

Can Mating Preferences Explain Changes in mtDNA Haplotype Frequencies?

SINGH and HALE (1990) propose that differences in mating preference and mating speed of *Drosophila pseudoobscura* females from Bogota and California may explain the frequency changes of mitochondrial DNA (mtDNA) haplotypes that we (MACRAE and ANDERSON 1988) observed in population cages. In the course of considering various aspects of mating behavior as possible causes for the frequency changes in mtDNA variants, we cited PRAKASH (1972) as having reported no mating preferences between *D. pseudoobscura* strains from Bogota and North America. We failed to cite SINGH (1983), who reported a strong mating preference of Bogota females for North America males, as well as an advantage in mating speed for the Bogota females, and we apologize for this oversight. We do not believe that mating preferences and mating speed can account for our results, however, and we outline our reasons below.

1. We chose mtDNA variants from Bogota and California for our experiments in order to compare conspecific haplotypes that were as different as possible. SINGH and HALE (1990) treat the Bogota and North America subspecies as if they were distinct species, but we feel they are properly described as conspecific. The genetic differences between them justify their description as subspecies (AYALA and DOBZHANSKY 1974; SINGH 1983), but they are separated by a partial reproductive isolation only. They can mate and produce fertile offspring.

2. It is worth recounting some puzzling aspects of the sterility of F_1 hybrid males from crosses of Bogota females to North American males. The Bogota strain we employed, BogER, was one of several brought back from Bogota in 1979 by JEFFREY POWELL and one of us (W.A.). It is the same strain used by ORR (1989) in his genetic analysis of sterility in Bogota/North America hybrids, but it is different from the strain SINGH (1983) used in his study of mating preference. POWELL (1982) reported that crosses between females from these new Bogota strains and males from North America *did* yield quite a few fertile F_1 male offspring when the crosses were made only two generations after the new strains were obtained. This F_1 male fertility was increased sixfold when the food medium contained tetracycline, leading POWELL (1982) to suggest that a microbial symbiont might be involved in the F_1 male sterility. F_1 male sterility had increased considerably after four or five generations of laboratory propagation but was still not complete, even in crosses with no tetracycline treatment. Five years after the new strains were brought back from Bogota, all F_1 males from crosses of the Bogota fe-

males to North American males were sterile, with or without tetracycline in the food. POWELL's (1982) results show that the F_1 male sterility is not simply a matter of genetic differentiation between *D. pseudoobscura* populations in Bogota and North America. A related observation was made by PRAKASH (1972), DOBZHANSKY (1974), and ORR (1989), all of whom found a strong maternal effect of the Bogota cytoplasm on F_1 male fertility; that is, both cytoplasm and nuclear genotype of Bogota females affect the fertility of their offspring produced from matings with North American males.

3. Our first population cage, the one in which the Bogota mtDNA variant rapidly increased in frequency, was begun with the male and female offspring of *reciprocal* crosses between Bogota and California strains. These males and females used to begin our population were placed together in bottles for 2 days before they were added to the cage, and few, if any, should have been unmated; our experience is that virtually all females mate under these conditions. Since both males and females were added to the cage, any unmated females would have had further opportunities to mate in the cage. Mitochondria are transmitted maternally, so that the mtDNA variant transmitted by a mother to her offspring is the same regardless of which male haplotype was her mate. Mating preference would not have altered the frequency of mtDNA haplotypes so long as the proportion of unmated females was the same for the Bogota and California strains. In addition, mating speed would not have affected the frequency of initial (generation 0) females who mated, since these females had an ample opportunity to mate in bottles before being added to the cage. Repeated mating by the females in the cage will not alter mtDNA frequencies either, again because the mtDNA haplotype of the male parent does not affect the haplotype of his offspring. We verified that our input frequencies of mtDNA were indeed 30% Bogota and 70% California, by Southern blotting of 142 adults. The frequency of Bogota mtDNA in this sample was 0.296.

4. Thus, neither mating preference nor mating speed can account for the increase in frequency of the Bogota mtDNA from 30% to 46% in the first generation of our cage. Moreover, we do not believe these mechanisms could account for the frequency changes in mtDNA haplotypes in later generations, either. Most offspring in the first generation were F_1 hybrids, with some parental genotypes produced by remating of females to males of their same strain. The F_1 genotype is composed of equal contributions from the

Bogota and California strains, and on average will be the same for all the F_1 's. Any genetic differences in mating preference or mating speed between the strains are likely due to nuclear genes, and thus we do not expect hybrid F_1 flies with either of the two mtDNA haplotypes to differ in their mating behavior.

