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A Study of Key Characteristics for Distinguishing Several Drosophila affinis Subgroup Species, with a Description of a New Related Species

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ABSTRACT: Certain characteristics of *Drosophila affinis* subgroup species reported as useful for species identification were reinvestigated in *D. affinis, algonquin, athabasca, azteca, narragansett,* and *tolteca,* and new criteria for species separation were sought. Both laboratory strains (in some cases kept at different temperatures) and wild specimens were studied. Special attention was paid to sex combs and other male foretarsal characteristics. Number of sex comb teeth is very useful, even though not 100% reliable, for distinguishing *D. affinis* from *D. algonquin.* The "sex comb index" (length of first tarsal segment divided by length of longest sex comb tooth) and the "tarsal segment index" (length of first tarsal segment divided by length of second tarsal segment) were found to be very effective for separating *D. affinis* from *athabasca* and appear likely to be useful for separating *D. azteca* from *tolteca.* Number of primary clasper teeth appears likely to be helpful (but not 100%) for distinguishing *D. athabasca* (8 or more) from *azteca* (usually 6 or less); these two species are often poorly distinguishable on the basis of mesonotum striping. Testis shape would seem to be a good basis for distinguishing *D. algonquin* (less coiled) from *tolteca* (more coiled). A new species, *D. novitskii,* is described from collections in Colorado. A modified key for separating American *D. affinis* subgroup species is presented.

INTRODUCTION

The *Drosophila affinis* subgroup consists of nine very similar species, eight of them native to the Americas. The American species are: *D. affinis* Sturtevant (1916), *D. algonquin, athabasca, azteca, narragansett,* and *seminole* (all Sturtevant and Dobzhansky, 1936); *D. dobzhanskii* Patterson (1943); and *D. tolteca* Patterson and Mainland (1944). The single European member of the subgroup is *D. helvetica* Burla (1948). The subgroup is included in the OBSCURA group of the subgenus *Sophophora* (Patterson and Stone, 1952).

The known geographical distributions of the American members of the subgroup were summarized by Miller (1958). The aggregate distribution of these species virtually covers North America and extends into Central and northern South America. Over much of this territory two or more of these species occur together. In the United States east of the Rocky Mountains four species, *D. affinis, algonquin, athabasca,* and *narragansett,* coexist widely, though *D. algonquin* appears to be absent from the extreme southeastern states (Georgia and Florida), and *D. athabasca* is mostly restricted to the

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northeastern states (northern Great Plains, Great Lakes region, Appalachians, and Atlantic coast north from Virginia.) In Mexico and Central America *D. azteca* and *tolteca* are found together over a large territory. On the other hand, certain species appear to be the sole representative of the subgroup in some regions. *D. athabasca* is the only member known throughout the western part of its range, i.e., from Alaska and western Canada southward to Oregon on the Pacific coast and to New Mexico in the Rockies. *D. azteca* appears to exist apart from its close relatives in the northwestern part of its range, in northern California, southern Arizona and New Mexico, western Texas, and northwestern Mexico. The incompleteness of collecting records has left room for doubt that *D. azteca* is actually separated from *D. athabasca* in northern California and in the mountains of New Mexico (Dobzhansky and Epling, 1944; but see new collecting records given below). Also, as indicated by Patterson and Wagner (1943), *D. azteca* has been obtained close to collecting sites of *D. affinis* and *algonquin* in western Texas. The geographical range of *D. azteca* has recently been extended southward; Dr. Alice Hunter of the University of the Andes (Bogotá, Colombia) has provided us with a culture of this species derived from a collection she made near Volcan Irazú, Costa Rica, in 1961. However, in northern South America (Colombia, Venezuela, Bolivia) *D. tolteca* remains the only known *D. affinis* subgroup species. In addition, the range of this species has recently been extended to the West Indies. In the summer of 1959 Dr. H. L. Carson of Washington University (St. Louis) furnished us with specimens collected by Mrs. Meredith Carson at Kenscoff, Haiti, and these were judged to be *D. tolteca* on the basis of morphological characteristics and breeding performance.

Certain characteristics of males of *D. affinis* subgroup species are useful for species recognition and have been incorporated into keys for identification of Drosophilas (Sturtevant and Dobzhansky, 1936; Sturtevant, 1942; Patterson, 1943; Patterson and Mainland, 1944; Strickberger, 1962). Outstanding are features of the sex combs. Males of the *D. affinis* subgroup differ from those of other OBSCURA group species in having a well-developed sex comb only on the first tarsal segment of each foreleg (rather than on both first and second tarsal segments). A single sex comb tooth is borne on the second tarsal segment of each foreleg in most *D. affinis* subgroup species. However, *D. tolteca* lacks such a tooth, and the European member of the subgroup, *D. helvetica,* has two or three teeth on the second segment. The first tarsal segment sex comb of *D. affinis* subgroup males varies in absolute and relative size in the different species. It has few teeth (reportedly no more than five) in *D. affinis, athabasca, azteca, helvetica, narragansett,* and *seminole.* On the other hand, *D. algonquin, dobzhanskii,* and *tolteca* have distinctly more teeth (reported as eight or more), and in these species the sex comb is oriented more nearly parallel to the axis of the tarsal segment than in the

others. Among those with few sex comb teeth, *D. athabasca, azteca,* and *narragansett* have small sex comb teeth, whereas *D. affinis* has teeth of relatively greater size.

Certain other features of males are also used for species recognition. Males of *D. narragansett* may be separated from those of *D. athabasca* by the fact that in *D. narragansett* the top of the head presents a silvery pollinose appearance when viewed obliquely. It is reported (Sturtevant and Dobzhansky, 1936) that *D. azteca* males are separable from those of *D. athabasca* by a difference of mesonotum striping, which consists of four distinct stripes in *D. azteca* and two less distinct ones in *D. athabasca.* Other less obvious features included in the descriptions of these species may conceivably be used to help distinguish them (such as the shape and thickness of the border of the eighth abdominal sternite of males, illustrated in Sturtevant and Dobzhansky, 1936). Females of the *D. affinis* subgroup are less readily distinguished as to species, and it is often practical to tell them apart only by permitting them to breed and examining their male offspring. However, certain differences of pigmentation and abdominal banding are sometimes useful for separating females, as in the case of *D. affinis* and *athabasca,* in which females of the former species have a distinctly banded abdomen in contrast to those of the latter, in which the abdomen may present a continuously pigmented appearance when viewed from above.

