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Genetics

Interspecific cytoplasmic gene flow in the absence of nuclear gene flow: Evidence from *Drosophila*

(mtDNA/species concept/evolutionary genetics)

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ABSTRACT **mtDNA polymorphism has been studied by restriction endonuclease site variation in *Drosophila pseudoobscura* and its sibling species *D. persimilis*. Eight enzymes have been used to study 54 isofemale strains from areas where the two species are sympatric and *D. pseudoobscura* is allopatric. Where sympatric, 75–80% of the strains have mitochondrial genomes found in both species. Where allopatric, *D. pseudoobscura* has diverged to the point where none of the strains have mtDNA in common with *D. persimilis*. The most likely explanation for this observation is that where sympatric the two species hybridize frequently enough to keep their mtDNA from diverging. However, hybridization has not prevented their nuclear genomes from diverging, perhaps due to selection against nuclear gene introgression contrasted with little or no selection against mtDNA introgression. These observations suggest that nuclear and cytoplasmic genomes have different evolutionary dynamics.**

The biological species concept states that species are groups of populations reproductively isolated from other such groups—i.e., they do not exchange genetic material (1, 2). For diploid sexually reproducing organisms, this concept has become generally accepted by evolutionists and systematists. The importance of the definition in an evolutionary view is that, in the absence of gene exchange, species are genetically independent evolutionary units. The most important observation that led Dobzhansky to articulate this species concept was the recognition of sibling species, species that are morphologically identical (or nearly so) yet are reproductively isolated by one or several mechanisms (3). The species studied by Dobzhansky that were instrumental in the development of these ideas were *Drosophila pseudoobscura* and its sibling *D. persimilis* (4). Vigorous hybrids between these species can be made in the laboratory; F_1 females are fertile while F_1 males are sterile (5). Furthermore, the mating behaviors of the species are such that only intraspecific matings are observed in nature and even in confined laboratory environments, when given a choice, a female will almost always mate with a male of her species (6). Further observations of fixed chromosome differences between species (7) as well as complete or nearly complete differentiation at protein-coding loci (8–10) leave little doubt that *D. pseudoobscura* and *D. persimilis* are good species. That is, they exchange nuclear genetic material not at all or so rarely that for all practical purposes they are independent evolutionary units.

In recent years, new technology has allowed evolutionary geneticists to study variation in cytoplasmically inherited genetic material—i.e., the DNA in mitochondria and plant plastids (11). This genetic material is maternally inherited in the egg cytoplasm and thus one can use variation in this DNA to follow maternal lineages. While the amount of DNA in cytoplasmic organelles is very small compared with the amount in nuclear chromosomes, it does play an important role and variation in organellar DNA can have profound effects (12, 13).

The most commonly used method to study mtDNA polymorphism is to isolate the DNA and cut it with restriction endonucleases that recognize a specific set of four to six contiguous nucleotides (14). The fragments generated are separated by size by gel electrophoresis. Because mtDNA is a circular molecule, the number of fragments is equal to the number of endonuclease recognition sites in the molecule. A variant mtDNA that has lost or gained a recognition site will generate a different fragment pattern. In addition, variant molecules may have large enough deletions or insertions of DNA to be detectable by electrophoresis.

I summarize here variation in mtDNA from 54 strains of *D. pseudoobscura* and *D. persimilis* as detected by eight restriction endonucleases. Each strain was begun by a single female captured in nature. Because of homogeneity of mtDNA within individuals (8, 9) and maternal inheritance, such strains should be homogeneous for mtDNA; no evidence of heterogeneity within a strain has been observed.

MATERIALS AND METHODS

Fifty-four strains of *D. pseudoobscura* and *D. persimilis*, each begun by a single inseminated female from a natural population, have been studied. The *D. pseudoobscura* strains came from Mather, CA, near Yosemite National Park (11 strains); Santa Cruz Island, about 40 km off the coast at Santa Barbara, CA (14 strains); Sabinas Hidalgo, 85 km north of Monterrey, Mexico (7 strains); and Bogotá, Colombia (7 strains), a geographically isolated population that is also partially reproductively isolated (15, 16) (Fig. 1).

