Letters to the Editor

Are Mitochondrial DNA Variants Selectively Non-Neutral?

MACRAE and ANDERSON (1988) have recently presented experiments purporting to indicate the action of natural selection on mitochondrial DNA (mtDNA) variants in a cage population of *Drosophila pseudoobscura*. Mated flies from reciprocal crosses between *D. pseudoobscura* from California and a population from Bogota, Colombia, were placed in population cages in numbers designed to give a predetermined initial frequency of Bogota mtDNA. In one experiment, the mtDNA increased in frequency over 10 generations from 30% to 80%, and then stabilized. After a perturbation of the Bogota mtDNA frequency by adding more *D. pseudoobscura* flies to the cage, the frequency returned to the pre-perturbation equilibrium within two generations. Other cages had different initial frequencies of Bogota mtDNA, but did not reproduce the dramatic alterations in mtDNA frequency. MACRAE and ANDERSON (1988) suggest that specific conditions can lead to "sporadic" selection that will favor one mtDNA type over others, likely as a result of cytonuclear interactions. These results have important bearing on the utility of mtDNA as a neutral marker. We discuss the validity of these results and offer an alternative explanation that does not involve selection on the mtDNA variants.

The mtDNA variants used by MACRAE and ANDERSON (1988) in their selection experiment were not conspecific and came from two different subspecies, *D. p. pseudoobscura* and *D. p. bogotana,* which are partially reproductively isolated (PRAKASH 1972; AYALA and DOBZHANSKY 1974). *D. bogotana* females crossed to *D. pseudoobscura* males produce sterile males but fertile females; the males and females from the reciprocal cross are fully fertile. MACRAE and ANDERSON (1988) discussed the role of differential mating of females as a possible explanation of their results but discarded it by pointing out that there appear to be no mating preferences of flies from Bogota for flies from other locations (PRAKASH 1972). In fact, we have found a strong mating preference of *bogotana* females for *pseudoobscura* males (SINGH 1983). In a no-choice experiment, using equal numbers of sexually mature males and females, 85% of *bogotana* females had mated with *pseudoobscura* males in the first five minutes whereas only 50% of *pseudoobscura* females had mated with *bogotana* males in the same period; 100% mating occurred between males and females from within species in the same time period. These results show evidence for isolation between *pseudoobscura* females and *bogotana* males which is also present in PRAKASH's results, although it was statistically not significant. In contrast to PRAKASH's results, we found that the mating preference of *bogotana* females was much more biased in a choice experiment. We used allozyme markers to distinguish offspring from the various types of mating (using equal numbers of males and females from both species), the mating was continued for 5–10 days, and on each subsequent day flies were changed to a new food bottle. From the genotypes of the F_1 offspring produced we inferred that for the first 2 days all mating occurred between *bogotana* females and *pseudoobscura* males, and no mating occurred between *bogotana* females and males. However, from the 3rd day onward, *bogotana* females appeared to have mated with equal frequency with both types of males. In contrast, *pseudoobscura* females appeared to be out-competed in mating with their own males and inhibited from mating with *bogotana* males as no offspring of *pseudoobscura* females appeared until day four of the mating. Based on a total of 318 offspring produced from matings over 5 days, the various types of mating were in these proportions: *bogotana* females × *pseudoobscura* males (67%); *bogotana* females × *bogotana* males (27%); *pseudoobscura* females × *bogotana* males (2%); and *pseudoobscura* females × *pseudoobscura* males (4%). Exactly the same type of results were obtained from mating between *D. bogotana* and *D. persimilis,* a sibling species of *D. pseudoobscura,* except that in this case 50% of the matings were between *persimilis* females and *bogotana* males and 43% between *bogotana* females and *persimilis* males; very little homogametic mating occurred in this experiment. Thus, in a choice situation *bogotana* females appear to prefer *pseudoobscura* males over their own males. In addition to the mating preference of *bogotana* females, we noticed a tendency for *bogotana* flies to reach sexual maturity sooner and mate faster than *pseudoobscura* flies. Our results differ from those reported by PRAKASH (1972) but they were obtained in a manner that make them applicable to the results obtained by MACRAE and ANDERSON.

The significance of our mating results for the mtDNA results of MACRAE and ANDERSON (1988) becomes obvious when we examine their experimental design. Their cage experiments did not start with F_1 hybrids but with females and males (500 each) of both species after the females of one species had been

mated with males of the other species and egg laying was allowed for 6 days. The results from our studies suggest that in the mtDNA experiment a bias due to mating preference may have occurred in several ways. First, it is very likely that remating occurred during the 6-day period of egg laying, and a large proportion of these rematings may have been between *bogotana* females and *pseudoobscura* males. This would have resulted in a higher starting frequency of *bogotana* mtDNA than that reported by the authors. Second, if some remating occurred between males and females of *bogotana* (remating has been observed to occur with a periodicity of 3–4 days in *D. pseudoobscura* and *D. bogotana*) (SINGH 1983), then this would have resulted in the production of some pure *bogotana* flies in the F_1 generation and their mating preference would have affected the mtDNA frequency in the F_2. Finally, although we do not have any data to bear upon this, if the mating preference of *bogotana* females were carried over to some extent to the F_2 or F_3 generations, then this would further extend the bias due to mating preference to the later generation until the genomes of all flies had become completely randomized.

