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GENETIC DIFFERENTIATION IN TWO MEMBERS OF THE *DROSOPHILA ATHABASCA* COMPLEX

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One central question of much interest to students of evolution is the amount of genetic change required for speciation. Situations which should provide information of particular interest are those where: (1) the level of phylogenetic relationship between the groups of organisms is unambiguously specified and (2) the process of speciation is not complete or is known to have been recently completed.

For this question the concern with partial or recent speciation arises because the divergence of species involves two sources of differentiation: (1) the genetic variation responsible for reproductive isolation may be accompanied by or follow genetic differentiation in ecological preferences or niches of the two incipient species and (2) genetic variation may arise within each species after speciation is complete. Measurement of variation in species which have long been isolated from one another cannot discriminate between the two sources of variation.

The application of electrophoresis to the detection of allozyme variability has made further study of speciation possible (see Ayala, 1975, and references therein). However, relatively few studies have been done using subspecific groups or incipient species. These include studies of subspecies of mice (Selander et al., 1969; Hunt and Selander, 1973), studies of subspecies and semispecies in *Drosophila* (Zouros, 1973; Richmond, 1972; Ayala et al., 1974; Prakash, 1972; Ayala and Dobzhansky, 1974), and studies of species of pocket gophers (Nevo et al., 1975).

The *D. athabasca* complex was used in the present study. It is a member of the affinis subgroup which in turn is part of the obscura group of *Drosophila* (Patterson and Stone, 1952). The division of the complex into three groups ("western-northern," "eastern A," and "eastern B") is based on several lines of evidence (Miller and Voelker, 1972). The groups are morphologically indistinguishable, but they exhibit premating isolation (Miller, 1958; Miller and Westphal, 1967), differences in the pulse repetition rate of males during courtship (Miller et al., 1975), unequal mean copulation times (Miller and Westphal, 1967), and chromosomal differences. Three of the seven chromosome arms contain different, diagnostic orders of salivary chromosomal bands in "western-northern" and "eastern" *D. athabasca*. In addition, "eastern A" and "eastern B" can usually be distinguished by the sequences of both arms of the X chromosome and both arms of the B chromosome (Miller et al., 1975).

Laboratory crosses between members of different groups have yielded fertile, viable offspring. The ranges of "western-northern" and "eastern A" *D. athabasca* and of "eastern A" and "eastern B" *D. athabasca* do overlap. However, the maintenance of distinct chromosomal sequences in each group, even in areas of overlap, indicates that the forms interbreed infrequently, if at all, in nature. This distinctness implies that the flies are separate genetic entities. Since sampling of "eastern" and "western-northern" *D. athabasca* has been very limited, the maintenance of reproductive isolation

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TABLE 1. *Number of "western-northern" and "eastern A"* D. athabasca *isofemale lines collected at each site.*

Site	Time of collection	Total number	"Western-northern"	"Eastern A"	Unclassified	Collector
Old Mill State Park (OM) (Thief River Falls, Minn.)	July 1973	36	34	1	1	D. Johnson
Lake Itasca State Park (LI) (Minn.)	July 1973	41	33	5	3	D. Johnson
Brule, Wisc. (B)	July 1973	19	6	12	1	D. Johnson
St. Croix State Park (SC) (Sandstone, Minn.)	August 1974	30	1	29	0	D. Johnson
Maplewood State Park (M) (Fergus Falls, Minn.)	August 1974	30	0	30	0	D. Johnson
Sibley State Park (SIB) (Willmar, Minn.)	July 1973	36	0	36	0	D. Johnson
Lytton, Quebec (L)	July 1973	17	2	14	1	L. H. Throckmorton
Saranac, New York (S)	July 1973	19	0	18	1	L. H. Throckmorton
		228	76	145	7	

across their total range of overlap has not been established. Therefore, they have been recognized as semispecies. The two eastern groups may also each represent semispecies (Miller and Voelker, 1972).

The *D. athabasca* complex is a promising one for the study of the genetic differentiation required for speciation since the forms cross in the laboratory but do have a definite degree of reproductive isolation in nature. Two of the three groups have been used in this study. They are *D. athabasca* "western-northern" and *D. athabasca* "eastern A" (hereafter referred to as *D. athabasca* "eastern").

METHODS AND MATERIALS

Samples of D. athabasca

Professor Dwight D. Miller of the University of Nebraska kindly provided a number of stocks which he had maintained in his laboratory. The stocks represented *D. athabasca* "western-northern" and *D. athabasca* "eastern A" and "eastern B" semispecies. These stocks were used for (1) the testing and modification of protein assays and (2) standards of reference of protein mobility in the assays. In addition, eight natural populations of the *D. athabasca* complex were sampled (Table 1).

Classification of the isofemale lines as "western-northern," "eastern A" or "eastern B" was based on the sequence of the long arm of the X chromosome in female larvae. The order of the bands can distinguish "eastern" and "western-northern" flies and will usually distinguish "eastern A" and "eastern B" flies (Miller and Voelker, 1969*a*). The larvae were dissected in Shen's solution, and the glands were stained with aceto orcein. Slides with the long arm of the X chromosome extended were scored and made permanent by the technique of Baker (1952).

