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Speciation via hybrid dysgenesis: negative evidence from the *Drosophila affinis* subgroup

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Abstract

With the realization that some transposable elements cause hybrid dysgenesis in *Drosophila melanogaster*, have come proposals that speciation may be one result of their action. One characteristic of hybrid dysgenesis is that the progeny of dysgenic individuals exhibit an elevated mutation rate. Dysgenic speciation models were tested by studying hybrids of four interfertile species and semispecies of the *Drosophila affinis* subgroup. A study of the progeny of all possible mating pairs found no evidence for an increased visible mutation rate.

Introduction

Hybrid dysgenesis is a term collectively applied to several genetic phenomena that occur in the germ line of *Drosophila melanogaster* individuals that are the hybrid progeny of two different types of strains (Kidwell, Kidwell & Sved, 1977; Picard *et al.*, 1978). Features associated with dysgenesis include: increased rates of visible mutation, lethal mutation, and chromosomal rearrangement; segregation distortion; male recombination; and hybrid sterility. Though specific mechanisms are not understood, transposable elements have been clearly implicated as the cause of dysgenic mutations. One element, called the P element, is known to move in the germ line of P-M dysgenic individuals and cause mutations (Bingham *et al.*, 1982). The I element plays a similar role in the I-R system of hybrid dysgenesis (Bucheton *et al.*, 1984). Recently, a third system of hybrid dysgenesis has been described (Yannopoulos *et al.*, 1987) in which the active transposon is the *hobo* element.

Because the syndrome occurs in hybrids and is associated with sterility, it has been proposed that transposable elements may cause speciation (Bingham *et al.*, 1982; Rose & Doolittle, 1983; Ginzburg *et al.*, 1984). The basic model (Bingham *et al.*, 1982) considers a species divided into two allopatric populations. When a dysgenic transposable element is introduced into either population, it quickly spreads via replicative transposition and mendelian transmission. In this fashion each population may undergo successive invasions of different elements. If the allopatric populations come into contact, the hybrids will be dysgenic for several elements and be completely sterile.

This paper reports the results of a search for an increased visible mutation rate in hybrids among members of the *Drosophila athabasca* species complex and between these semispecies and *Drosophila algonquin*. Fertile hybrids can be produced in the laboratory despite significant premating barriers to hybridization (Miller, 1950; Miller, 1958; Miller *et al.*, 1975). It is shown that

dysgenic transposons do not appear to have contributed to isolation in this group.

Material and methods

Drosophila lines

Four iso-female lines were used in the study. *Drosophila algonquin* (DA) and *D. athabasca* Eastern-A (EA) lines were collected at the Edmund Niles Huyck Preserve in Rensselaerville, New York near Albany. *D. athabasca* Western-Northern (WN) was kindly provided by A. Beckenbach from British Columbia. *Drosophila athabasca* Eastern-B (EB) was collected in Miller Place, Long Island, New York.

The separation of *Drosophila algonquin* from the members of the *D. athabasca* complex is straightforward because of distinct morphological differences. However, no morphological differences have been identified among members of the *D. athabasca* semispecies. The identification of these semispecies were made on the basis of known geographic limits (Miller *et al.*, 1975), Y chromosome types (Miller & Westphal, 1967), and allozymes (Jaenike *et al.*, 1978; Johnson, 1985).

Mating protocol

For each mating, four or five males were placed in a vial of food with one to five virgin females and placed at 19 °C under high relative humidity (70%–80%) and constant light. Each individual cross was transferred to a replicate vial of fresh food every week until the flies died or ceased to produce progeny.

All F_1 progeny were collected as virgins and backcrossed to virgins of one of the parental species. Backcrosses were labeled so that progeny were collected blind; that is without knowledge of the sex of their hybrid parent. All progeny were closely examined for visible aberrations. Those flies that appeared unusual in any respect were mated with virgins of the species or semispecies to which they were most closely related. The progeny of these crosses were inbred for two generations to monitor the reappearance of the original phenotype. All flies were reared using the food and protocol of Hey and Houle (1985).

Expectations under hybrid dysgenesis

To choose between the null hypothesis (that the mutation rate to sex-linked visible mutations occurs at non-dysgenic levels) and the alternative (that the mutation rate is equal to or greater than that expected of one or more families of dysgenic transposable elements) an expected mutation rate under dysgenesis is required. Because screening for visible mutations can be a subjective process, any expected rate must be applied with caution. Several distinct estimates have been considered in this study.