Thus, the mating preference of *bogotana* females as well as their faster mating ability can adequately explain the rapid increase of *bogotana* mtDNA in the first few generations of the cage experiment reported by MACRAE and ANDERSON. Nonrandom mating may also lie behind the dramatic return of mtDNA frequency after the perturbation. It is important to note that the preference of *bogotana* females for *pseudoobscura* males would not affect the genomic components of the two species in the hybrids, and so the lack of change in the third chromosome inversion frequencies in the mtDNA experiment is consistent with our explanation.

The utility of animal mitochondrial DNA (mtDNA) as a tool in evolutionary biology is based on several features that set it apart from the nuclear genes, including strictly maternal inheritance, lack of recombination, and rapid evolution (reviewed in WILSON *et al.* 1985; AVISE *et al.* 1987). An additional assumption of neutrality is especially important if mtDNA variants are to be used as (neutral) markers in studies of mtDNA polymorphism, population structure, species' genetic boundaries, hybrid zones, and gene flow. It is assumed that any substitution in the gene product that affect metabolic function will have sufficiently deleterious effects on the organism that they will be rapidly eliminated. Support for this view came from the observation of a great preponderance of synonymous over nonsynonymous nucleotide substitution in comparisons of closely related species (BROWN and SIMPSON 1982). However, interspecific comparisons of species that are not evolutionarily close indicate that (1) amino acid substitution into mitochondrially encoded polypeptides are not rare, and (2) the rate of nucleotide and amino acid substitution is variable between mitochondrial genes (BROWN 1983; CLARY and WOLSTENHOME 1985), indicating that some gene products are less conserved than others.

Selection of *intraspecific* mtDNA variants has been inferred for mtDNAs varying in *size* in the major noncoding region. In studies of Gryllus crickets heteroplasmic for size variant mtDNAs, RAND and HARRISON (1986) noted a consistent transmission bias of the smaller types in succeeding generations. HALE and SINGH (1986) noted that the population frequency distribution of mtDNA size classes in *D. melanogaster* was strongly skewed to the smaller class, despite the obviously very high rate of size mutation as evidenced by the presence of size variants in every restriction site haplotype. These data strongly indicate a selective advantage toward smaller mtDNAs. The "selection" in this case is more likely intracellular transmission bias, possible due to a replication advantage of smaller sized molecules, and therefore not operative at the level of the organism. However, restriction site variants arising in larger sized molecules would have greater chance of being eliminated due to hitchhiking effect.

Mitochondrially coded proteins have one feature which allows some room for directional or balancing selection. Most of them are subunits of enzymes for which the other subunits are encoded in the nucleus. This leaves the obvious potential for strong cytonuclear interactions based on the association between subunits. Much effort has gone into describing the theoretical effects of such interactions (CLARK 1984; GREGORIUS and ROSS 1984; ASMUSSEN, ARNOLD and AVISE 1987; ARNOLD, ASMUSSEN and AVISE 1988), but empirical studies are still few. CLARK (1985) and CLARK and LYCKEGAARD (1988) have studied cytoplasmic effects of second chromosome segregation in strains of *D. melanogaster* from geographically diverse populations. They observed a significant cytoplasmic effect on chromosomal transmission when diverse lines are crossed, but not when lines from the same population are crossed. Even when there is an effect it is not consistent as the maternal cytoplasms can favor the chromosome from its own population or from the other. CLARK and LYCKEGAARD (1988) point out that "cytoplasmic effects" are not necessarily a product of organelles or their DNA. They could not definitively ascribe the effects they say to the restriction variants of mtDNA.

An example of such cytoplasmic effects not ascribable to mtDNA has been described by HOFFMANN, TURELLI and SIMMONS (1986) and HOFFMANN and TURELLI (1988). Naturally occurring strains of *D.*

simulans associated with a cytoplasmically borne microorganism show asymmetric fertility when crossed with noncarried strains, in the direction that F_1s should mainly harbor the mtDNA of the affected strain. Restriction analysis of mtDNAs confirm this (L. R. HALE and A. A. HOFFMANN, unpublished data). Therefore, the mtDNA associated with the affected strain is being driven to a higher frequency as a consequence of a cytoplasmic mating incompatibility system, and not because of selection for mtDNA (T. PROUT, personal communication). Directional mating incompatibility or mating bias may also occur in the absence of cytoplasmic effects, particularly when closely related species are hybridized. As discussed, we think this is probably the basis for the results obtained by MACRAE and ANDERSON.

What is bothersome about the mtDNA selection experiment is that the results obtained in the initial experiment showing relative fitnesses as high as $W = 1.81 - 2.09$ were essentially nonrepeatable in the later experiments; the selection outcomes varied not only between experiments but between replicates. MACRAE and ANDERSON (1988) explained the varied and unpredictable outcomes of their selection experiments by suggesting that selection could be of sporadic nature and may depend on initial conditions involving cytonuclear interaction. Sporadic selection in mtDNA is a real possibility with highly diverged mtDNA variants and has important consequence for introduction of mtDNA among isolated populations and across species boundaries. However, unless the interacting genetic systems can be identified, use of the sporadic selection argument to explain variable results would be unwise as there is a danger of confusing real results due to cytonuclear interaction and spurious results due to some uncontrolled aspects of the biological system. Most species show plenty of mtDNA variation involving site as well as size changes. It is important that any attempt to understand role of selection on mtDNA variants should first begin with simpler conspecific variants rather than with interspecific variants.

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