It has been our impression, on the basis of numerous wild and laboratory-bred specimens of most of these species, that some existing key characteristics for the *D. affinis* subgroup are distinctly inadequate. Firstly, certain allusions to difference of size and proportion in keys are misleading because of an insufficient or inappropriate reference for comparison. For instance, in the keys of Sturtevant (1942) and Patterson (1943) it is indicated that *D. affinis* has "teeth of sex comb distinctly longer than greatest diameter of tarsal segment" while *D. athabasca* has "teeth of sex comb scarcely longer than greatest diameter of tarsal segment." As pointed out by Miller (1955), actual measurements of sex comb lengths and tarsal segment diameters and calculations of the indicated ratio showed a broad overlap of the two species, both for wild and laboratory-bred specimens. Instead, it was shown that these two species could almost always be distinguished by the ratio of first tarsal segment *length* divided by sex comb length (the so-called sex comb index), the values of this ratio being less than 2.5 in *D. affinis,* more than 3.0 in *D. athabasca.* Secondly, the range of variation of some of the attributes used in key separation sometimes transgresses the limits stated in descriptions and keys. For example, data on *D. algonquin,* to be presented below (Tables 1 and 2), show the number of sex comb teeth of this species to range from five to eleven, rather than the eight to ten of the original description (Sturtevant and Dobzhansky, 1936). Thirdly, some characteristics, particularly those of pigmentation, have proven to be elusive, being not only hard to assess in wild specimens but

difficult, if not impossible, to recognize in laboratory cultures of the species. For example, Sturtevant and Dobzhansky (1936) described the eastern subspecies *mahican* of *D. athabasca* on the basis of its relatively light coloration; however, laboratory strains of *mahican* were reported by Novitski (1946) to lack distinguishing coloration, and so he decided to disregard the subspecies designation altogether. We have encountered another example. Laboratory specimens of *D. azteca* stocks (California, Mexico, Costa Rica) have commonly been found to be indistinguishable from those of *D. athabasca,* since the difference of mesonotum striping reported for members of these species has been found indistinguishable.

Such a state of affairs is, of course, neither unique nor surprising for a group of closely related species. Nevertheless, it has seemed to us to warrant special consideration. Since the *D. affinis* subgroup species are widespread in the Americas and often widely overlap each other in geographical range, and since they constitute an assemblage of forms very closely related to each other but yet distinct, they are potentially very useful for studies of population and species similarities and differences, including comparisons of ecological characteristics. Consequently, it is a matter of importance that collectors be able, inasmuch as possible, to distinguish these species. On this account it seemed that a worthwhile study might be made of the extent of variation that could be found in certain of the key characteristics reported for the separation of *D. affinis* subgroup species, particularly those involving the sex combs. Admittedly, the exploration of variation in species is an endless task, considering the wide range of genetic and environmental factors presumably capable of influencing it. Nevertheless, it was expected that, even with a moderate number of cultures and specimens and a limited range of environmental conditions, it would be possible to show that described characteristics do transgress reported limits and that the species do overlap. In addition, it was hoped that additional, more reliable traits for distinguishing these species might be found.

The following is an account of a study of certain characteristics alleged to distinguish several of the *D. affinis* subgroup species or suspected of being useful for this purpose. This investigation, which has lasted about eight years, has been complicated and limited by the varying degrees of availability of the different species of the subgroup. These have been very unequally represented by numbers of laboratory strains and wild specimens (entirely lacking for some species). Moreover, some strains have been lost during the period of investigation and others acquired late, making direct comparisons of all such strains for every characteristic impossible. Nevertheless, the results do provide the basis for some conclusions and suggestions for future investigations when more material becomes available. The description of a new species of the subgroup is included, and a modified key for the American members of the *D. affinis* subgroup is presented.

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VARIATION IN SEX COMBS AND IN TARSAL SEGMENT DIMENSIONS

Materials and Methods

The strains used for the part of this study concerned with variation under laboratory conditions were as follows: *D. affinis,* Alice (Texas), and Staten Island (New York); *D. algonquin,* Iron River (Wisconsin); *D. athabasca,* two strains from Matanuska Valley (Alaska), and one from Laurentides Park (Quebec); *D. azteca* Mather (California); and *D. tolteca* Santa Maria de Ostuma (Nicaragua). These strains had been established by breeding wild females (one or several for each strain) inseminated in nature before capture. The duration of laboratory culture of the strains varied widely, from less than one year (in the case of the Alaskan strains of *D. athabasca*) to more than a decade (as with the Alice, Texas, strain of *D. affinis*).

Each of these strains was raised at two different temperatures because of the expectation that greater variability for the characters to be studied might be induced. (Other strains were represented only by specimens reared at the lower temperature of the stock room.) Numerous other studies have, of course, indicated that temperature may influence the phenotype of a *Drosophila* species. A relevant example is that of Combs (1937). His studies showed that in a single generation temperature differences could bring about non-inheritable variation in the number of sex comb teeth and also in the length of the first tarsal segments of *D. melanogaster* males. The temperatures used in the present study were 18 ± 2 C and 27 ± 4 C. The lower temperature range was maintained by an electrically controlled "cool room," and the higher range was afforded by an electric incubator located in the cool room.

The culture medium used was standard cornmeal, agar, molasses medium to which mold inhibitor (tegosept M) was added. For strains reared at both temperatures, to prevent excessive crowding, females were not allowed to oviposit directly in the culture bottles. Rather, eggs were collected in plastic spoons filled with food medium, the eggs counted from the spoons every 24 hours, and 50 eggs placed

in each culture bottle. In other cases (distinguished in the tables), specimens were taken directly from stock bottles kept at 18 C.

Adult males and females were etherized and placed in 95% alcohol for at least 36 hours. They were then removed to microscopic slides where the right prothoracic leg was removed from each specimen. Creosol was used both as clearing agent and mounting medium. After clearing for at least 12 hours, the tarsi and sex combs (of the males) were studied under a compound microscope. Measurements were made with an eyepiece micrometer, one unit of which corresponded to about 3.3 μ at the magnification used (ca. 200X).

The following measurements were made: length of the first tarsal segment of the right prothoracic leg (f); greatest diameter of the first tarsal segment (d); second tarsal segment length (s); and, for males, the length of the longest tooth in the sex comb (c). Determination was also made of the number of teeth in the right sex comb of each male and of the number of second tarsal segment teeth. Using the measurements as a basis, the following ratios were computed: 1) length of the sex comb divided by the diameter of the first tarsal segment (c/d, after Sturtevant, 1942); 2) length of the first tarsal segment divided by the length of the longest tooth in the sex comb (f/c, a modification of the "sex comb index" of Miller, 1955, necessary because of variation of sex comb tooth number); 3) first tarsal segment length divided by the length of the second tarsal segment (f/s). The third ratio was also computed for 20 females of each strain raised at each of the temperatures.

Some of these measurements were also made on certain other laboratory strains and wild-caught specimens. These included a strain of *Drosophila narragansett* (Lebanon, N. Y.), which was, unfortunately, not sufficiently vigorous to provide enough individuals for study at the two temperatures. These specimens and their treatment will be discussed in the sections which follow.

Variation in Numbers of Sex Comb Teeth

Sturtevant (1942), in his "Key to North American Species of *Drosophila*," describes *D. algonquin* as having "proximal sex comb with 8 to 10 teeth, nearly parallel to the axis of the tarsus." Thus, the species is separated from *D. affinis, athabasca* and *azteca* which are said to have "proximal sex comb with 4 to 6 teeth, more oblique." Sturtevant and Dobzhansky (1936) describe *D. affinis, athabasca,* and *azteca* as having four to six, four, and three to five sex comb teeth respectively. Patterson and Mainland (1944), in their "Key to Mexican Species of the Genus Drosophila" state that *D. azteca* can be distinguished from *D. tolteca* because the former has four or five sex comb teeth while the latter has six or seven. It can also be noted, that, although not included in key separations, key criteria indicate that *D. azteca* (with four to five teeth), *athabasca* (with four teeth) and *algonquin* (with eight to ten teeth) could all presumably be separated from *tolteca* (with six to seven teeth) because of sex comb size dif-

ferences. However, as reported by Miller (1955), the number of teeth for *D. athabasca* has been found to range from three to five in a laboratory stock (New Jersey) and from three to six in wild specimens identified as *D. athabasca* (both Michigan and Wyoming).

