

# REPRODUCTIVE ISOLATION BETWEEN *DROSOPHILA* *TOLTECA* AND RELATED SPECIES<sup>1</sup>

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*Drosophila tolteca* Patterson and Mainland 1944 is a member of the *D. affinis* subgroup (Subgroup b) of the *D. obscura* group of the subgenus *Sophophora*. It is distributed from Mexico to Bolivia (Miller, 1958b). Patterson and Mainland (1944) suggested, on the basis of morphological characters, that it is more closely related to *D. azteca* than to any of the other members of the *D. affinis* subgroup.

This study was undertaken to determine to what extent reproductive isolation exists between *D. tolteca* and other members of the *D. affinis* subgroup.

## LABORATORY STRAINS

Single locality *Drosophila* strains used in this research were: *D. affinis*, Nebraska (Huskerville); *D. algonquin*, Nebraska (Humboldt); *D. athabasca*, Quebec (Laurentides Park); *D. athabasca*, Wyoming (Jackson Hole mix); *D. azteca*, California (Mather); *D. narragansett*, Nebraska (Halsey); and three strains of *D. tolteca*, Bolivia (Coroicó), Colombia (Villavicencio), and Nicaragua (Santa Maria de Ostuma). The strains designated "mix" were derived from two or more wild caught specimens, while all others were derived from single females isolated after capture.

In addition, certain strains were derived by crossing individuals from strains of different geographical origins. The Wy-

oming-Quebec (LP) strain of *D. athabasca* was established by crossing females from Jackson Hole, Wyoming, with males from Laurentides Park, Quebec. This strain was genetically marked by the sex-linked, recessive mutant gene "bright eyes" (Miller, 1958a). *D. athabasca*, Michigan-Quebec (SA), was started by mating females from the University of Michigan Biological Station by males from Sainte Anne de Bellevue, Quebec. The Colombia-Nicaragua strain of *D. tolteca* was derived by crossing females from a Villavicencio, Colombia, strain with males from a Santa Maria de Ostuma, Nicaragua, strain.

## MATINGS

An attempt was made to determine the extent of sexual isolation existing between *D. tolteca* strains and strains of five other species by use of the "no-choice" mating technique. This involved combining males of one species with females of their own species, determining the insemination frequency, and comparing this figure with the insemination frequency obtained by the combining of males of this same species with females of a different species.

Newly-emerged flies were placed together and allowed to cohabit for ten or eleven days before the seminal receptacles of the females were dissected out and examined microscopically for the presence of sperm. The bottles in which the flies cohabited were kept in an incubator where the temperature was usually between 23° C and 28° C.

The results of this work are presented in tables 1 and 2. These tables show

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TABLE 1. Insemination frequencies of combinations involving *D. tolteca* and certain other members of the *D. affinis* subgroup. The following strains were used: *Nebraska affinis*, *Nebraska algonquin*, *California azteca*, *Nebraska narragansett*, and *Nicaragua tolteca*

	Females inseminated	
	Fraction	Per cent
Intraspecific Combinations:		
<i>D. affinis</i> ♀ × <i>D. affinis</i> ♂	104/109	95.5
<i>D. algonquin</i> ♀ × <i>D. algonquin</i> ♂	110/125	88.0
<i>D. athabasca</i> ♀ × <i>D. athabasca</i> ♂	98/109	89.9
<i>D. azteca</i> ♀ × <i>D. azteca</i> ♂	101/105	96.0
<i>D. narragansett</i> ♀ × <i>D. narragansett</i> ♂	54/59	91.5
<i>D. tolteca</i> ♀ × <i>D. tolteca</i> ♂	104/109	95.5
Interspecific Combinations:		
<i>D. tolteca</i> and <i>D. affinis</i>		
<i>tolteca</i> ♀ × <i>affinis</i> ♂	25/105	23.8
<i>affinis</i> ♀ × <i>tolteca</i> ♂	31/107	29.0
<i>D. tolteca</i> and <i>D. algonquin</i>		
<i>tolteca</i> ♀ × <i>algonquin</i> ♂	12/104	11.5
<i>algonquin</i> ♀ × <i>tolteca</i> ♂	1/104	1.0
<i>D. tolteca</i> and <i>D. athabasca</i>		
<i>tolteca</i> ♀ × <i>athabasca</i> ♂	60/102	58.9
<i>athabasca</i> ♀ × <i>tolteca</i> ♂	19/118*	16.1
<i>D. tolteca</i> and <i>D. azteca</i>		
<i>tolteca</i> ♀ × <i>azteca</i> ♂	58/109	53.2
<i>azteca</i> ♀ × <i>tolteca</i> ♂	35/115**	30.4
<i>D. tolteca</i> and <i>D. narragansett</i>		
<i>tolteca</i> ♀ × <i>narragansett</i> ♂	13/98	13.3
<i>narragansett</i> ♀ × <i>tolteca</i> ♂	2/64	3.1

\* Larvae produced.