Methods Used to Detect Allozyme Variation

Allozyme variation was measured at 17 loci (Table 2). These 17 loci were chosen because the assays for them yielded clean, well-defined, repeatable patterns for the members of the *D. athabasca* complex. All such readable assays were crosschecked to see if two supposedly different assays were actually staining the same protein. This

TABLE 2. *Allele frequencies of "eastern A" and "western-northern"* D. athabasca. *N* = estimated number of genomes. For locations, see Table 1. w = "western-northern," e = "eastern A." Lines over these symbols = average for semispecies.*

		Location														
Locus	Allele	OM w	OM e	LI w	LI e	B w	B e	SC w	SC e	M e	SIB e	L w	L e	S w	$\bar{w}$	$\bar{e}$
Tetra-	*.61*	—	—	—	—	—	—	—	0.01	—	—	—	—	—	—	<0.01
zolium	*1.00*	>0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.99	0.99	1.00	1.00	1.00	1.00	>0.99	0.99
Oxidase	*1.44*	<0.01	—	—	—	—	—	—	—	0.01	—	—	—	—	<0.01	<0.01
(*To*)	*N**	76.5	2.7	75.3	8.0	15.5	31.7	2.7	77.3	80.0	88.9	4.3	36.0	40.0	174.3	361.9
Larval	*.97*	—	—	—	—	—	—	—	—	—	0.01	—	—	—	—	<0.01
Protein 1	*1.00*	1.00		1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	>0.99
(*Lp-1*)	*N**	60.0		56.0	6.0	10.0	20.0	3.0	84.0	90.0	60.0	4.0	24.0	30.0	133.0	294.0
Larval	*.96*	0.01		—	—	—	—	—	0.01	0.01	0.01	—	—	—	0.01	<0.01
Protein 2	*1.00*	0.97		1.00	1.00	1.00	1.00	1.00	0.96	0.99	0.98	1.00	1.00	1.00	0.98	0.98
(*Lp-2*)	*1.05*	0.02		—	—	—	—	—	0.02	—	0.01	—	—	—	0.01	0.01
	null	—		—	—	—	—	—	0.01	—	—	—	—	—	—	<0.01
	*N**	58.0		56.0	6.0	10.0	20.0	3.0	84.0	90.0	60.0	4.0	24.0	30.0	131.0	294.0
Larval	*.96*	—		—	—	—	—	—	—	0.01	—	—	0.01	—	—	<0.01
Protein 3	*1.00*	0.98		1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00	0.99	0.96	0.99	0.98
(*Lp-3*)	*1.03*	0.02		—	—	—	—	—	—	—	—	—	—	0.04	0.01	0.01
	null	—		—	—	—	—	—	—	0.01	—	—	—	—	—	<0.01
	*N**	58.0		56.0	6.0	10.0	20.0	3.0	84.0	90.0	60.0	4.0	24.0	30.0	131.0	294.0
Larval	*1.00*	0.97		0.92	1.00	1.00	0.98	1.00	0.95	1.00	0.98	1.00	1.00	0.98	0.98	0.99
Protein 4	*1.03*	0.03		0.08	—	—	0.02	—	0.05	—	0.02	—	—	—	0.02	0.01
(*Lp-4*)	*N**	58.0		56.0	6.0	10.0	20.0	3.0	84.0	90.0	60.0	4.0	24.0	30.0	131.0	294.0
Larval	*.95*	—		—	—	—	—	—	—	—	0.01	—	0.02	0.42	—	0.05
Protein 5	*1.00*	0.97		0.92	1.00	1.00	1.00	1.00	1.00	0.98	0.99	1.00	0.96	0.58	0.96	0.95
(*Lp-5*)	*1.04*	0.03		0.08	—	—	—	—	—	0.02	—	—	0.02	—	0.04	<0.01
	*N**	58.0		56.0	6.0	10.0	20.0	3.0	84.0	90.0	60.0	4.0	24.0	30.0	131.0	294.0
Phospho-	*.84*	0.04		—	0.23	—	—	—	0.02	0.05	0.02	—	—	0.08	0.02	0.04
gluco-	*1.00*	0.96		1.00	0.77	1.00	0.87	1.00	0.84	0.85	0.88	1.00	0.93	0.77	0.98	0.85
mutase	*1.13*	—		—	—	—	0.13	—	0.14	0.09	0.10	—	0.07	0.15	—	0.11
(*Pgm*)	*1.21*	—		—	—	—	—	—	—	0.01	—	—	—	—	—	<0.01
	*N**	55.0		44.0	6.5	11.5	22.0	2.5	67.5	73.5	68.5	2.5	27.5	36.5	115.5	302.5