Table 1 shows the range of sex comb tooth number found in the laboratory strains raised at 27 and 18 C. In contrast to published reports, the number in all five species reared at 27 C overlapped at five teeth. Moreover, except for the almost complete distinctness of *D. athabasca* from *algonquin* and *tolteca,* the overlapping is considerable. Considering only those species separated in keys by tooth number differences, *D. affinis* and *azteca* overlapped the tooth number range of *D. algonquin,* with some specimens of each having five, six and seven teeth. Similarly, *D. tolteca* specimens overlapped those of *D. azteca.* In general, a greater range in tooth number for each species than is indicated in the keys is evident, and in some instances a relatively large number of the specimens examined had sex combs with more or fewer teeth than reportedly possible for the species. For example, over half (27 out of 50) of the *D. algonquin* specimens reared at 27 C had fewer than the eight sex comb teeth reported as minimum for the species, and at this temperature 13 *D. tolteca* individuals out of 50 were found with more than the reported maximum of seven teeth for that species.

The difference in the numbers of sex comb teeth of *D. affinis*

TABLE 1.—Numbers of sex comb teeth (first segment of right foretarsus) of laboratory strains of five *D. affinis* subgroup species raised at 27C and 18C

Laboratory strains	Temp. (°)	Number of teeth 3	4	5	6	7	8	9	10	*n*	Mean ± S.E.
D. athabasca											
Matanuska Valley	27	9	28	3						40	3.9±0.087
Alaska	18	6	32	12						50	4.1±0.085
Laurentides Park,	27	4	42	4						50	4.0±0.057
Quebec	18	3	34	13						50	4.2±0.072
D. azteca											
Mather, Calif.	27		31	13	4	2				50	4.5±0.116
	18	1	31	13	5					50	4.4±0.101
D. affinis											
Staten Island, N.Y.	27		7	39	4					50	4.9±0.067
	18		3	35	12					50	5.2±0.072
Alice, Texas	27		2	15	29	4				50	5.7±0.095
	18		5	26	16	2	1			50	5.4±0.114
D. tolteca											
Nicaragua	27			1	5	31	8	5		50	7.2±0.119
	18					17	24	9		50	7.8±0.101
D. algonquin											
Iron River, Wis.	27			1	4	22	18	5		50	7.5±0.121
	18					9	17	17	7	50	0.4±0.134

strains from Alice, Texas (mean number of 5.7), and Staten Island, New York (mean number of 4.9), reared at 27 C is indicative of intraspecific genetic variation possible for this characteristic. The "t test" of significance computed for the difference revealed it to be significant at the 0.05 level of rejection.

It is noteworthy that at the lower temperature, 18 C, there was little overlapping of the number of sex comb teeth for the species usually distinguished by this difference. However, three specimens of *D. affinis* (Alice, Texas) having seven or eight teeth overlapped nine *D. algonquin* individuals with seven. Also, although seven teeth per sex comb is given as the maximum in the description of *D. tolteca*, over half (33) of the specimens raised at 18 C had eight or more teeth, so the tooth number range overlapped that of *D. algonquin* as broadly as when *D. tolteca* was raised at 27 C.

The mean number of sex comb teeth for certain species was quite different depending on which of the two temperatures was used. All but two of the seven strains (the exceptions being *D. affinis* from Alice, Texas, and *D. azteca*) had a greater mean number at 18 C. Since this resulted in the reportedly separable species being generally more distinct as to sex comb tooth number at the cooler temperature (and probably more nearly normal for all species, judging from their ease of culture at this temperature), it was wondered to what extent overlapping of tooth number might be shown to exist among wild-caught flies.

Table 2 presents data on a number of wild-caught specimens judged at the time of collecting to be either *D. affinis* or *D. algonquin*. As is customary, individuals were assigned to species on the basis of inspection at the relatively low magnification of a dissecting microscope (no more than 30X), at which general size and orientation of sex combs could be determined but *not* number of teeth per sex comb. (It should be added that this sort of species identification may partly depend on additional unrecognized criteria incapable of objective evaluation, which the examiner more or less unconsciously applies in his identifications — e.g., involving size, proportions, pigmentation.) To determine numbers of sex comb teeth, specimens were put in 95% alcohol and later transferred to creosol, as previously described for laboratory-reared specimens. Right prothoracic legs were then removed and numbers of sex comb teeth determined at higher magnification.

It may be seen from these data that specimens classed as *D. algonquin* had numbers of sex comb teeth that ranged from 6 through 11. Although, according to Sturtevant's key, specimens with six teeth should be *D. affinis*, one of the Michigan specimens called *D. algonquin* had six teeth, as did two of those so classified from Minnesota, where, in addition, one specimen called *D. affinis* also had six teeth. Such identifications could, of course, be considered mistakes. However, the data from the laboratory strain of *D. algonquin* given in Table 1 show that this species may sometimes have as few as five

or six teeth in a sex comb and that *D. affinis* may have as many as seven or eight. All of the specimens with seven teeth were originally classified as *D. algonquin*. Actually, seven teeth is not within the range of sex comb tooth number for either *D. affinis* or *algonquin* according to published keys. However, laboratory strains of both of these species reared at 27 and 18 C were found to have some members with this number of teeth (Table 1). Therefore, based on sex comb tooth number, the species status of wild specimens having six or seven teeth must be considered as uncertain. Sturtevant's key describes the angle of the sex comb as being more oblique in relation to the axis of the tarsus in *D. affinis* than in *algonquin*. In our examinations, the obliqueness, though not actually measured, appeared to be such that large sex combs were more nearly parallel to the tarsal axis and small ones more oblique in both species.

Comparison of Sex Comb Length with Tarsal Segment Dimensions

Sturtevant's (1942) key characterizes *D. affinis* as having "teeth of sex comb distinctly longer than greatest diameter of tarsal segment" while *D. athabasca* and *azteca* are said to have "teeth of sex comb scarcely longer than greatest diameter of sex comb." Miller (1955) showed that individual *D. affinis* and *athabasca* males could not be distinguished by such a difference. Similarly, the present study of *D. affinis* and *azteca* did not reveal a distinguishing difference in this

TABLE 2.—*Drosophila* males identified by inspection as either *D. affinis* or *D. algonquin* on basis of relative size and orientation of sex combs and later determined as to actual number of sex comb teeth on right sex comb

Collection place (date)	Number of teeth 3	4	5	6	7	8	9	10	11	*n*	Mean ± S.E.
Lincoln, Nebr. (1953)											
D. affinis	1	25	85	24						135	4.5±0.0356
D. algonquin					5	25	24	19	3	76	8.9±0.114
Schenectady, N.Y. (1953)											
D. affinis		9	33	8						50	5.0±0.076
D. algonquin					3	7	6	1		17	8.3±0.210
Univ. Mich. Biol. Sta. (1954)											
D. affinis		1	11							12	4.9±0.087
D. algonquin				1	19	101	80	32	7	240	8.6±0.062
Halstad, Minn. (1957)											
D. affinis	1	27	35	1						64	4.6±0.087
D. algonquin				2	11	40	42	26	4	128	8.7±0.090

relationship for these two species. For example, calculation of the ratio, sex comb length (c) divided by first tarsal segment diameter (d) for the Alice, Texas, strain of *D. affinis* and the Mather, California, strain of *D. azteca* (50 specimens of each reared at 27 C) showed that *D. affinis* ranged from c/d equals 1.20 to 1.58 and *D. azteca* had values of c/d from 1.20 to 1.80. It would, therefore, not have been possible to distinguish male specimens of these incubated strains by a comparison of the diameter of the first tarsal segment to the length of the sex comb.