Details of the procedures used to isolate mtDNA will be presented elsewhere. Nuclei were removed from a homogenate of flies by using differential centrifugation. DNA was then isolated from a pellet of the remaining organelles. Evidence that this procedure does isolate mtDNA, or at least enriches for it to such an extent that nuclear contamination is a weak background, includes the following: (*i*) the resulting DNA produces a single band of about 17 kilobase pairs as sized by circular markers on agarose electrophoresis gels, fragments generated by restriction endonuclease digestion total about 17 kilobase pairs as sized by linear markers, (*ii*) crosses between strains having different patterns of digested fragments indicate that the DNA is maternally inherited, (*iii*) the *Eco*RI and *Hin*dIII digestion patterns of a strain of Oregon R *D. melanogaster* are identical to published descriptions of the mtDNA this strain isolated from cesium chloride gradients (17, 18).

Eight restriction endonucleases were used to digest mtDNA from all 54 strains; these were *Eco*RI, *Hin*dIII, *Pvu* II, *Taq* I, *Hin*cIII, *Ava* II, *Bst*NI, and *Hae* III. They were obtained from New England BioLabs and Bethesda Research Laboratories;

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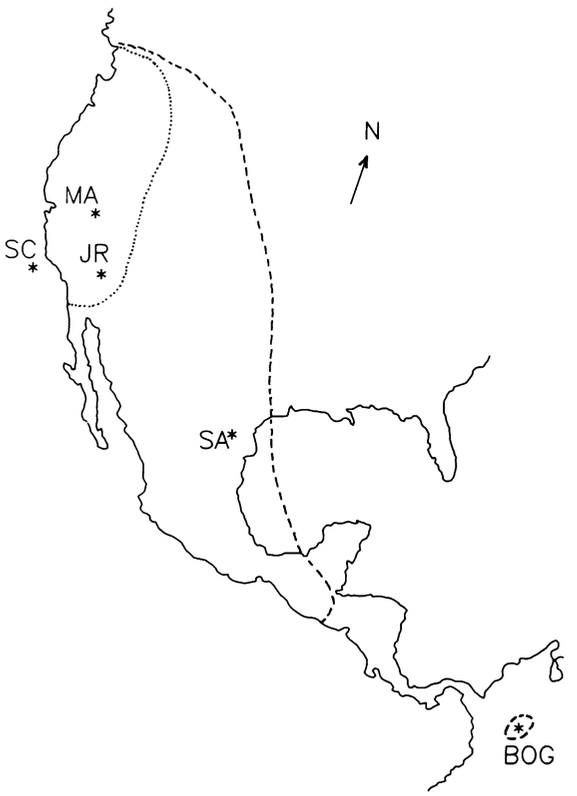

FIG. 1. Distributions of species and origin of samples. ---, *D. pseudoobscura*; ·····, *D. persimilis*. Sample locality abbreviations: MA, Mather; SC, Santa Cruz; JR, James Reserve; SA, Sabinas; BOG, Bogotá.

digestion conditions were those suggested by the suppliers. Digestion fragments were separated by agarose gel electrophoresis (0.9%) in a Tris borate/EDTA buffer. Bacteriophage λ DNA digested with *Hin*dIII served as size markers. DNA fragments in gels were detected by ethidium bromide staining.

RESULTS

For each restriction endonuclease, one pattern, the most common in this sample of strains, is designated A. Patterns that deviate from A are denoted by succeeding letters; in almost all cases, the different patterns can be derived from the A pattern by a single loss or gain of a recognition site. In addition, some strains were found in which the mtDNA gave the A pattern for all eight endonucleases, except that one fragment was consistently about 500 base pairs larger than the corresponding fragment in the A pattern. This is interpreted as representing a single insertion of DNA. A sample of the data is shown in Fig. 2.