An estimate can be made from the ratio of visible to lethal mutations observed under different types of mutagenesis for the X chromosome of *D. melanogaster*. For EMS induced mutations the ratio of 1 : 6.6 (visibles : lethals) has been reported (Kaplan *et al.*, 1970); and for X-ray induced mutations Muller (1954) reported the ratio to be about 1 : 7 or 1 : 8. It is known in *D. melanogaster* that the rate to sex-linked lethals via P-M hybrid dysgenesis is between 1.5% (Eanes *et al.*, 1988) and 3% (Kidwell, Kidwell & Ives, 1977). If this rate is doubled for the larger X chromosome of *D. affinis* subgroup species (Sturtevant & Novitski, 1941) and divided by 7, we expect a visible rate between 0.43% and 0.86%.

A different approach is suggested by Kidwell's (1983) proposal that P elements have swept through natural populations of *D. melanogaster* within the last 30 years. In accord with this is the report by Berg (1982) of very high rates of mutation in the germ line of wild males in the period 1967–1974. It is likely that this period of high mutation rates was caused by the spread of P elements through the study populations. In a study of the progeny of these putatively dysgenic wild males, sex-linked visible mutations were observed to arise at a rate of 2.04% (Berg, 1974). If similar

events had occurred in the *affinis* subgroup species, we would expect a rate of about 4% because of the larger X chromosome.

A third expectation of rate comes from a study of hybrids of *D. pseudoobscura* and *D. persimilis* (Sturtevant, 1939). Hybrids and their progeny were backcrossed with parental stocks. In the male progeny of the second backcross generation, sex-linked visible mutations were observed at a rate of 0.56%. These mutations were nearly all identical to previously recognized mutations. All of these visible mutations occurred in the descendants of progeny of *D. persimilis* males and *D. pseudoobscura* females. The reciprocal cross did not yield any visible mutations in the second backcross generation. This nonreciprocal increase in mutation rate is expected if *D. persimilis* males carried dysgenic transposable elements that were absent in *D. pseudoobscura*. If we consider only that cross yielding visible mutations, then the rate is 0.96%.

Miller (1950) performed an experiment similar to that of Sturtevant (1939) in which hybrids between *D. algonquin* and *D. athabasca* were backcrossed with *D. algonquin* males. The resulting progeny exhibited abnormalities at a rate of 3.4% in females and 7.8% in males. The abnormalities included rough eyes, missing bristles, wings held out, and abnormal abdomen. Although Miller did not examine the inheritance of these aberrations, the circumstances of their occurrence, the description of their appearance, and the higher rates in males (expected if many aberrations have a basis in sex-linked recessive mutations) suggest that they were dysgenic mutations.

Results

Out of 84 crosses between EA females and EB males, 10 were successful (12%). For the reciprocal mating, 77 crosses were made and 10 were successful (13%). Between EA females and WN males, 50 crosses yielded not a single success, while the reciprocal mating yielded 2 successful crosses out of 23 attempts (9%). Matings between WN females and EB males had a high success rate, 53 out of 85 crosses (64%). The reciprocal cross yielded 14 successes in 55 attempts (25%). These observations fit quite closely the data of Miller (1958) and Miller and Westphal (1967), further confirming the semispecies designations. As expected from the report of Miller (1950), the matings between *D. algonquin* and *D. athabasca* semispecies were successful only when DA females were used. These yielded the following success rates: with EA males, 6 out of 37 crosses (16%); with EB males, 10 out of 176 crosses (6%); and with WN males, 4 out of 100 crosses (4%). With the exception of female progeny of the DA female/EA male crosses, all hybrid progeny of DA females appeared sterile.

In Table 1 are shown the numbers of flies collected of each sex from each type of backcross. Backcross type HD involved female hybrids and males from a parental strain. The male progeny of these crosses are expected to reveal visible sex-linked recessive mutations if they arose in the germline of hybrid females. Backcross type CN involved hybrid males and females from parental strains. Table 1 also shows the numbers of aberrant progeny of different phenotypic classes.

All aberrant flies were backcrossed with the parental strain to which they were most closely related. For males, two generations of crosses are necessary to determine inheritance of sex-linked recessive mutations. For females showing aberrations, a single generation is required assuming dominance of the mutation. Unfortunately, the majority of the crosses to determine heritability were unsuccessful. Many aberrant flies died soon after they were collected, and many of the survivors appeared to be sterile by virtue of failing to produce progeny. Of the 98 aberrant male progeny from type HD backcrosses, only 17 could be checked for heritability. Of a total of 163 aberrant females, 63 where checked. Not a single aberration examined for inheritance, proved heritable in a Mendelian fashion.