Table 3 summarizes data on right prothoracic first tarsal segment (f) and sex comb (longest tooth, c) lengths and the ratio of these for various strains of laboratory stocks of *D. affinis, athabasca, azteca,* and *narragansett*. Strains reared at 18 C only are represented by males taken directly from stock bottles. Strickberger (1962) presents a key in which *D. affinis* is indicated as having "large teeth on sex comb" while *D. athabasca, azteca, narragansett,* and *seminole* have "small teeth on sex comb." That the length of the sex combs (rather than teeth within sex combs) does not completely separate *D. affinis* and *D. athabasca* was shown by Miller (1955), who found overlapping in both laboratory-reared and wild specimens. Such overlapping was also encountered for values of the longest sex comb tooth (c) of *D. affinis, athabasca, azteca,* and *narragansett* in the present study, though mean values were distinctly greater for *D. affinis* than for the other species, as may be seen from values of c in Table 3.

The sex comb index was proposed by Miller (1955) for the separation of male *D. affinis* and *athabasca*. The values of this ratio (first tarsal segment length divided by sex comb length, l/s) were said to be usually 2.5 or less for *D. affinis* and usually 3.0 or more for *D. athabasca*. Miller measured the length subtended by the *whole sex comb* along a line parallel to the sex comb teeth while in the present study the measurements were made of the *longest sex comb tooth*. Our results, however, were similar in that the ratio values were always greater for *D. athabasca* than for *affinis* (Table 3). All of the *D. athabasca* males had first tarsal segments at least three times as long as the longest tooth in the sex comb, the lowest value being 3.2. Only two *D. affinis* specimens were found for which the modified sex comb index values equaled or exceeded 3.0; both of these flies were raised at 18 C. The findings differed from the earlier ones in that many of the ratio values given here for *D. affinis* are between 2.5 and 3.0. In the Staten Island strain raised at 18 C, 46 of the 50 specimens had ratios (f/s) exceeding 2.5. The discrepancy between the earlier study and this one is probably mostly due to the different way of measuring the sex combs. From the standpoint of collectors it is important to note that the sex comb index, either in its original or modified form, is a relatively easy and reliable way to distinguish individual *D. affinis* males from male *athabasca* under a dissecting microscope (ca. 30X).

Preliminary comparisons of male *D. affinis* and *azteca* indicated

TABLE 3.—*D. affinis, athabasca, azteca* and *narragansett* laboratory-reared males. Mean values (eyepiece micrometer units) of first tarsal segment length (f), right sex comb length (c), and the ratio of these with its standard error. Limits of dimensions and ratios beneath corresponding means

Species and strains:	Temp. (°)	n	f	c	f/c
D. affinis					
Alice, Texas	27	50	40.5	18.6	2.18±0.021
			(35-45)	(17-22)	(1.78-2.50)
	18	50	47.8	19.5	2.45±0.045
			(42-57)	(18-22)	(2.10-3.16)
Austin, Texas	18	10	44.8	18.5	2.42±0.023
			(40-50)	(17-20)	(2.20-2.64)
Halsey, Nebraska	18	10	45.3	18.9	2.40±0.023
			(40-50)	(17-20)	(2.20-2.56)
Hopkinsville, Kentucky	18	10	48.1	19.6	2.46±0.059
			(45-52)	(18-21)	(2.19-2.63)
Huskerville, Nebraska	18	10	49.1	19.1	2.57±0.043
			(45-55)	(18-21)	(2.24-2.75)
Keokuk, Iowa	18	10	48.7	19.7	2.47±0.026
			(45-55)	(17-21)	(2.25-2.65)
Kushla, Alabama	18	10	50.3	20.0	2.51±0.034
			(47-56)	(18-21)	(2.19-2.70)
Little Falls, Minnesota	18	10	46.5	18.9	2.46±0.029
			(42-51)	(18-21)	(2.21-2.72)
Nacogdoches, Texas	18	10	47.1	20.4	2.31±0.092
			(40-53)	(18-21)	(1.86-2.61)
Staten Island, N. Y.	27	50	47.4	19.7	2.40±0.026
			(43-54)	(18-21)	(2.04-2.84)
	18	50	53.1	20.1	2.64±0.019
			(45-57)	(19-22)	(2.04-3.00)
D. athabasca					
Laurentides Park, Quebec	27	50	58.2	15.4	3.78±0.029
			(53-64)	(14-17)	(3.29-4.57)
	18	50	64.1	15.8	4.06±0.035
			(54-70)	(15-17)	(3.60-4.60)
Matanuska Valley, Alaska	27	40	61.8	15.3	4.04±0.030
			(58-67)	(14-16)	(3.66-4.46)
	18	50	65.9	15.6	4.23±0.031
			(63-69)	(15-17)	(3.70-4.60)
D. azteca					
Durango, Mexico	18	40	60.0	17.1	3.51±0.018
			(55-65)	(15-19)	(3.11-4.07)
Mather, California	27	50	56.8	16.9	3.36±0.040
			(54-62)	(14-18)	(3.00-4.35)
	18	50	60.6	17.7	3.42±0.037
			(57-65)	(16-19)	(3.00-3.87)
D. narragansett					
Lebanon, New York	18	10	62.4	15.7	3.98±0.081
			(53-68)	(15-17)	(3.53-4.33)

that the first tarsal segments of the forelegs of *D. azteca* are much like those of *D. athabasca* in that they are relatively long compared to those of *D. affinis* when these lengths are compared to the length of the sex comb. Measurements and calculations of f/c values proved this to be generally true for the strains studied (Table 3). No values for the ratio of less than 3.0 were found for *D. azteca*. Since two specimens of *D. affinis* had values for this ratio of 3.0 or slightly greater, the distinction of the two species was not quite complete. The meager data from *D. narragansett* support the expectation that it, like *D. athabasca* and *azteca* could be separated from *D. affinis* by the large values of its sex comb index (in all cases distinctly greater than 3.0).

That temperature is capable of influencing the sex comb index is indicated by the fact that differences in sex comb index values between the strains raised at the two temperatures (27 and 18 C) were statistically significant for both of the strains of *D. affinis* and *athabasca* reared at the two temperatures. In each case the value of the ratio was inversely related to temperature, being higher at 18 than at 27C. The Mather strain of *D. azteca* showed a similar variation of sex comb index with temperature, though the difference was not statistically significant.