TABLE 2. *Continued.*

Locus	Allele	Location														
		OM w	OM e	LI w	LI e	B w	B e	SC w	SC e	M e	SIB e	L w	L e	S w	$\bar{w}$	$\bar{e}$
Xanthine	*.95*	0.01	—	0.01	—	—	—	—	—	0.03	0.01	—	—	—	0.01	0.01
Dehydro-	*.97*	0.18	—	0.08	—	—	0.02	—	0.05	0.04	0.06	0.25	0.03	0.06	0.12	0.05
genase	*.99*	0.14	0.50	0.07	—	0.16	—	1.00	0.13	0.09	0.06	—	—	—	0.12	0.07
(*Xdh*)	*1.00*	0.19	—	0.42	0.50	0.53	0.43	—	0.35	0.35	0.31	0.25	0.27	0.39	0.32	0.34
	1.01	0.07	—	0.13	—	—	—	—	0.11	0.15	0.10	0.50	0.32	0.02	0.10	0.11
	1.02	0.21	0.50	0.19	0.50	0.31	0.55	—	0.34	0.34	0.39	—	0.33	0.48	0.20	0.39
	1.04	0.04	—	0.01	—	—	—	—	—	—	—	—	0.05	0.02	0.02	0.01
	1.05	0.09	—	0.04	—	—	—	—	—	—	0.07	—	—	—	0.05	0.02
	1.06	0.02	—	0.01	—	—	—	—	—	—	—	—	—	—	0.02	—
	1.07	0.04	—	0.01	—	—	—	—	—	—	—	—	—	0.03	0.02	<0.01
	1.09	—	—	0.01	—	—	—	—	—	—	—	—	—	—	0.01	—
	null	0.01	—	0.02	—	—	—	—	0.02	—	—	—	—	—	0.01	<0.01
	*N**	78.0	3.0	81.0	6.0	19.0	28.0	3.0	90.3	85.0	84.3	6.0	34.7	45.0	189.0	376.3
Alcohol	*1.00*	—	1.00	—	1.00	0.15	1.00	—	1.00	1.00	1.00	—	1.00	1.00	0.02	1.00
Dehydro-	*1.10*	1.00	—	1.00	—	0.85	—	1.00	—	—	—	1.00	—	—	0.98	—
genase	*N**	80.3	3.0	76.7	9.0	16.0	19.0	2.7	77.7	80.0	81.7	5.7	33.0	40.0	181.7	343.0
(*Adh*)																
Malic	*.92*	0.04		—	—	—	—	—	—	—	—	—	—	—	0.02	—
Enzyme	*1.00*	0.96		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.93	0.98	0.99
(*Me*)	*1.08*	—		—	—	—	—	—	—	—	—	—	—	0.07	—	0.01
	*N**	79.3		71.3	5.3	16.0	22.3	2.7	76.7	80.0	79.3	2.7	33.3	37.3	167.7	351.7
Esterase	*.86*	—		0.03	—	—	—	—	0.02	—	—	—	—	—	0.01	<0.01
(*Est*)	*.90*	0.04		—	—	—	—	—	—	—	—	—	—	0.04	0.02	<0.01
	.92	0.03		0.04	0.45	—	0.59	—	0.64	0.53	0.64	—	0.68	0.81	0.01	0.63
	.96	0.08		0.06	0.36	—	0.21	—	0.22	0.27	0.24	—	0.21	0.10	0.06	0.21
	.98	0.03		0.03	—	—	—	—	—	—	—	—	—	—	0.02	—
	1.00	0.78		0.76	0.19	1.00	0.11	1.00	0.12	0.20	0.11	1.00	0.11	0.05	0.83	0.13
	1.06	0.03		0.02	—	—	0.09	—	—	—	—	—	—	—	0.01	<0.01
	null	0.01		0.06	—	—	—	—	—	—	0.01	—	—	—	0.04	0.01
	*N**	72.5		70.0	5.0	13.5	22.0	2.5	74.0	78.5	62.5	3.0	31.0	38.5	151.0	318.5

TABLE 2. *Continued.*

Locus	Allele	Location																	
		OM w	OM e	LI w	LI e	B w	B e	SC w	SC e	M e	SIB e	L w	L e	S w	w	e
Leucine-Amino-Peptidase (*Lap*)	*.92*	—		—	—	—	—	—	—	—	0.01	—	—	—	—	<0.01
	.94	0.04		0.11	—	0.09	—	—	—	—	0.02	—	0.02	0.02	0.09	0.02
	.97	0.47		0.13	0.06	0.04	0.32	—	0.01	0.03	0.09	—	0.06	0.14	0.24	0.02
	1.00	0.45		0.70	0.94	0.87	0.64	1.00	0.98	0.87	0.82	1.00	0.88	0.77	0.60	0.84
	1.03	0.04		0.06	—	—	0.04	—	0.01	0.10	0.06	—	0.04	0.09	0.07	0.05
	1.06	<0.01		—	—	—	—	—	—	—	—	—	—	—	<0.01	—
	*N**	85.3		74.0	10.7	15.0	23.7	2.7	83.5	80.0	99.0	5.3	35.0	45.3	193.0	401.0
Octanol Dehydrogenase (*Odh*)	*.83*	—	—	—	—	—	0.02	—	—	0.02	—	—	—	—	—	<0.01
	1.00	0.96	1.00	0.99	0.78	1.00	0.98	1.00	0.97	0.93	0.89	1.00	1.00	0.98	0.98	0.93
	1.14	—	—	—	0.11	—	—	—	—	—	0.11	—	—	0.02	—	<0.01
	1.23	0.04	—	0.01	0.11	—	—	—	0.03	0.04	—	—	—	—	0.02	0.05
	1.33	—	—	—	—	—	—	—	—	0.01	—	—	—	—	—	0.01
	*N**	87.7	3.0	74.3	12.3	13.3	28.8	2.7	84.5	80.0	80.0	5.7	34.7	45.0	185.3	357.1