Flies that were missing scutellar bristles (class D, Table 1, resembling scute of *D. melanogaster*) appeared at frequencies near 1% in the progeny of EB/EA, EA/EB, and EB/WN crosses.

They occurred in males and females and in the progeny of both types of backcross. The heritability tests revealed the majority of the scute aberrations to be partially heritable. Regardless of whether the aberration occurred in a male or female, the first generation of the heritability test often revealed a minority of the progeny to exhibit the trait. However, thirty separate attempts to further isolate the trait failed. Despite repeated attempts at crossing males and females with the trait and crossing flies with the trait against full sibs, not once could the frequency of the trait in the progeny be increased above ten percent. This pattern of inheritance was repeated in the progeny of two females that exhibited small wing blisters and one female with bent dorsal thoracic bristles.

Table 1. Frequencies of aberrations.
HD refers to female hybrids backcrossed to males from a parental strain. CN refers to hybrid males backcrossed to females from parental strains. Class A of visible aberrations includes most types of dorsal bristle aberrations, including singed bristles, bent bristles, and cases were several bristles pointed in unusual directions. Class B consists of wing aberrations including folded, ruffled, and drooping wings. Class C includes swollen abdomens, distorted tergites, and bent tarsae. Class D consists solely of cases of missing scutellar bristles. Class E includes small flies with short bristles. These aberrations closely resembled Minute mutations of *D. melanogaster* (Lindsley Grell, 1968). Class F is made up of flies that revealed blisters or bubbles in one or both wings.

Hybrid cross (female/male)	Backcross Type	Progency	Classes of Aberrations A	B	C	D	E	F
EB/EA	HD	male 1007	1	1	5	3	–	–
		female 1109	3	3	3	19	–	–
	CN	male 327	–	–	–	8	–	–
		female 334	2	1	1	5	–	–
EA/EB	HD	male 1871	3	3	5	6	1	4
		female 1889	3	4	3	26	–	2
	CN	male 747	2	2	2	2	–	–
		female 761	2	3	3	–	–	–
WN/EB	HD	male 675	–	8	2	2	14	4
		female 734	2	6	–	1	14	6
	CN	male 183	2	–	–	1	–	–
		female 142	1	–	–	–	–	–
EB/WN	HD	male 845	1	10	1	8	13	1
		female 958	–	7	2	9	20	5
	CN	male 39	–	–	–	–	–	–
		female 50	–	–	–	–	–	–
WN/EA	HD	male 337	1	1	–	–	–	–
		female 459	–	–	–	–	–	1
	CN	male 18	–	–	–	–	–	–
		female 188	1	–	–	–	–	–
DA/EA	HD	male 132	–	–	–	–	–	–
		female 161	–	–	3	2	–	–

The rate of appearance of males and females that appeared to carry Minute (class E) was about 2% in the type HD backcrosses of EB/WN and WN/EB hybrids. They did not appear in the type CN backcrosses, though sample sizes were smaller in these cases. All class E flies seemed sterile.

Of the numerous wing aberrations (class B) from WN/EB and EB/WN crosses, it was possible to check only 7 females for inheritance. None proved heritable.

There was no overall tendency for backcross type HD males to have a higher frequency of visible aberrations than backcross type CN males (2.01% vs. 1.45%; $G = 1.91$, $p > 0.5$). Backcross type HD females actually had a higher frequency of aberrations than did backcross type HD males (2.71% vs. 2.01%; $G = 5.38$, $p < 0.025$). Clearly, there is no overall tendency for more aberrations in backcross type HD males, as would be expected if many aberrations were recessive mutations caused by hybrid dysgenesis. A minimum estimate of the number of loci at which mutations should have high penetrance and viability is available from lists of visible markers in *D. affinis* (Sturtevant, 1940) and *D. pseudoobscura* (Anderson & Norman, 1977; Sturtevant & Novitski, 1941). These loci are: cut, scute, forked, singed, vermilion, white, scarlet, sepia, veinlet, dusky, lozenge, and yellow. With the exception of scute, as discussed above, none of the aberrations recovered in backcross type HD males resembled any of these previously described sex-linked mutations. In particular, the visibles reported in the hybrid study of Sturtevant (1939) (forked, singed, cut, scutellar, dusky, bobbed, ascute, slender, and short) were not observed. One apparent singed male (which died shortly after recovery) occurred in the type CN backcross of EB/EA hybrids.