Comparison of First and Second Tarsal Segment Lengths

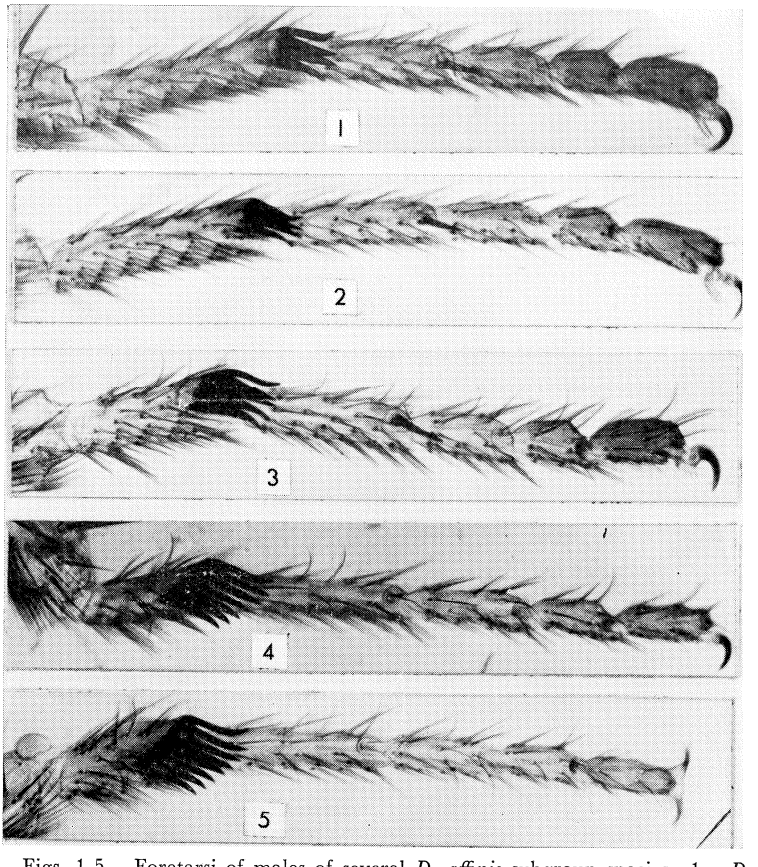
Interspecific variation exists in the *affinis* subgroup with regard to the relative lengths of the first two prothoracic tarsal segments. The mean lengths of these segments and their mean ratios (first tarsal segment length divided by the length of the second tarsal segment, f/s) are given in Table 4. The six species studied can be divided into two groups. In one group the first tarsal segment was always longer than the second (*D. athabasca, azteca,* and *narragansett*) and in the other group the second tarsal segment was nearly always the longer (*D. affinis, algonquin,* and *tolteca*). This difference for five of the species (except for *D. narragansett*) is shown in figures 1-5. Of the species regularly having a longer second tarsal segment, the only exceptions were found in *D. affinis*. A single specimen of the Alice, Texas, strain of *D. affinis* raised at 18 C had first and second tarsal segments of equal lengths, and four males of the Staten Island strain at 27 C were found with ratio values of 1.0 or greater, the highest being 1.08. In general, the first and second tarsal segment lengths of *D. affinis* were found to be more nearly equal in length than in the other four species examined. Yet no *D. affinis* individuals overlapped the ratio values of *D. athabasca* for which the lowest f/s value was 1.12. Therefore, the only overlapping of ratios of the two groups was between *D. affinis* and *azteca* since a few specimens of the latter had f/s values of only slightly over 1.00. The temperature at which the flies were raised had an influence on the ratio of tarsal segment lengths which, although small, was statistically significant for

TABLE 4.—*D. affinis, algonquin, athabasca, azteca, narragansett* and *tolteca* laboratory-reared males. Mean values of first tarsal segment length (f), second tarsal segment length (s) (eyepiece micrometer units), and the ratio of these with its standard error. Limits of dimensions and ratios beneath corresponding means

Species and strains:	Temp. (°)	n	f	s	f/s
D. affinis					
Alice, Texas	27	50	40.4	45.7	0.885±0.0094
			(34-45)	(43-48)	(0.739-0.977)
	18	50	48.2	55.1	0.875±0.0090
			(41-56)	(51-64)	(0.763-1.000)
Staten Island, New York	27	50	47.0	50.5	0.930±0.0089
			(41-54)	(43-57)	(0.630-1.08)
	18	50	53.0	61.6	0.860±0.0052
D. algonquin			(45-56)	(58-65)	(0.762-0.932)
Iron River, Wisconsin	27	50	52.3	62.6	0.836±0.0070
			(46-59)	(54-68)	(0.750-0.933)
	18	50	57.6	70.0	0.823±0.0057
			(50-62)	(67-74)	(0.735-0.886)
Humboldt, Nebraska	18	50	55.2	67.0	0.823±0.0097
D. athabasca			(48-62)	(60-75)	(0.725-9.000)
Laurentides Pk., Quebec	27	50	57.9	47.2	1.22 ±0.0083
			(53-64)	(44-51)	(1.16 -1.39)
	18	50	64.0	51.3	1.24 ±0.0087
			(54-70)	(45-57)	(1.12 -1.39)
Matanuska Valley, Alaska	27	40	61.5	49.9	1.23 ±0.0111
			(55-67)	(46-55)	(1.12 -1.31)
	18	50	65.7	53.9	1.22 ±0.0057
D. azteca			(63-69)	(48-57)	(1.12 -1.27)
Mather, California	27	50	57.0	49.1	1.16 ±0.0035
			(51-62)	(45-53)	(1.07 -1.44)
	18	50	60.6	53.6	1.13 ±0.011
			(57-65)	(51-57)	(1.03 -1.22)
Durango, Mexico	18	40	60.0	54.0	1.11 ±0.00487
D. narragansett			(55-65)	(50-58)	(1.02 -1.20)
Lebanon, New York	18	10	62.4	47.1	1.32 ±0.0104
D. tolteca			(53-68)	(39-50)	(1.26 -1.42)
Santa Maria de Ostuma, Nicaragua	27	50	41.0	49.8	0.823±0.0045
			(39-44)	(47-54)	(0.754-0.875)
	18	50	42.0	53.7	0.799±0.0050
			(40-45)	(50-56)	(0.732-0.880)
Chapulhuacán, Mexico	18	15	42.8	55.8	0.767±0.0159
			(39-45)	(53-59)	(0.714-0.818)
Coróico, Bolivia	18	15	44.2	54.1	0.817±0.0179
			(40-50)	(50-60)	(0.072-0.920)
Medellín, Colombia	18	15	47.7	60.8	0.785±0.012
			(45-52)	(57-67)	(0.701-0.879)
Mérida, Venezuela	18	15	45.1	56.9	0.792±0.0159
			(41-48)	(52-60)	(0.716-0.870)
Villavicencio, Colombia	18	15	44.6	55.5	0.804±0.0016
			(39-48)	(50-60)	(0.714-0.882)

D. affinis (Staten Island but not Alice), *algonquin* (Iron River) and *tolteca* (Santa Maria de Ostuma).

It is proposed that the relationship, first tarsal segment length divided by second tarsal segment length (f/s) be termed the *tarsal segment index*. Our data, presented in Table 4, show that values of tarsal segment index ranged as follows (species in order of increasing values of means): *D. tolteca* (0.714 to 0.920), *algonquin* (0.725 to 0.933), *affinis* (0.630 to 1.08), *azteca* (1.02 to 1.44), *athabasca* (1.12 to 1.39), and *narragansett* (1.26 to 1.42). Several complete separations of these species would have been possible, namely those of *D. tolteca* and *algonquin* on the one hand, from *D. athabasca*, *azteca*,