\*\* Adult hybrids obtained.

that, while sexual isolation did exist between the strains of *D. tolteca* and the other members of the *D. affinis* subgroup, the isolation was not absolute. *D. tolteca* mated with each of the other species, although with varying frequencies. Differences were also noted in the insemination frequencies of all reciprocal combinations of *D. tolteca* with each of the other species.

#### STUDIES OF HYBRIDS

A few larvae were observed in two of the 15 culture bottles containing combinations of Wyoming *D. athabasca* females and Nicaragua *D. tolteca* males.

As hybrids of *D. athabasca* and *D.*

*tolteca* had not previously been reported, further attempts to obtain adult hybrids were made using mixed strains which were descendants of intraspecific matings of strains of different geographical origins. Females of one such mixed locality strain—Wyoming-Quebec (LP) *athabasca*—which was genetically marked by “bright eyes,” a sex-linked recessive mutant gene, were combined with Colombia-Nicaragua *tolteca* males. From one mating bottle 27 female and 25 male hybrids were recovered. The hybrid nature of these individuals was evident as the females had normal eye coloration and the males, although they had bright red eyes, possessed morphological

TABLE 2. Insemination frequencies of combinations involving *D. tolteca* and two strains of *D. athabasca*, mixed locality strains of both species being used

	Females inseminated	
	Fraction	Per cent
Intraspecific Combinations:		
<i>D. athabasca</i> ♀ × <i>D. athabasca</i> ♂ Michigan-Quebec (SA)	57/62	91.9
<i>D. athabasca</i> ♀ × <i>D. athabasca</i> ♂ Wyoming-Quebec (LP)	47/54	87.0
<i>D. tolteca</i> ♀ × <i>D. tolteca</i> ♂ Colombia-Nicaragua	60/68	88.2
Interspecific Combinations:		
<i>D. tolteca</i> , Colombia-Nicaragua, and <i>D. athabasca</i> , Michigan-Quebec (SA) <i>tolteca</i> ♀ × <i>athabasca</i> ♂	17/60	28.3
<i>athabasca</i> ♀ × <i>tolteca</i> ♂	8/66	12.1
<i>D. tolteca</i> , Colombia-Nicaragua, and <i>D. athabasca</i> , Wyoming-Quebec (LP) <i>tolteca</i> ♀ × <i>athabasca</i> ♂	34/53	64.2
<i>athabasca</i> ♀ × <i>tolteca</i> ♂	21/59*	35.6

\* Adult hybrids obtained.

characteristics intermediate between those of the parental species.

The hybrids (four to six in each combination) were tested in  $F_1 \times F_1$  matings and in back crosses of both sexes with each of the parental strains, the experimental subjects being allowed to cohabit for a period of three weeks. In none of these combinations were any larvae produced, and no eggs were produced by the hybrid females. On the basis of these observations, both sexes of the hybrids were considered to be sterile.

The male hybrids appeared normal in size and general appearance except that the testis pigmentation was not so conspicuous through the ventral abdominal body wall as is usual in males of the *D. affinis* subgroup. At the end of the three-week cohabitation period, the reproductive tracts of the hybrids were examined and, although associated structures appeared to be normal, the testes were found to be extremely small compared with those of males of the parental species. The testes of some of the hybrid males were examined microscopically and

no sperms or spermatocytes were observed in any of the specimens examined.

Since the first tarsal segments of the prothoracic legs of males bear sex combs which are important as a means of separating species in the *D. affinis* subgroup (Sturtevant and Dobzhansky, 1936; Patterson, 1943), these structures were studied in some detail in the hybrids. For purposes of comparison, prothoracic legs from Colombia-Nicaragua *tolteca* males and from Wyoming-Quebec (LP) *athabasca* males were also studied.