The Bogota and California strains carry different inversions of the third chromosome, TL in Bogota and ST in California, and we used heterozygosity for these gene arrangements to measure the frequency of hybrids in the F_1 generation of our first cage. The TL and ST gene arrangements carry different alleles at the amylase locus, and by acrylamide electrophoresis for amylase we were able to determine the frequencies of genotypes in adults sampled from this population. In the F_1 , 88.8% of a sample of 107 adults were ST/TL heterokaryotypes, and hence hybrids. The remaining 11.2% of the adults were ST/ST homokaryotypes and thus had to be the progeny of rematings by California females to California males. There were no TL/TL homokaryotypes in the sample and consequently no females with the full nuclear genome from Bogota, who, in keeping with SINGH's (1983) results, might have a mating preference for California males. Thus, the 17% increase in Bogota mtDNA frequency in our first cage between the F_1 and F_2 generations was not due to a mating preference or differential mating speed of Bogota females in the F_1 , as SINGH and HALE (1990) suggest, since there were few, if any, such Bogota females in the F_1 generation.

The sterility of F_1 hybrid males from crosses of Bogota females to California males should not affect the frequencies of the mtDNA variants, since males do not transmit mitochondria to their offspring. The only factor that matters is having a sufficient number of males to inseminate the females. A loss of approximately half the males in the F_1 generation to hybrid sterility should not reduce the fraction of females that mate, at least not in large cages like ours.

5. In the F_2 and later generations, the nuclear genotypes of flies in our cage were mixtures of the Bogota and California genotypes, and we would not expect the mtDNA haplotypes to remain associated with any genes for mating behavior. Consequently, there is no reason to expect that differences in mating preference or mating speed in the initial strains would produce changes in mtDNA frequency in the F_2 and later generations.

Mating preference cannot explain the apparent equilibrium for mtDNA frequencies reached in several of our cages, nor can it explain the return of mtDNA haplotype frequencies to their previous values when we perturbed haplotype frequencies in our first population at generation 22. Any effect of hitchhiking due to mating preferences should have ended long before this time.

For these reasons, most important among them the maternal inheritance of mitochondria, we do not feel that mating preferences and differences in mating speed can account for the frequency changes in mtDNA haplotypes observed in our first population.

6. SINGH and HALE (1990) discuss the possibility that cytoplasmic effects not ascribable to mtDNA might affect the frequency of mtDNA haplotypes. T. PROUT has kindly informed us of the study L. NIGRO and he have conducted on a mating incompatibility that is mediated by cytoplasmic rickettsia in *D. simulans*, and of the pattern of selection on mtDNA variants that can result. POWELL (1982) previously suggested that a cytoplasmic endosymbiont might be involved in the one-way F_1 hybrid sterility of males in crosses between Bogota and North American *D. pseudoobscura*, although how the system might function is not clear. It is possible that a cytoplasmic microorganism was responsible for the mtDNA frequency changes in our cages, but two sets of tests argue against this hypothesis. First, when we fed the flies tetracycline at the F_{31} generation, at the concentration given in HOFFMAN and TURELLI (1988), there was no change in mtDNA frequency. This led us to reject provisionally the microorganism hypothesis.

Second, we subjected the entire initial cage (cage I in MACRAE and ANDERSON 1988) to heat shock treatments at 37° for 50 to 120 min on 3 successive days during egg laying in the F_{34} generation. Similar treatment conditions were used by EHRMAN (1967), who hypothesized that "heat shocks depress the growth or multiplication of the cytoplasmic factor which causes the maternally transmitted sterility of the hybrid males" in *D. paulistorum*. Thus, heat shocks could, in principle, induce changes in the composition of enteric cytoplasmic bacteria in *D. pseudoobscura*. This in turn could change mtDNA frequencies, if selection (or incompatibility) were in fact operating on a cytoplasmic microorganism rather than on mtDNA. The heat shock treatments, however, produced no significant change in mtDNA frequency. This led us to conclude that either there were no detectable changes in enteric bacterial composition as a result of heat shock, or that the mtDNA frequency changes were not due to selection on cytoplasmic factors susceptible to heat shock treatments.

7. Like ORR (1989) and SINGH (1983), we have also examined possible causes of the male hybrid sterility that exists in one direction of Bogota × North America matings. Our aim was to elucidate the role that mtDNA haplotypes might play in the hybrid sterility.

In tests with three different Bogota stocks, including BogER, we confirmed PRAKASH's (1972) result that the F_1 male offspring from crosses of Bogota females to North American males were all sterile.