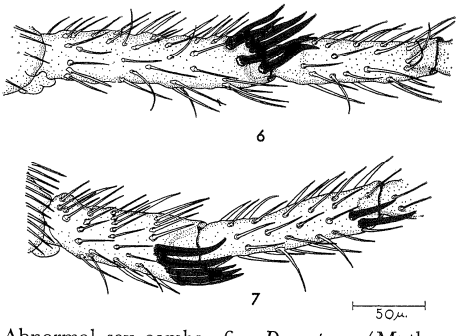
Figs. 1-5.—Foretarsi of males of several *D. affinis* subgroup species. 1.—*D. athabasca* (Matanuska Valley, Alaska). 2.—*D. azteca* (Mather, California). 3.—*D. affinis* (Staten Island, N. Y.). 4.—*D. tolteca* (Santa Maria de Ostuma, Nicaragua). 5.—*D. algonquin* (Iron River, Wisconsin).

and *narragansett* on the other. Potentially most useful among those would be the separation of the widely sympatric *D. tolteca* and *azteca.*

The tarsal segment index may be useful for the separation of females of certain *affinis* subgroup species. Measurements and calculations revealed, however, that the f/s ratio values for females were greater than 1.00 for all of the species. Ranges of female f/s values at both 18 and 27 C (20 at each temperature, except for only 17 of *D. azteca* at 18 C) were as follows: *D. tolteca* (Santa Maria de Ostuma), 1.13 to 1.27; *algonquin* (Iron River), 1.19 to 1.42; *affinis* (Alice, Staten Island), 1.10 to 1.40; *azteca* (Mather), 1.34 to 1.53; and *athabasca* (Laurentides Park, Matanuska Valley), 1.30 to 1.60. Thus the range of *D. tolteca* did not overlap those of *D. azteca* and *athabasca.* However, the maximum of *D. tolteca* approached closely the minima of *D. azteca* and *athabasca;* considering the paucity or absence of other criteria in females (e.g., regarding sex combs) it seems doubtful that the separation of females of these species by the tarsal segment index would be easy. Nevertheless, further investigation of the usefulness of the tarsal segment index for separating *D. azteca* and *tolteca* in their zone of overlap would seem to be worthwhile when additional stocks, and especially wild specimens, become available.

Abnormalities of Sex Combs and of Second Tarsal Segment Tooth Number

Most males observed in this study had sex combs in which the teeth were arranged in a single row, but in a few laboratory and wild-caught specimens the sex comb teeth were more or less in two tiers. In the *D. azteca* strain raised at 27 C, eight of the fifty specimens examined had sex combs which were abnormal in this way (Fig. 6). In addition, two *D. affinis* (Alice) males, one at each of the temperatures, were so characterized. The tiers were not always distinct; the larger row was usually quite regular, the smaller row less definite.



Figs. 6-7.—Abnormal sex combs. 6.—*D. azteca* (Mather, California) with first tarsal segment comb in two tiers, second tarsal segment tooth lacking. 7.—*D. affinis* (Alice, Texas) with two teeth on second tarsal segment.

D. tolteca normally lacks a tooth on the second tarsal segment which is found in the other American species of the *affinis* subgroup, and the European species, *D. helvetica,* possesses two or three such teeth. In exceptional cases individuals belonging to other species may also lack, or possess two, second tarsal segment teeth. Miller (1955) found that members of a *D. affinis* strain from Alice, Texas, frequently had two teeth on their right second foretarsal segment. The same strain in the present study produced 12 such specimens of 50 raised at 27 C, and 7 among the 50 raised at 18 C (Fig. 7). A single wild-caught male judged to be *D. affinis* from Halstad, Minnesota, had a duplication of the second tarsal segment tooth, as did two males from the same locality classified as *D. algonquin.* The same duplication was found for one specimen of *D. algonquin* (Iron River) raised at 27 C. Among male *D. algonquin* raised at 18 C, at least five specimens were found with possible duplicated teeth. In this case it was difficult to distinguish the teeth from large hair-like structures found on the tarsus. Four *D. algonquin* individuals collected at the University of Michigan Biological Station in 1954 had a similar tooth duplication, and numerous cases were borderline.

As expected, all of the *D. tolteca* males studied lacked the second tarsal segment tooth. It was also found, however, that some specimens of *D. affinis, algonquin, athabasca,* and *azteca* lacked this structure. Four *D. azteca* males raised at 27 C lacked the tooth, while one *D. affinis* (Alice) specimen raised at 18 C and two at 27 C were so characterized. None of the laboratory *D. athabasca* lacked the tooth in question, but among 11 specimens collected in Halstad, Minnesota, and classified as *D. athabasca,* a single male without the tooth was noted. *D. algonquin* males frequently lacked a second tarsal segment tooth, as illustrated by the foreleg shown in Figure 5. Twenty-two of 128 individuals classed as *D. algonquin* from the University of Michigan Biological Station (1954 collection) exhibited the absence. The Iron River laboratory strain of *D. algonquin* had a much higher incidence of tooth absence when raised at 18 C than when raised at 27 C. Of the 50 males raised at the higher temperature, only four lacked the tooth, while at 18 C, 20 individuals were found with this abnormality. This difference is statistically significant at the 0.05 level.

The occasional absence of a second tarsal segment tooth in American *affinis* subgroup species other than *D. tolteca* is of importance from the standpoint of species separation in that the other subgroup species, especially *D. algonquin,* cannot be considered completely distinct from *D. tolteca* with respect to this character.

DIFFERENCE IN GENITAL CLASPER TOOTH NUMBER BETWEEN D. ATHABASCA AND AZTECA

Since *D. athabasca* and *azteca* are very similar as to number of sex comb teeth, sex comb index, and tarsal segment index, and the difference of mesonotum striping reported between these species has

seemed to us unreliable, additional ways of distinguishing these two species were especially sought. Preliminary investigation revealed a potentially useful basis for separating males of these species, namely difference of number of teeth in the primary clasper combs.

Hsu (1949) studied the genital apparatus of male *Drosophila* including certain *affinis* subgroup species (ie., *D. affinis, algonquin, azteca,* and *narragansett*). The *D. azteca* from Durango, Mexico, which Hsu investigated had from four to six "primary teeth" on the "primary clasper" of the hypopygium; the other *affinis* subgroup species had more such teeth. The hypopygium of *D. athabasca* was not described by Hsu. However, a few examinations revealed that in *D. athabasca* the primary clasper comb has a relatively large number of teeth.

Examinations of the genital apparatus were subsequently made on two strains of *D. azteca* and eight of *D. athabasca*. The specimens were taken from stock bottles and then placed in alcohol for at least 24 hours. The posterior portion of each male was removed in creosol, and after clearing, the number of primary clasper teeth determined for each specimen under a compound microscope (ca. 200X). The results of the determinations for right claspers are summarized in Table 5. The range of clasper tooth number found for *D. azteca* was five to six, while *D. athabasca* males had from eight to eleven teeth. Typical *D. azteca* and *athabasca* primary claspers are shown in Figures 8 and 9 respectively.