In 25 Colombia-Nicaragua *tolteca* males investigated by the author, the range of the sex comb tooth number was from six to eight, with an average of  $6.7 \pm 0.13$  teeth per sex comb. This was a larger range than the six to seven reported for *D. tolteca* by Patterson and Mainland (1944).

*D. athabasca* may have as few as three and as many as six sex comb teeth (Miller, 1955), although the species was originally described as having four (Sturtevant and Dobzhansky, 1936). In 23 Wyoming-Quebec (LP) males of this

species, the author found a range of from three to five, with an average of  $4.1 \pm 0.11$ .

In the 22 *athabasca-tolteca* hybrid males examined, the number of sex comb teeth was five in nineteen of the specimens and four in the remaining three, with an average of  $4.9 \pm 0.08$ . Thus the hybrid average lies between the average numbers found in the *D. tolteca* and *D. athabasca* males examined.

A single sex comb tooth occurs on the second tarsal segment of the prothoracic leg in most of the *D. affinis* subgroup species (Patterson, 1943). It is almost always present in *D. athabasca*, but it is always lacking in *D. tolteca*. In the hybrids between the two species, the second tarsal segment tooth was present in 17 of the 22 males examined.

Sulerud (1958) suggested that the ratio of the first tarsal segment length over the second tarsal segment length (the f/s ratio) may be used to distinguish *D. athabasca* from *D. tolteca* males. In his study he found no overlapping of this ratio in the two species.

The average f/s ratio in the 22 *athabasca-tolteca* hybrid males which were examined in this study was  $1.00 \pm 0.013$ , with a range of from 0.85 to 1.13. Comparable numbers of males of the parental species were similarly investigated by the author. Twenty-three Wyoming-Quebec (LP) *athabasca* males had an f/s ratio of  $1.24 \pm 0.012$ , with a range of from 1.13 to 1.36. Twenty-five Colombia-Nicaragua *tolteca* males averaged  $0.81 \pm 0.008$ , ranging from 0.74 to 0.88. The average f/s ratio of the hybrids thus fell roughly midway between the values for the parental strains.

The hybrid females appeared normal in body size and proportions. The ovaries were dissected out and were found to be extremely small compared to ovaries of the parental species.

#### OBSERVATIONS OF MATING BEHAVIOR

Mating behavior was observed in *D. tolteca* and the other *D. affinis* subgroup

species. A preliminary study showed that the optimal conditions for observing copulation involved aging the flies for fourteen days in the incubator at 23° C to 28° C before making the observations.

The observations were made in chambers consisting of sections of glass tubing 20 mm high with an inner diameter of 12 mm. An agar plug 10 mm high filled the bottom of the chamber. The chamber was divided in half by a vertical piece of cardboard. A small quantity of food was smeared on the divider and a drop of yeast suspension placed on the food.

In order to prepare the flies for the observations, adults were separated according to sex within 24 hours after emerging and were aged in shell vials containing food slants.

After being aged for fourteen days, the flies were etherized and pairs to be observed were transferred to observation chambers, the male being placed on one side of the divider and one female on the other. Cotton plugs were put in place to restrict the flies to the chamber, and the chamber was placed on a glass slide.

Four to eight hours elapsed before the observations on mating began. This interval seemed desirable in order to give the flies time to recover from the effects of the ether. Sufficient humidity within the observation chamber was maintained by periodically adding a drop of water to the base of the chamber.

When the observations were to be made, the divider and the cotton were carefully removed, a  $\frac{7}{8}$  inch square cover slip was placed over the top of the chamber, and the assembly was placed under the low magnification (13 $\times$ ) of a dissecting microscope.

To provide illumination, light from an incandescent source was passed through a round bottom flask filled with water. A sheet of onion skin paper between the flask and the observation chamber served to diffuse the light, bringing about conditions which seemed most favorable

to courtship and mating for the largest numbers of species. The light intensity at the observation chambers was about 18 foot candles as measured by a photometer.

Each pair was observed for thirty minutes. During this time the general pattern of courtship was studied and recorded and the duration of any copulation was timed with a stop watch. Ten to thirteen pairs were observed for each combination.