The relationships among the 18 different mtDNAs from these 54 strains are given in Table 1. The composite designations (for all eight enzymes) are roman numerals; designations II–XII denote patterns derivable from designation I by a single event while designations XIII–XVIII denote patterns two or three changes away from designation I. (All patterns will be illustrated in another publication.) The frequencies of the 18 mtDNA genomes in the six population samples are given in Table 2.

DISCUSSION

The first point of note is the large amount of variation in mtDNA in these species: 18 different mitochondrial genomes in a sample of 54 strains. This level of variation is consistent with the previously published survey of variation in *Drosophila* mtDNA in

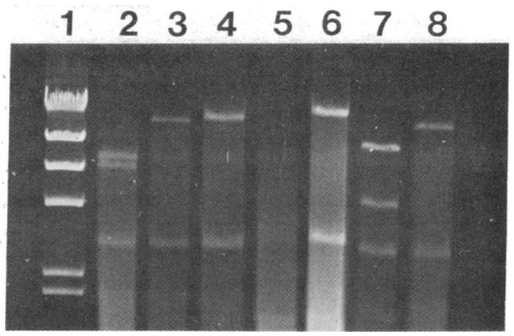

FIG. 2. *Hae* III digestion of *D. pseudoobscura* and *D. persimilis* mtDNA. Electrophoresis is from the top; fragments were visualized by ethidium bromide staining. Lanes: 1, *Hin*dIII-digested λ DNA size standards (from top to bottom, 23, 9.5, 6.7, 4.3, 2.25, and 1.95 kilobase pairs); 2, pattern B; 3 and 8, pattern A; 4 and 6, pattern A with a single insert in the larger fragment; 7, pattern E. Patterns B and E can be derived from pattern A by the addition of a recognition site in the larger fragment of A.

which three different mtDNA genomes were detected in a sample of 10 *D. melanogaster* strains (19).

The point emphasized here is the striking similarity in mtDNA in the two species where they are sympatric and the dissimilarity in areas where *D. pseudoobscura* is allopatric. The most common mtDNA (composite pattern I) is the same in both species where they are sympatric. Even some of the relatively rare variant patterns (XII and XIII) are shared by the two species. Of the seven different mtDNAs detected in the sample of 15 strains of *D. persimilis*, four also exist in the sample of 25 sympatric strains of *D. pseudoobscura*. Eighty percent (12/15)

Table 1. Designation of the 18 mtDNA genomes found in the 54 strains of *D. pseudoobscura* and *D. persimilis*

Composite designation	Endonuclease	Variation from all-A pattern
I	—	None
II	*Bst*NI	B
III	*Hinc*II	C
IV	*Hae* III	B
V	*Hae* III	G
VI	*Hae* III	E
VII	*Ava* II	B
VIII	*Pvu* II	C
IX	*Hae* III	C
X	*Taq* I	E
XI	*Eco*RI	D
XII		Single insert
XIII	*Hin*dIII	D
	Hae III	B
XIV	*Pvu* II	C
	Hae III	C
XV	*Pvu* II	C
	Taq I	D
XVI	*Hinc*II	B
	Hae III	C
XVII	*Pvu* II	B
		Single insert
XVIII	*Eco*RI	C
	*Hin*dIII	D
	Taq I	F

Strains having mtDNA that produces the most common digestion pattern for all eight restriction endonucleases, all pattern A, are designated I. Variants from this are denoted by subsequent roman numerals. (All patterns will be illustrated in another publication.)