The genital clasper teeth are small and their numbers can be determined only when examined under rather high magnification. However, the more crowded appearance of the clasper teeth in *D. athabasca* as compared with *D. azteca* and the somewhat more elongated shape of the *D. athabasca* claspers can be distinguished at a magnification of about 100X or less. The criterion cannot be considered an easy way to distinguish living specimens of the two species, but the complete distinctness of the few strains of *D. athabasca* and

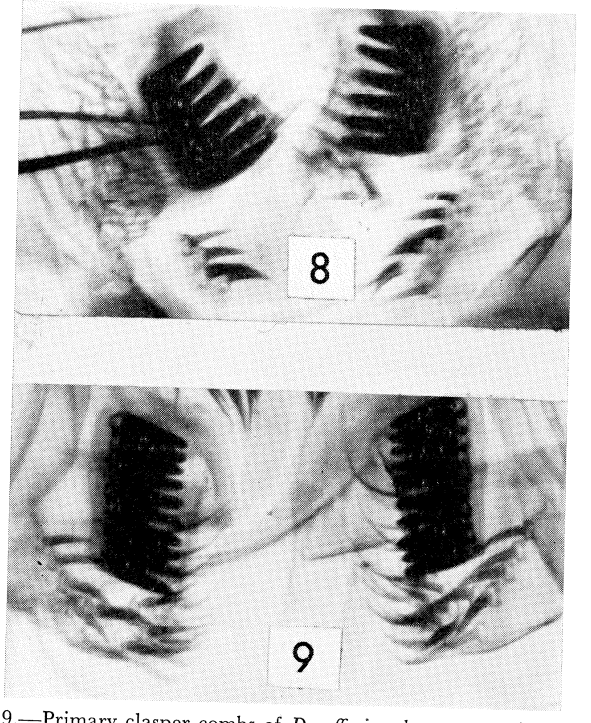
TABLE 5.—Numbers of right clasper comb teeth of males of laboratory strains of *D. athabasca* and *D. azteca* raised at 19C

Species and strains:	Number of teeth 5	6	7	8	9	10	11	n	Mean
D. athabasca									
Algonquin Park, Ontario				3	7			10	8.7 ±0.153
Cheboygan, Michigan				4	5	1		10	8.7 ±0.214
Cold Spring Harbor, N.Y.				6	4			10	8.6 ±0.245
College, Alaska				2	3	5		10	9.3 ±0.260
Gatineau Park, Quebec				7	3			10	8.3 ±0.149
Iron Mountain, Michigan				6	3	1		10	8.5 ±0.224
Jackson Hole, Wyoming				1	5	4		10	9.3 ±0.211
Matanuska Valley, Alaska					2	7	1	10	9.9 ±0.182
D. azteca									
Durango, Mexico	29	1						30	5.03±0.0339
Mather, California	4	26						30	5.53±0.0637

azteca at first examined suggested it to be of considerable value in the absence of a better means of separation.

Following this preliminary investigation, wild specimens and newly established stocks likely to be *D. athabasca* and/or *D. azteca* became available from collections made in the summers of 1963 and 1964 in the western United States and British Columbia (collections by one of us, D.D.M., supplemented by those of Miss Judith Barkley of the University of Oregon and of Professor Th. Dobzhansky of the Rockefeller Institute). Wild male specimens from British Columbia, Washington, Oregon, California, and Colorado were preserved in alcohol, cleared in creosol, and examined for clasper comb tooth numbers. In addition, newly established laboratory cultures from Arizona and New Mexico served as a source of males for which clasper comb tooth numbers were similarly determined. Data on these are presented in Table 6, which unlike Table 5 gives numbers of teeth on *pairs* of clasper combs rather than from right claspers only.

Table 6 shows that specimens from British Columbia, Washington, Colorado, and New Mexico appear to have been exclusively *D. athabasca* on the basis of the assumption that males classifiable as



Figs. 8-9.—Primary clasper combs of *D. affinis* subgroup species males. 8.—*D. azteca* (Mather, California). 9. *D. athabasca* (Matanuska Valley, Alaska).

TABLE 6.—Numbers of clasper comb teeth of males of *D. athabasca* and/or *azteca* collected in western localities (and some cultured from recently established stocks). Pairs grouped according to total numbers of teeth with number of each kind of pair beneath it in parentheses

	TOTAL NUMBER OF TEETH														
	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
A.	WILD SPECIMENS														
	British Columbia (Victoria) and *Washington* (several collecting sites in Baker National Forest, Olympic National Forest, San Juan Islands). (n = 30)														
								8-8	8-9	8-10	9-10		10-11		
								(6)	(3)	(1)	(7)		(2)		
										9-9					
										(13)					
	Oregon														
	Eugene (n=9)								8-9	9-9	9-10				
									(5)	(2)	(2)				
	Eel Creek Camp, Siuslaw National Forest (n = 59														
	5-5	5-6					7-8	8-8	7-10	8-10	9-10	10-10	10-11		11-12
	(6)	(2)					(2)	(3)	(1)	(1)	(16)	(2)	(1)		(1)
									8-9	9-9					
									(14)	(10)					
	California														
	Six Rivers National Forest, (n = 55)														
	Panther Flat Camp														
4-5	5-5	5-6	6-6												
(1)	(32)	(16)	(6)												
	Rockport (n = 20)														
4-5	5-5	5-6	6-6												
(2)	(13)	(4)	(1)												
	Gurnsey Campground, Lassen National Forest (n = 4)														
		5-6	6-6				7-8								
		(1)	(1)				(2)								

TABLE 6.—(continued)

							TOTAL NUMBER OF TEETH							
9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Colorado														
Rocky Mt. Biological Laboratory, Gunnison National Forest (n = 22)														
								8-9 (5)	9-9 (9)	9-10 (3)	10-10 (5)			
B. LABORATORY STOCK SPECIMENS														
New Mexico														
Raton Pass (n = 16)							8-8 (4)	8-9 (6)	9-9 (3)	9-10 (1)				
									8-10 (2)					
Ruidoso (n = 5)											10-10 (2)		11-11 (1)	11-12 (2)
Arizona														
Southwestern Research Station. Chiricahua Mountains (n = 59)														
4-5 (2)	5-5 (35)	5-6 (6)	6-6 (14)	6-7 (1)	7-7 (1)									

belonging to one or the other of these species and having eight or more teeth per clasper comb are *D. athabasca*. The identification of stock specimens from Ruidoso, New Mexico, as *D. athabasca* is noteworthy inasmuch as it confirms earlier reports of the presence of this species in south-central New Mexico, not far (about 150 miles or less) from reported collecting sites of *D. azteca* in southwestern New Mexico and western Texas (Dobzhansky and Epling, 1944).