occurred with the assays for aldehyde oxidase (*AO*) and xanthine dehydrogenase (*XDH*). The *XDH* assay yielded a more readable pattern so it was retained.

Nine assays, which revealed variation at 14 loci, were performed by electrophoresis in vertical acrylimide gels. The general techniques are described elsewhere (Hubby and Lewontin, 1966), but some required modification. The altered ones are: alcohol dehydrogenase (*ADH*)—the current was reversed since the *ADH* of *D. athabasca* migrates toward the cathode; octanol dehydrogenase (*ODH*)—the gel concentration was 6% acrylamide and electrophoresis was performed for 2½ h; leucine-amino-peptidase (*LAP*)—the gel concentration was 6% acrylamide; malic enzyme (*ME*)—electrophoresis was performed in the manner described for the malate dehydrogenase (*MDH*) assay of Hubby and Lewontin (1966), but the stain is that cited in Richmond (1972); tetrazolium oxidase (*TO*)—electrophoresis and stain citation were the same as for *ME*.

Three assays were performed in starch. Horizontal starch gel electrophoresis was performed at 400 v and 10 to 20 ma for 16 h. The gels were composed of 45 g of electrostarch in 450 ml of 0.1 M tris-borate-EDTA buffer pH 8.9. This buffer was used in the gel chambers also. The stains for the assays were cited in the following papers: phosphoglucomutase (*PGM*)—Richmond (1972), α-glycerophosphate dehydrogenase (*α-GPDH*)—Hubby and Lewontin (1966), fumarase (*FUM*)—Zouros (1973).

No age related variation in allozymic pattern was found for flies aged zero to 10 days after emergence; therefore, flies of indeterminate ages were used for all assays of adult proteins. Most flies were aged 10 days or less when they were prepared for use in protein assays. Extracts of third instar larvae were assayed for larval hemolymph proteins (*LP*). Live adults were used for assays of *EST* and *ODH* and for most assays of *XDH*. Adults, frozen at −27 C, were used for the remaining assays.

The results of the protein assays were recorded as follows. Extracts of two or more flies from stocks fixed for one electrophoretic allele at the locus being assayed were placed on every gel as a standard. The mobilities of the proteins of flies from other stocks were compared to them. The most common variant at each locus was designated by 1.00. The variants exhibiting different mobilities were symbolized by the mobility of those proteins relative to the 1.00 standard.

Two F_1 offspring of each isofemale line were sampled for each protein assay in most cases. This method of sampling requires estimation of the number of different genomes sampled. Since sibs were used, the actual number of different genomes assayed was less than two times the number of individuals sampled. Cotterman's weighting system (1947, 1954) was used for estimation of sample sizes of sibs. The weighted sum of the genomes will reflect the possibility of counting the same parental genome more than once. Each genome was multiplied by $2/(s + 1)$ where s is the number of sibs scored. Two exceptional cases existed. Two loci, *Est* and *Pgm*, are sex-linked. The weighted sum of the chromosomes observed was estimated to be 2.5 whether for samples of two females or of one female and one male. In some cases three F_2 individuals were sampled. Here each genome was given a weight of one-half.

RESULTS

Variation in D. athabasca *"Western-Northern"*

The distribution of "western-northern" isofemale lines is given in Table 1. Variant frequencies for "western-northern" flies from each sample site are given in Table 2. The average frequencies are also in this table. (Four loci, *Lp-O, Fum, Mdh,* and *α-Gdph* were monomorphic in all samples of both "eastern A" and "western-northern" *D. athabasca* and are not included in the table.)

Patterns of variation in *D. athabasca* "western-northern" can be categorized as follows:

1) Monomorphic loci: These include *Fum, α-Gpdh, Mdh, Lp-O, Lp-1,* and *Adh* (for all western lines except those from Brule).

2) Loci at which the frequency of one variant is greater than 0.95: The criterion is applied to the Old Mill and Lake Itasca collections where the sample size was always greater than 15. The loci are *Pgm, Odh, Me, To, Lp-2,* and *Lp-3* (Table 2).