The several predictions of a dysgenic sex-linked

visible mutation rate yield a range of values from 0.43% to 2% (not including the very high rates reported by Miller (1950)). If it is assumed that sex-linked dysgenic mutations occur independently, then the Poisson distribution can be used as a test of mutation rates. Table 2 lists the numbers of backcross type HD males and the mutation rates that can be rejected assuming a Poisson distribution. In actuality, dysgenic mutations can occur in clusters and the resulting distribution of mutation frequencies should have a variance greater than expected under the Poisson model. However, studies in *D. melanogaster* reveal that the large majority of mutations appear to arise independently (Green, 1977; Simmons & Lim, 1980). Thus the Poisson expectation is probably slightly over-stringent, but remains a useful criterion.

It is clear from Table 2 that the expected range of mutation rates (0.43% to 2%) is generally rejected. In those crosses that yielded fewer than 700 progeny, the power of rejection is lessened. The simplest interpretation is that dysgenic transposable elements do not separate these species and semispecies. Clearly, if several dysgenic elements separate each of these species and semispecies pairs (as expected under the models of Bingham *et al.* (1982) and Ginzburg *et al.* (1984)) their action would have been evident. However, if we consider the possibility that some subtle visible mutations were not detected, or if we consider the possibility that some of the aberrations for which the heritability could not be checked actually represented sex-linked recessive visible mutations, then it is possible that dysgenesis is occurring to some small degree. These qualifiers are most appropriate for the hybrids of DA females and EA males from which few progeny could be reared.

Table 2. Tests of visible mutation rates.

Hybrid female (Female parent/ Male parent)	Number of sons	Mutation rate to be rejected at $p = 0.05$
EB/EA	1007	> = 0.30%
EA/BA	1871	> = 0.16%
WN/EB	675	> = 0.44%
EB/WN	845	> = 0.35%
WN/EA	337	> = 0.89%
DA/EA	132	> = 2.27%
Total	4867	> = 0.06%

Discussion

The species and semispecies studied are closely related but exhibit many clear differences. *D. algonquin* is quite distinct from the *D. athabasca* complex on the basis of morphological differences, allozyme comparisons (Lakovaara *et al.*, 1976), chromosomal inversions (Miller & Sanger, 1968; Miller, 1977), and mating songs (Chang & Miller, 1978). Within the *D. athabasca* complex there is also considerable evidence for genetic divergence. The semispecies are very nearly parapatric or allopatric (Miller *et al.*, 1975). Divergence has occurred at several allozyme loci (Johnson, 1978; Johnson, 1985). Each of the semispecies possesses several apparently unique chromosomal inversions (Miller & Voelker, 1968; 1969a; 1969b; 1972). Several different forms of Y chromosome also serve to separate the semispecies (Miller & Westphal, 1967). Finally, it appears that the mating songs of the three semispecies are quite distinct (Miller *et al.*, 1975).

In contrast to the well documented genetic divergence among these species, this study finds no evidence for transposable elements which are mobilized in interspecific hybrids. Consideration of several independent studies provide a range of expected visible mutation rates under hybrid dysgenesis (0.43% to 2.0%). Not a single visible mutation was recovered, and the expected mutation rate is clearly rejected for most crosses. The simplest interpretation following from these data is that dysgenic elements have not contributed to the divergence of these species and semispecies.

It should be stressed that the lack of hybrid sterility *per se* does not obviate the occurrence of elevated mutation rates. This is clear from the general success of dysgenic crosses with *D. melanogaster* and by the partial independence of the

effects of temperature on mutation rates and sterility (Engels, 1981).

These results support the work of Coyne (1986) who was led to reject the occurrence of hybrid dysgenesis by the absence of high levels of recombination in male hybrids of *D. pseudoobscura* and *D. pseudoobscura bogotana, D. simulans* and *D. sechellia*, and *D. virilis* and *D. lummei*. Male recombination is a marked phenomenon in P element dysgenic hybrids of *D. melanogaster* (see e.g. Bregliano & Kidwell, 1983).

The data reported here are in conflict with the observations of Miller (1950), in which male progeny of *D. algonquin/D. athabasca* hybrids exhibited abnormalities at a rate of 7.8%. All of the 132 male progeny of DA/EA females that could be produced in this study appeared normal (Table 1), and the reason for the discrepancy is not clear.

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