that all of the variation in θ is due to variation in N_e, because mutation rates also vary among these species. Moreover, θ will accurately reflect differences in population size only under a neutral model. Under a slightly deleterious model, selection will be more effective in larger populations; the removal of deleterious mutations in large populations may depress linked, silent variation via background selection (Charlesworth, 1994; Charlesworth, Morgan & Charlesworth, 1993; Charlesworth, Charlesworth & Morgan, 1995).

One of the chief difficulties for any model of deleterious mutations is the narrow range of $N_e s$ under which the models are expected to exhibit their unique behavior. If $|N_e s| < 1$, mutations will behave as neutral mutations, and if $|N_e s| >> 1$, substitutions will stop (Gillespie, 1994, 1995). In particular for the data summarized here, the challenge is to reconcile one model with the range of values of N_e that presumably exist among organisms as diverse as those in Tables 1 and 2. Although these results are in qualitative agreement with the hypothesis that many mitochondrial amino acid polymorphisms are deleterious, specific models that incorporate both polymorphism and divergence are needed to assess whether these data will fit quantitative predictions.

Non-neutral mtDNA evolution

The results reported here attest to the generality of non-neutral evolution in animal mitochondrial DNA. These findings indicate that the patterns first reported for *Drosophila* (Ballard & Kreitman, 1994; Rand, Dorfsman & Kann, 1994) and house mice (Nachman, Boyer & Aquadro, 1994) are not unique to those organisms, but extend to species that are not commensal with humans. Non-neutral patterns have been detected in different mitochondrial genes (COI, COII, cytb, ND3, ATPase6), but individual genes can also show quite different patterns in different species. At ND3 in *Drosophila*, N.I. = 1.0, but in mice this same gene shows N.I. = 9.7 (Table 2). It also remains unclear why mitochondrial DNA evolution appears neutral in some species but not in others. Given the limited statistical power to detect deviations from neutral expectations, differences among species cannot be assumed to reflect differences in the evolutionary process. If many of the deviations are due to the presence of a large class of deleterious mutations, however, then some of the differences among species may reflect differences in N_e.

It is also unclear whether the patterns reported here are unique to mitochondrial DNA. The only species for which McDonald-Kreitman tests have been performed at multiple nuclear genes are *D. melanogaster* and *D. simulans*. Neither species has many genes with high values of N.I. In humans, however, a large survey of nuclear genes revealed a high ratio (11:15) of replacement to silent polymorphisms (Li & Sadler, 1991). This raises the possibility that the patterns reported here for mtDNA may also be found at some nuclear loci.

Acknowledgements

I thank J.B. Walsh and H. Akashi for suggestions and D.M. Rand for comments on the manuscript.

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