3) Polymorphic loci with similiar patterns of variation: *Lp-4* and *Lp-5* each have one variant which is in a frequency greater than 0.90 in all "western-northern" samples. Eight variants have been found at the *Est* locus. However, *Est 1.00* is the most common in the two large samples, and it is the only variant found in the small ones (Table 2).

4) Polymorphic loci with different patterns of variation: Two loci show marked variation in variant frequencies in the Old Mill and Lake Itasca samples. They are *Xdh* and *Lap* (Table 2).

Variation in D. athabasca *"Eastern"*

Patterns of variation in *D. athabasca* "eastern" were similiar to those of the "western-northern" form.

1) Monomorphic loci: No variants were observed at the *Fum, α-Gpdh, Mdh, Lp-O,* and *Adh* loci.

2) Loci at which the frequency of one variant is greater than 0.95: Four loci meet this criterion in the six largest collections of "eastern" flies. These are *To, Lp-1, Lp-2,* and *Lp-3* (Table 2).

3) Polymorphic loci with similiar patterns of variation: The *Me, Lp-4, Odh,* and *Pgm* loci each have one variant which is most common or fixed in each "eastern" sample. All "eastern" samples exhibit similiar frequencies of the three most common forms at the *Est* locus.

4) Polymorphic loci with different patterns of variation: Two loci, *Lap* and *Lp-5,* each have one allele which is the most fre-

quent variant in all samples. However, the range in frequency of the common form is very large in both cases. The *Xdh* locus has two common variants. $Xdh^{1.00}$ is the most frequent form found in two samples while $Xdh^{1.02}$ is the most frequent form found in the others (Table 2).

Comparison of "Eastern" and "Western-Northern" Populations

Eleven of 17 loci carry the same or similiar arrays of variants both within and between the two forms. Five loci, *Fum, Mdh, Lp-O,* α-*Gpdh,* and *Lp-4* have identical electrophoretic forms in both groups. At six more loci, *Lp-1, Lp-2, Lp-3, Me, To,* and *Lp-5,* the same common form is shared by both groups. Variants unique to "eastern" or "western-northern" samples are also found at each of these loci. However, with the exception of *Lp-5,* the unique forms are all rare. The unique $Lp\text{-}5^{.95}$ was found only in *D. athabasca* "eastern" and it was common only in one of the three collections where it was found. At the six polymorphic loci, 11 of 19 electrophoretic variants are shared by both groups. "Western-northern" flies have one unique variant and "eastern" flies have seven, two of which are null variants (Table 2).

The remaining six loci, *Adh, Est, Odh, Lap, Xdh,* and *Pgm* all exhibit heterogeneous allele frequencies either within or between the groups.

The situation at the *Adh* locus is clearly defined. *D. athabasca* "eastern" samples carried only one form at this locus, $Adh^{1.00}$. Sixty-two of 63 "western-northern" isofemale lines carried $Adh^{1.10}$. The one "western-northern" line with $Adh^{1.00}$ was collected at Brule, a site where overlap of the two forms occurs. The variants of the *Adh* locus would serve as highly diagnostic characteristics for the two groups. The method of Ayala and Powell (1972) was used to estimate the probability of correct diagnosis of the form of a single individual with a known genotype at the *Adh* locus. It is greater than 0.999.

The *Est* locus also exhibits marked differences in variant frequencies between "eastern" and "western-northern" flies. The most common in *D. athabasca* "western-northern," $Est^{1.00}$, has an average frequency of 0.83. In "eastern" flies its average frequency is 0.13. The most frequent *Est* allele in the "eastern" flies is $Est^{.92}$ with an average frequency of 0.63. In "western-northern" flies the average frequency of this variant is 0.01. The differences in allele frequencies are obviously statistically significant. A contingency χ^2 test was performed on the three largest collections of "western-northern" flies, and another was performed on the six largest collections of "eastern" flies to determine if there were significant differences in frequencies between populations of each group. None was found (Table 3).

χ^2 tests were performed using the average frequencies at the remaining four loci to determine if statistically significant differences existed between the groups. If they did, *G* tests (Sokal and Rohlf, 1969) were used to group the samples by gene frequencies (Table 3). The *G* test was modified to compensate for the use of sibs in the data by basing it on the multinomial dirichlet likelihood (Sing and Rothman, 1975). By this test, homogeneous combinations of samples could be determined; that is, combinations where allele frequency differences are less than the level of statistical significance.

The *Odh* locus does not exhibit significant heterogeneity in frequencies between "eastern" and "western-northern" flies even though "eastern" flies have five variants at the locus as compared to "western-northern" flies' two variants (Table 2).