The mating behaviors of the six species were studied in conspecific crosses. From this it was possible to determine the gen-

eral courtship pattern, although individual differences brought about exceptions to the behavior of the flies as given in this description. The major aspects of courtship under the conditions of this experiment and the number and duration of copulations observed in single locality strains are presented in table 3. The major features of *Drosophila* courtship have been described elsewhere (Miller, 1950b; Spieth, 1952) and will not be described in detail here.

Concurrently with the investigations of mating behavior in conspecific crosses, studies were carried out on the mating

TABLE 3. Observations of mating behavior in conspecific combinations of six species of the *D. affinis* subgroup. The following strains were used: *Nebraska affinis*, *Nebraska algonquin*, *Wyoming athabasca*, *California azteca*, *Nebraska narragansett*, and *Nicaragua tolteca*. Figures under "tapping" indicate the proportions of the pairs in which tapping was observed

Species	Angle of approach	Behavior			Copulations	
		Tapping	Wing movement	Circling	Number	Duration
<i>affinis</i>	45° from behind	6/11 55%	Both wings spread apart 90° and vibrated, also often vibrated one wing extended 60°	Circled only to rear from angle of approach	10	48''-2'35'' ( $\bar{X}$ = 1'40'')
<i>algonquin</i>	Directly in front of female	10/12 83%	Extended both wings at an angle of 40° from body and vibrated them, also one wing extended 30°, then to 90°, vibrated, and back to 30°	Yes	7	3'5''-7'2'' ( $\bar{X}$ = 6'3'')
<i>athabasca</i>	Directly in front of female	9/12 75%	Unilateral; vibrated wing at 40° or at 90° or extended it 40° with no vibration	Yes	4	5'19''-7'5'' ( $\bar{X}$ = 6'20'')
<i>azteca</i>	Directly in front of female	7/10 70%	Vibrated one wing extended at 45° angle, then while circling vibrated one wing held at 90° angle	Yes	8	4'30''-7'55'' ( $\bar{X}$ = 5'52'')
<i>narragansett</i>	Directly in front of female	7/11 64%	Flicked one wing held 35° to 90° from body and elevated 20° to 30°, simultaneous attenuated movement of other wing	Yes	11	10'24''-19'39'' ( $\bar{X}$ = 13'13'')
<i>tolteca</i>	45° from behind	11/12 92%	Extended one wing 90°, depressed posterior edge, sometimes vibrated the wing	Circled only to rear from angle of approach	10	2'45''-9'59'' ( $\bar{X}$ = 6'4'')

TABLE 4. Observations of mating behavior in interspecific combinations of *D. tolteca* females with males of five other species of the *D. affinis* subgroup. The following strains were used: Nebraska affinis, Nebraska algonquin, Wyoming athabasca, California azteca, Nebraska narragansett, and Nicaragua tolteca. Figures under "tapping" indicate the proportion of the pairs in which tapping was observed

Species combination	Angle of approach	Tapping	Wing movement	Behavior			
				Circling	Att. cops.	Number	Duration
<i>tolteca</i> ♀ × <i>affinis</i> ♂	45° from behind	6/10 60%	Both wings spread apart 90° and vibrated	No	0	0	
<i>tolteca</i> ♀ × <i>algonquin</i> ♂	Directly in front of female	7/10 70%	One wing extended 30°, then 90°, then back to 30°	Yes	52	0	
<i>tolteca</i> ♀ × <i>athabasca</i> ♂	Directly in front of female	10/10 100%	One wing extended 40°, usually vibration of wing	Yes	2	2	7'30''-8'27'' ( $\bar{X}$ = 7'43'')
<i>tolteca</i> ♀ × <i>azteca</i> ♂	Directly in front of female	9/10 90%	Vibrated one wing held at 45° angle from body, then while circling vibrated one wing held at 90° from body	Yes	60	2	4'36''-5'22'' ( $\bar{X}$ = 4'59'')
<i>tolteca</i> ♀ × <i>narragansett</i> ♂	Directly in front of female	10/11 91%	Bilateral flicking followed by unilateral flicking	Yes	73	1	10'25''

behavior in interspecific combinations of *D. tolteca* with five other species. These data are presented in tables 4 and 5. Females in these interspecific combinations displayed such mating deterrents as decamping, pushing against the males with their mesothoracic legs, fluttering their wings, extrusion of the vagina, and raising, twisting or lowering the abdomen.