We used a test involving replacement of the nuclear

background to examine the influence of mtDNA on F₁ hybrid male sterility. We compared the fertility of two types of hybrid males; F₁ males with *Bogota ER mtDNA* but half their nuclear genes from Bogota and the other half from California; and backcrossed males with *Bogota ER mtDNA* and about 99% of their nuclear genes from California. These backcrossed hybrids were produced by crossing California males to Bogota ER females, and then backcrossing the resulting female progeny to highly inbred, nearly homozygous California males. The backcrossing was then repeated for 12 generations. F₁ males with a 50% California/50% Bogota nuclear background were all sterile, whereas almost all of the backcrossed hybrid males, 99% of whose nuclear background had been replaced by California genes, were fertile. Thus, the Bogota mtDNA is not by itself responsible for the F₁ male sterility.

8. As we stated in our paper, the large mtDNA frequency changes we observed cannot be explained in terms of simple, constant, repeatable selection acting on haploid genomes. For the reasons discussed above, we do not think that hitchhiking with strain differences in mating behavior can explain the changes in mtDNA haplotype frequencies that we observed. Our experiment was conceived as a test of the hypothesis that mtDNA variants are selectively neutral, and our paper was titled to reflect the finding that they were not. It was only by the elimination of other nonselective explanations, and our inability to accept a simple form of constant selection as an explanation, that we came to interpret our data in terms of sporadic selection on mtDNA variants. Thus, sporadic selection was invoked as a "last resort" explanation.

There are several reasons why we considered cytonuclear interactions as one possible mechanism of selection: first, all cages reached internal equilibrium mtDNA frequencies; second, there were no significant mtDNA frequency changes on a controlled, homozygous nuclear background; and third, our "triple mtDNA cages" went to different final mtDNA frequencies after 20 generations. Other authors have also recently suggested that there can be large cytonuclear effects on fitness. For example, ZEVIANI *et al.* (1989) found large mtDNA deletions in humans with mitochondrial myopathy, and their studies indicated that the mtDNA deletions were produced by an autosomal dominant mechanism.

AMY F. MACRAE* and
WYATT W. ANDERSON†

* Department of Botany and Plant Sciences
University of California
Riverside, California 92521

† Department of Genetics
University of Georgia
Athens, Georgia 30602

LITERATURE CITED

- AYALA, F. J., and TH. DOBZHANSKY, 1974 A new subspecies of *Drosophila pseudoobscura* (Diptera:Drosophilidae). *Pan-Pacif. Entomol.* **50**: 211-219.
- DOBZHANSKY, TH., 1974 Genetic analysis of hybrid sterility within the species *Drosophila pseudoobscura*. *Hereditas* **77**: 81-88.
- EHRMAN, L., 1967 A study of infectious hybrid sterility in *Drosophila paulistorum*. *Proc. Natl. Acad. Acad. Sci. USA* **58**: 195-198.
- HOFFMAN, A. A., and M. TURELLI, 1988 Unidirectional incompatibility in *Drosophila simulans*: inheritance, geographic variation, and fitness effects. *Genetics* **119**: 435-444.
- MACRAE, A. F., and W. W. ANDERSON, 1988 Evidence for non-neutrality of mitochondrial DNA haplotypes in *Drosophila pseudoobscura*. *Genetics* **120**: 485-494.
- ORR, H. A., 1989 Genetics of sterility in hybrids between two subspecies of *Drosophila*. *Evolution* **43**: 180-189.
- POWELL, J. R., 1982 Genetic and nongenetic mechanisms of speciation, pp. 67-74 in *Mechanisms of Speciation*, edited by C. BARIGOZZI. Alan R. Liss, New York.
- PRAKASH, S., 1972 Origin of reproductive isolation in the absence of apparent genic differentiation in a geographic isolate of *Drosophila pseudoobscura*. *Genetics* **72**: 143-155.
- SINGH, R. S., 1983 Genetic differentiation for allozymes and fitness characters between mainland and Bogota populations of *Drosophila pseudoobscura*. *Can. J. Genet. Cytol.* **25**: 590-604.
- SINGH, R. S., and L. R. HALE, 1990 Are mitochondrial DNA variants selectively non-neutral? *Genetics* **124**: 000-000.
- ZEVIANI, M., S. SERVIDEI, C. GELLERA, E. BERTINI, S. DIMAURO and S. DIDONATO, 1989 An autosomal dominant disorder with multiple deletions of mitochondrial DNA starting at the D-loop region. *Nature* **339**: 309-311.