The *Lap* locus exhibits considerable heterogeneity in variant frequencies, even after excluding small samples (*N* less than 15). There are significant differences between the average variant frequencies of "eastern" and "western-northern" flies. However, the *G* tests indicate that within group differences account for much of that heterogeneity. Neither all "western-northern" nor all "eastern" samples can be combined without significant increases in het-

TABLE 3. *Tests for heterogeneity of gene frequencies of populations of* D. athabasca *"western-northern" and* D. athabasca *"eastern A."*

1 Contingency χ^2 statistics[1] for gene frequencies of the *D athabasca* complex

Locus		*df*
Est ("western-northern")	3.03[2]	8
("eastern")	24.84[2]	8
Odh	4.75	2
Lap	52.62**	5
Pgm	6.5*[2]	2
	4.6[3]	2
Xdh	31.86**	7

2 Groupings of populations of *D. athabasca* complex which exhibit homogeneous allele frequencies

Locus	Group	*G*[4] Value	*df*
Lap	1. Lake Itasca ("western-northern"), Brule ("western-northern" and "eastern"), Sibley ("eastern"), Lytton ("eastern"), Saranac ("eastern")	23.28	15
	2. St. Croix ("eastern"), Maplewood ("eastern")	5.08	3
	3. Old Mill ("western-northern")	—	—
Pgm	1. Old Mill ("western-northern"), Lake Itasca ("western-northern"), Brule ("western-northern")	not signif. (by inspection)	
	2. Brule ("eastern"), St. Croix ("eastern"), Maplewood ("eastern"), Sibley ("eastern"), Lytton ("eastern"), Saranac ("eastern")	2.43	5
Xdh	1. St. Croix ("eastern"), Maplewood ("eastern"), Sibley ("eastern"), Saranac ("eastern")	1.46	15
	2. St. Croix ("eastern"), Maplewood ("eastern"), Sibley ("eastern"), Lytton ("eastern")	23.06	15
	3. Old Mill ("western-northern")	—	—
	4. Lake Itasca ("western-northern")	—	—

[1] The χ^2 values are multiplied by ⅔ in order to correct for the fact that the observations were not independent because sib pairs were sampled
* $0.01 < P < 0.05$
** $P < 0.01$
[2] Values for females only
[3] Values for males only
[4] Corrected *G* values (See text.)

erogeneity. As is shown in Table 3, homogeneous combinations of certain "eastern" and "western-northern" samples can be made, however.

The *Xdh* locus also exhibits large differences in frequencies between samples. As with the *Lap* locus, a χ^2 test of frequencies of "eastern" and "western-northern" flies was highly significant (Table 3). *G* tests were performed on samples of *N* greater than 15. Significant differences exist between variant frequencies in the two larger "western-northern" samples as well as between frequencies in "eastern" samples. Two homogeneous groupings of "eastern" samples are given in Table 3.

Finally, allele frequency differences at the *Pgm* locus were marginally significant at the 0.05 level. The *G* tests indicated two groupings can be formed (Table 3). One consists of the "western-northern" samples and the other of the "eastern" ones. The primary differences between the forms is the presence of a unique variant, $Pgm^{1.13}$, in "eastern" collections with frequencies ranging from 0.07 to 0.15.

Genetic similarities (Nei, 1972) were calculated over all loci for samples of "eastern" and "western-northern" *D. athabasca* ($N > 15$) (see Table 4). The average *I* (similarity index) over all loci for average allele frequencies of "western-northern" and "eastern" *D. athabasca* is 0.90. The values for 14 of the 17 loci are

TABLE 4. *Genetic similarity between samples of "western-northern" and "eastern A"* D. athabasca.

	Old Mill (west.)	Lake Itasca (west)	Brule (east)	St. Croix (east)	Maple-wood (east)	Sibley (east)	Lytton (east)	Saranac (east)
Old Mill (western)		0.990	0.896	0.885	0.895	0.890	0.887	0.866
Lake Itasca (western)			0.900	0.901	0.908	0.901	0.901	0.877
Brule (eastern)				0.990	0.991	0.994	0.989	0.983
St. Croix (eastern)					0.998	0.997	0.996	0.982
Maplewood (eastern)						0.998	0.997	0.981
Sibley (eastern)							0.997	0.984
Lytton (eastern)								0.982

greater than 0.90. The differentiation within each form is less than that between them. The average I values between populations of "western-northern" *D. athabasca* and between populations of "eastern A" *D. athabasca* are each 0.99.

DISCUSSION

The relationship of "eastern A" and "western-northern" D. athabasca.—"Western-northern" and "eastern" *D. athabasca* do have ranges which overlap. The boundaries of the region in which they are sympatic are not known. It is possible that the area which they both occupy may be up to 100 mi in width in Minnesota. Miller and Westphal (1967) have found that up to 10% of females placed in non-choice mating situations can be inseminated by males of a form other than their own. However, no evidence is seen for such mixing in nature.