#### DISCUSSION

The insemination frequencies observed between *D. tolteca* and other species of the *D. affinis* subgroup indicate that partial sexual isolation exists between *D. tolteca* and the other species studied. The fairly high frequencies of inseminations, however, were accompanied by few, or no hybrids. A situation of this nature in combinations of *D. affinis* females and *D. athabasca* males has been reported previously by Miller (1950a). It is noteworthy that *D. tolteca* exhibited

some mating with each of the other species of the *D. affinis* subgroup, although other studies have shown little such interspecific mating between other members of the subgroup, e.g., Miller (1950a), Ensign and Miller (1957), Miller (1958a).

The hybrids obtained from *D. athabasca* females and *D. tolteca* males raise to seven the number of hybrids reported in the *D. affinis* subgroup.

The role of tapping in courtship behavior in the *D. affinis* subgroup species is an interesting one. Spieth (1952) in his studies of *D. affinis*, *D. algonquin*, *D. athabasca* and *D. azteca* stated that he had observed no instance in which courtship in these species was initiated without at least one tapping movement on the part of the male. Miller (1950b) reported that he found no tapping in *D. affinis* and *D. algonquin* although he looked closely for it. It should be men-

TABLE 5. Observations of mating behavior in interspecific combinations of *D. tolteca* males with females of five other species of the *D. affinis* subgroup. The following strains were used: *Nebraska affinis*, *Nebraska algonquin*, *Wyoming athabasca*, *California azteca*, *Nebraska narragansett*, and *Nicaragua tolteca*. Figures under "tapping" indicate the proportion of the pairs in which tapping was observed

Species combination	Angle of approach	Tapping	Wing movement	Circling	Copulations	
					Att. Num-cops.	ber Duration
<i>affinis</i> ♀ × <i>tolteca</i> ♂	45° from behind	5/12 42%	Unilateral: extended one wing 90°, depressed posterior edge, sometimes vibrated wing; bilateral movement also	No	0	0
<i>algonquin</i> ♀ × <i>tolteca</i> ♂	45° from behind	9/10 90%	Same unilateral movement as above; almost no bilateral movement	Circled only to rear from angle of approach	65	0
<i>athabasca</i> ♀ × <i>tolteca</i> ♂	45° from behind	8/10 80%	Same unilateral movement as above; some bilateral movement	Rarely	0	0
<i>azteca</i> ♀ × <i>tolteca</i> ♂	45° from behind	9/10 90%	Same unilateral movement as above; almost no bilateral movement	Circled only to rear from angle of approach	54	0
<i>narragansett</i> ♀ × <i>tolteca</i> ♂	45° from behind	9/11 82%	Same unilateral movement as above; almost no bilateral movement	Yes	59	0

tioned, however, that Miller did not use a microscope for most of his observations. In the strains observed during this investigation, tapping was observed in all of the combinations studied but *not in all of the courtships*.

This study was limited in scope since it dealt with only a few strains of the species involved, and some of these strains were "unnatural" in that they were derived by hybridization of geographically different strains. Nevertheless, interspecific inseminations were observed in all possible species combinations with *D. tolteca* and, moreover, some species hybrids were obtained. These findings indicate that the species are really very similar and closely related, despite their evident differences of morphological characteristics and geographical distributions.

## SUMMARY

A study was made of reproductive isolation between *Drosophila tolteca* and a number of related species: *D. affinis*, *D. algonquin*, *D. athabasca*, *D. azteca*, and *D. narragansett*.

A series of "no-choice" mating experiments was conducted involving combinations of *D. tolteca* with each of the other species and intraspecific combinations of each of the six species. The insemination frequencies showed that sexual isolation is not complete in any combination, inseminations occurring in all combinations. However, some degree of isolation did exist between *D. tolteca* and each of the other species.

Hybrids were obtained from matings of *D. athabasca* females by *D. tolteca* males. Both sexes of the hybrids were

sterile, the gonads being poorly developed. The average ratio of the length of the first tarsal segment over the second tarsal segment of the prothoracic leg and the average sex comb tooth number in the hybrids were intermediate between those of the parental species.

Mating behavior was observed in *D. tolteca* and each of the other species. Courtship was initiated by tapping in some pairs in all the species studied, but not in every pair observed. Direct observations of mating behavior of *D. tolteca* with each of the five other species showed that courtship generally was not sustained up to the point of copulation.

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