Table 2. Number of strains from each locality having the different mtDNA genomes denoted in Table 1

	Composite pattern																	
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII
D. pseudoobscura																		
Sabinas (A)	—	—	—	—	—	—	—	3	1	—	—	—	—	2	—	—	—	—
Bogotá (A)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7	—	—
Mather (S)	7	1	1	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—
Santa Cruz (S)	6	2	—	1	1	1	1	—	—	—	—	1	—	—	—	—	1	—
D. persimilis																		
Santa Cruz (S)	5	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	1
James Reserve (S)	4	1	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—

(S), Area where *D. pseudoobscura* and *D. persimilis* coexist; (A), area where only *D. pseudoobscura* is found.

of the *D. persimilis* strains have mtDNA endonuclease patterns known in sympatric strains of *D. pseudoobscura*; 76% (19/25) of sympatric *D. pseudoobscura* have mtDNA patterns found in *D. persimilis*. Considering these rather small sample sizes, detecting this degree of sharing of mtDNA, including variants from the most common form, is remarkable.

In contrast to this interspecific similarity in sympatry, where *D. pseudoobscura* is allopatric (Sabinas and Bogota), there is complete divergence of mtDNA. None of the patterns in these two populations is found in *D. persimilis* nor in Mather or Santa Cruz *D. pseudoobscura*. Thus, there is more divergence between allopatric populations of the same species than between sympatric populations of different species.

A possible explanation for the mtDNA similarity between species is that the mtDNA is evolving very slowly. However, considering (*i*) the degree of polymorphism within populations, (*ii*) the differentiation of allopatric populations, and (*iii*) evidence that mtDNA evolves more rapidly than nuclear DNA in other organisms (20), this explanation seems unlikely. Furthermore, in preliminary studies in this laboratory, the closely related species *D. miranda* has been observed to have diverged considerably in mtDNA. *D. miranda* has a geographic range similar to that of *D. persimilis* and is thus sympatric with both other species. *D. miranda* can hybridize with both *D. pseudoobscura* and *D. persimilis* but with much greater difficulty; F_1 males are always sterile and F_1 females are either sterile or partially fertile, depending on the particular strains used (21, 22). No *D. miranda* hybrids have been observed in nature.

Invoking some type of selection to explain the results is likewise unappealing. These species diverged from each other long enough ago to allow their nuclear genomes to become completely divergent even where sympatric. What kind of ecological constraints could be acting at the nucleotide level of mtDNA and not affect the nuclear genome? Selection would have to be acting to keep mtDNA more similar between species than among different populations of the same species.

An alternative explanation consistent with the observations is that, where sympatric, *D. pseudoobscura* and *D. persimilis* hybridize frequently enough to keep their mtDNA from diverging. Keep in mind that F_1 hybrid females are fertile, the sex that must be fertile to pass cytoplasmic genes. Hybrids are known to occur in nature (ref. 23; B. C. Moore, personal communication). Three female hybrids have been unambiguously observed as they produced progeny with chromosomal complements that could only occur in backcrosses of F_1 hybrids. It is estimated that progeny from about 30,000 females from areas where the two species are sympatric have been analyzed for polytene chromosomes; thus, the unambiguous hybrid rate is about 3/30,000. More frequently, females from nature give rise to progeny having chromosomal complements of F_1 hybrids, but these are suspect as mating may occur in the confines of collecting vials. If these hybrid events represent potential episodes of interspecific gene flow, then this frequency of about 10^{-4} is great enough to keep large populations from randomly drifting apart (24).

This explanation raises the question: Why then have the nuclear genomes become so divergent? There may be selection against introgression of nuclear genes in backcross generations while little or no selection occurs against the mtDNA. Because of the mode of inheritance of mtDNA, all backcross offspring will have identical mtDNA so the only way to select against mtDNA gene flow is to select against backcross females altogether. However, because of recombination of nuclear genes, selection can act on backcross offspring having various degrees of nuclear genes of the two species. If the different genes of a species genome are coadapted with one another, as seems to be the case in these species of *Drosophila* (4), then it is conceivable that backcross offspring having more genes from a single species are favored over those having a mixture.

The conclusion reached here is not intended to call into question the species status of these siblings nor to question the validity of the biological species concept. Rather, it is to point out that the evolutionary biology of nuclear and cytoplasmic genomes may be different.

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