In this study no hybrids of "eastern" and "western-northern" sequences of the long arm of the X chromosome were observed (Table 1). One hundred thirty-seven of the lines sampled were collected in areas where both forms were found. The chromosomal data of Miller and Voelker (1968; 1969*a;* 1969*b;* 1972) show the "eastern" and "western-northern" forms to be distinct, with only one possible case of mixing of "eastern" and "western-northern" chromosome sequences in one isofemale line from a locality where both groups had been collected. These data are strong evidence that the two forms of *D. athabasca* are effectively isolated under natural conditions, but they have a limitation. The chromosome studies were performed on stocks which had been kept in the laboratory up to 18 months or more. Under laboratory conditions, selection against individuals with combinations of "eastern" and "western-northern" *D. athabasca* chromosomes might have taken place. Therefore lines would appear to be "eastern" or "western-northern" even though they had originated from a cross between members of different groups.

The allozyme data also indicate that effective reproductive isolation occurs between "western-northern" and "eastern" *D. athabasca*. (See the data on *Est, Adh,* and *Pgm* in Tables 2 and 3.) Further, they are not subject to the same criticisms as the inversion studies because the allozyme frequencies are based on samples of offspring of isofemale lines. In the vast majority of cases the samples were F_1 offspring of wild caught females. The *Adh* locus represents a case of near fixation of alternate alleles in the two groups. Since hybrids between "eastern" and "western-northern" *D. athabasca* are viable in the laboratory, progeny of hybrid crosses should have been detected at the *Adh* locus. None was found. Hence, interbreeding is not frequent under natural conditions, if it occurs at all.

This evidence strongly suggests that "eastern A" and "western-northern" *D. athabasca* are sibling species. The sample sizes involved in this study were relatively small; however, eight populations have been sampled. Six of these lie within a few hundred miles of each other, and two, Lytton and Saranac, are widely separated

from the others. This gives a basis for concluding that the significant differences observed between the two groups are not localized. Unfortunately, a considerable portion of the area of possible overlap has not been sampled. Therefore, the evidence for a complete lack of gene flow is compelling, but not apodictic.

Genetic Differentiation During Speciation

Apparently the development of reproductive isolation can involve few changes in the genome. Examples of such cases include the small differences in genetic similarity between members of subspecies and members of semispecies in the *D. willistoni* group (Ayala et al., 1974), the overlap in the range of I (similarity index) for comparisons of races and comparisons of sibling species in the *D. mulleri* group (Zouros, 1973), and the overlap in the range of I values for comparisons of subspecies and comparisons of species in the *D. bipectinata* group (Yang et al., 1972; note values given in Ayala et al., 1974). For example, the I value for two races of the *D. mulleri* group (0.88) is high, matched by comparisons of two sibling species of the group (0.88). The situation is similar in the *D. bipectinata* group with I of subspecies comparisons ranging from 0.90 to 0.92, whereas species comparisons range from 0.68 to 0.96. Genetic analysis also indicates that few genetic changes are involved in isolation. Prakash (1972) found that the reproductive isolation existing between mainland and Colombian populations of *D. pseudoobscura* was due to the effects of approximately four loci.

While these data show that the actual development of isolation requires relatively few genetic changes, one can still question whether isolation is always preceded by an accumulation of genetic differences which are not in themselves isolation mechanisms. One approach to this question is to compare the genetic similarity between species bearing in mind that the genetic differences responsible for isolation may be small.

Measures of similarity made between species must include the differences which occurred prior to, during, and after speciation. Therefore, minimum estimates provide the most reliable guide to the amount of differentiation necessary for speciation. It is noteworthy that the amount of similarity between the two forms of *D. athabasca* (I = 0.90) is quite high. This I value is considerably greater than values for comparisons between species of other members of the obscura group. These values range from 0.15 to 0.79. Comparisons of *D. athabasca* with *D. algonguin* and with *D. affinis* yield average I values of 0.52 and 0.48, respectively (Lakovaara et al., 1972). Many other species comparisons also produce I values which are considerably less than 0.90. (See Ayala et al., 1974 for a summary.) Other recent studies also compare species pairs in which little detectable genetic variation occurs. For example, there is greater similarity between certain populations of the Hawaiian *Drosophila* species *D. ochrobasis* and *D. setosimentum* (maximum I = 0.98) than between certain populations of the single species *D. ochrobasis* (minimum I = 0.79) (Carson et al., 1975). The overall similarity is high in other species of Hawaiian *Drosophila* also. The average I value for comparisons of *D. silvestris* and *D. heteroneura* is 0.94 (Sene and Carson, 1976) and it is larger for comparisons between three species, *D. cyrtoloma, D. ingenes,* and *D. melanocephala* (I = 0.96–0.99) (Johnson et al., 1974). The occurrence of high I values between species is not limited to *Drosophila*. A comparison of species in two genera of California minnows gave a value of 0.95 (Avise et al., 1975).

The pattern of the distribution of I values for within and between group comparisons in *D. athabasca* (Table 5) also indicates that the divergence between the two groups is small. Most values fall within the same range. Only comparisons of "western-northern" samples and "eastern" flies at the *Est* and *Adh* loci and of the Old Mill "western-northern" samples and

TABLE 5. *Frequency distributions of loci relative to genetic similarity in* Drosophila athabasca.

	Genetic similarity									
	0–.09	.10–.19	.20–.29	.30–.39	.40–.49	.50–.59	.60–.69	.70–.79	.80–.89	.90–1.0
Within group comparisons	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	7.7%	91.9%
Between group comparisons	5.9%	1.0%	3.9%	1.0%	0.0%	0.0%	0.5%	3.5%	4.4%	79.9%

"eastern" samples for *Lap* fall outside the range of within group comparisons. The bimodal distribution of *I* values which exists indicates that genetic changes which accompany speciation may involve only a portion of the genome. Such a bimodal distribution is often found in comparisons of different species. This type of distribution may reflect selective processes such as selection for different alleles at certain loci and/or for the maintenance of the same alleles at others (Ayala and Gilpin, 1974). At present one cannot distinguish between the effects of genetic drift and selection in the determination of the allele frequencies within species.

There are certain qualifications of the above conclusions reached from electrophoretic studies regarding differentiation and speciation. One involves the problem of electrophoretically silent alleles. It has long been recognized that one electrophoretic variant is not necessarily one protein (Henning and Yanofsky, 1963; Hubby and Throckmorton, 1965; Hubby and Lewontin, 1966). Additional variants have been found at several loci in *Drosophila* species (Bernstein et al., 1973; Singh et al., 1974, 1975) and butterflies (Johnson, 1975) using several methods. The increase in variability within a species is nearly two-fold. Identity in electrophoretic mobility is a phenotypic characteristic and does not guarantee genetic identity of variants. This fact means that allozyme data are more reliable when discontinuities are found between groups than when similarities are. For example, the issue of silent alleles does not affect the conclusion that the gene pools of "western-northern" and "eastern" *D. athabasca* are separate or nearly so. The marked differences in allele frequencies at the *Adh* and *Est* loci are good evidence for lack of gene flow. With gene flow, boundaries must be blurred irrespective of whether all alleles are detected.

The genetic identity and distance between groups cannot be measured precisely when measures are based on electrophoretic variant frequencies. As more variants are detected at loci, the values of *I* based on these loci will change. Conclusions which rely on electrophoretic studies will be robust only if *I* changes proportionally for both intra- and interspecific comparisons.

Several workers (i.e., Mayr 1970; Carson, 1975; Wilson et al., 1974; Praeger and Wilson, 1975) have suggested that changes in regulatory, not structural, genes provide the basis of speciation. If true, then no matter how representative the data on electromorphs are for structural loci as a whole, they may not reflect changes which are fundamental to the speciation process. Thus the differences we describe may only serve to mark the process of differentiation between the forms and not be causative agents.

The data from this and other studies support the concept that the genetic changes required for speciation can be few and not necessarily greater in number than those underlying within species differentiation. Some earlier models of speciation (Mayr, 1963; Dobzhansky, 1970; Carson, 1968) stressed large changes in the genome (i.e., a "genetic revolution") as a part of speciation; however, other workers have pointed out that speciation may involve relatively few changes (Hubby and

Throckmorton, 1965, 1968; Throckmorton, 1969, 1972; Dobzhansky, 1937, 1970; Metz and Bridges, 1917).

Ayala et al. (1974) and Avise (1976) have emphasized situations where speciation has involved significant genetic changes. However, their main concern is with average changes while mine has been with minimum changes. The importance of minimum values of genetic change is that they provide evidence that speciation does not necessarily have to depend upon the accumulation of major genetic changes prior to the development of reproductive isolation. A large genetic change is not prerequisite for each speciation event. It is reasonable to suppose that more than one mode of speciation exists (Bush, 1975); indeed, the description of all speciation events by one model is unduly restrictive. Further understanding of the genetic processes of speciation awaits studies which can assess the significance of patterns of variation relative to the speciation event.

Summary

Genetic variation in two members of the *D. athabasca* complex, "western-northern" and "eastern A" *D. athabasca*, has been assayed electrophoretically. These two forms are especially appropriate for studying the genetic differentiation required for speciation because they can produce progeny under laboratory conditions but exhibit reproductive isolation in nature.

Electrophoretically detectable variation at seventeen protein loci has been described for collections of *D. athabasca* from eight localities. Eleven of the loci have similiar frequencies of variants both within and between the two forms. Three loci exhibit significant differences in variant frequencies within groups. The remaining three loci, *Pgm, Est,* and *Adh* show evidence of differentiation between "western-northern" and "eastern A" *D. athabasca*. The most marked differentiation occurs in the variants at the *Adh* locus which could serve as diagnostic characteristics for the two groups. The differentiation is maintained within and outside regions of overlap of the groups.

The average genetic similarity between the two forms of *D. athabasca* is high (I = 0.90) while the data concerning the *Adh* locus and chromosomal polymorphisms indicate that the two forms are effectively reproductively isolated. This information as well as that in other studies cited is taken as evidence that the genetic differentiation required for speciation can be small.

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