The data on specimens from Oregon, California, and Arizona are interesting for two reasons. Firstly, collections in all three states provided individuals (or stocks) with values of clasper comb teeth numbers well within the range of *D. azteca* according to the data of Table 5. Secondly, each of these states provided some specimens with seven teeth per clasper comb, a value intermediate between those previously determined for *D. athabasca* and *azteca* and hence ambiguous. Moreover, seven teeth were found combined with six or with eight (and even ten) teeth on the opposite comb. The identity of wild individuals with seven teeth per comb must remain in doubt, and, of course, the possibility remains that even other values (e.g., six or eight) might exist in both species. Nevertheless, on the basis of some (or many) individuals with small numbers of clasper comb teeth (i.e., four through six) the conclusion seems warranted that *D. azteca* exists farther north along the Pacific coast than previously reported — specifically, in extreme northwestern California, at Panther Flat (about 25 mi NE of Crescent City), and in southwestern Oregon, at Eel Creek (about 15 mi SW of Reedsport). Moreover, at Eel Creek *D. athabasca* and *azteca* now appear to have been collected together; hence, the ranges of these two species must overlap in Oregon. In California, on the other hand, *D. athabasca* was not determined, barring the possibility that individuals with the 7-8 combination of clasper comb teeth collected at Gurnsey Campground in the Lassen National Forest represented this species rather than *D. azteca*.

At most localities females likely to be *D. athabasca* and/or *azteca* were also collected, and attempts were made to establish laboratory strains from such females (as indicated in Table 6, the material from Arizona and New Mexico consisted exclusively of such strains, provided by Professor Dobzhansky). Unfortunately, a number of such females failed to breed, and still others gave rise to strains that lasted only a short while. Work is still in progress on a few surviving strains. Nevertheless, some conclusions regarding the laboratory stocks may be presented. Ten strains from Eel Creek, Oregon, all appeared to be *D. athabasca*, including males with no fewer than eight clasper teeth (though only a few males from each strain have been available for examination). Twelve strains from Panther Flat, California, seem to be exclusively *D. azteca*, containing males with no more than six teeth per clasper comb. Unfortunately, no strains were established from the Rockport and Gurnsey Campground (Lassen National Forest) collecting sites. Fourteen strains from the Southwestern Research Station, Arizona, all seem to be *D. azteca*. Of these, all but

one had males with no more than six teeth per clasper comb. The remaining strain contained one individual with a 7-7 and another with a 6-7 combination of clasper comb teeth numbers, and, in addition, one male with the 5-5 combination (only three males altogether available for examination before the strain was lost). Since the strain seemed otherwise to be *D. azteca,* judging from general appearance (see below), this would seem to mean that *D. azteca* may contain individuals with as many as seven teeth per clasper comb.

In addition to clasper comb tooth number, certain other characteristics distinguished the newly established strains. Although older strains of *D. athabasca* and *azteca* have not seemed easily distinguishable to us, members of the newly established strains suspected of being *D. athabasca* and *azteca* do appear consistently different from each other in a number of ill-defined ways — e.g., as to degree and nature of thoracic pigmentation (those judged to be *D. azteca* appearing darker than *D. athabasca* and often somewhat striped), density and orderliness of rows of acrostichal hairs (somewhat more dense and regular rows in *D. azteca*), and in ease of culture (*D. azteca* generally breeding more vigorously). Regarding mesonotum striping, *D. azteca* of recently established stocks does sometimes show four broad dark longitudinal bands, two inside and two outside the dorsocentral bristle rows, such that one conspicuously light stripe often appears in the middorsal line and others coincide with the dorsocentral rows, whereas in *D. athabasca* there is no such evident striping. However, old specimens of *D. azteca* (even in recent stocks) often lack such distinct markings, and we have still had much uncertainty regarding the identity of wild specimens on the basis of coloration, etc.

Although most of the new cultures failed to survive long enough in our laboratory for breeding tests with other strains of the two species, some crosses have been performed. Both northern California (Panther Flat) and Arizona (Southwestern Research Station) stocks of *D. azteca* have proven fertile in combinations with each other and with a strain of this species from Mexico (Chilpancingo).

D. tolteca, like *D. athabasca,* was not among the species studied by Hsu (1949). Examination of a few males of our Nicaraguan strain of *D. tolteca* plus several from another strain from Bolivia (Coróico) showed clasper combs in this species to have 6 or 7 teeth, hence overlapping *D. azteca.* Due to the fewness and condition of available strains of *D. tolteca,* this character was not further explored in this species. Wild specimens in the region of coexistence of *D. azteca* and *tolteca* may possibly differ sufficiently in clasper comb tooth number to make it worthwhile to try to separate them on this basis.

DIFFERENCE OF TESTIS SHAPE BETWEEN D. ALGONQUIN AND TOLTECA

The description of *D. affinis* (Sturtevant, 1916) characterizes the species as having testes with one and one-half coils or gyres. Sturtevant and Dobzhansky (1936) further state that the testes of *D. affinis,*

algonquin, athabasca and *azteca* are spiral rather than ellipsoidal, but they do not indicate any testis shape difference between the species. *D. tolteca* is described by Patterson and Mainland (1944) as having "two inner and two and one-half outer coils or gyres." It would appear, therefore, that it should be possible to distinguish *D. tolteca* from *affinis, algonquin, athabasca* and *azteca* because of differences in the degree of testis coiling. The suggestion of the possible usefulness of testis shape differences for separating certain of these five species was also made to one of us (R. L. S.) by Dr. Colin S. Pittendrigh of Princeton University. Examinations were made of several strains of each of these species with the disclosure that although *D. athabasca* and *azteca* sometimes have testes similar to *D. affinis*, some specimens occur in which the testes are more highly coiled. *D. athabasca* and *azteca* appeared to be somewhat intermediate between *D. affinis* and *algonquin* on the one hand and *D. tolteca* on the other with regard to testis shape. *D. affinis* and *algonquin* were found consistently to have club-shaped testes with only about one and one-half gyres. Contrastingly, *D. tolteca* testes studied all appeared more highly coiled than those in the other species examined.

The criterion of testis shape was studied further as a means of distinguishing *D. algonquin* and *tolteca* because these species seem difficult to distinguish by other means. (Of course, according to present knowledge, these species have geographical ranges that are very distinct and nonoverlapping.) The general indistinctness of *D. algonquin* and *tolteca* on the basis of sex comb tooth number has been discussed as has the fact that *D. algonquin* often lacks the second tarsal segment tooth, which is characteristically absent in *D. tolteca*. In making testis examinations, the entire posterior abdominal contents were removed in Ringer's solution and the testis then separated from the extraneous material. The examinations were made under a dissecting microscope at a magnification of about 20X. For the determination of the number of gyres present, only one (right or left) testis of each pair was selected. No attempt was made to choose either right or left member because of difficulties in orientation. The length of each testis and its greatest width were also determined with an eyepiece micrometer, one unit being equal to about 0.033 mm. This permitted the computation of the ratio, testis length divided by width (l/w). All of the strains studied were from regular laboratory stocks raised at about 18 C. These strains and the results of the examinations are given in Tables 7 and 8.

All of the *D. tolteca* individuals had testes with more than two gyres, and more coiling was usually evident. Although the testes of *D. algonquin* usually had less than two gyres, there were two exceptions in the Humboldt, Nebraska, strain. However, the *D. algonquin* testes with two gyres appeared quite distinct from *D. tolteca* testes. That this distinction existed is shown by the l/w ratio difference for the two species with values in *D. algonquin* never exceeding 6.60 and never lower than 9.00 in *D. tolteca*. The ability to distinguish

individual living *D. algonquin* from *tolteca* by examination of the testis depends in large part on the age of the specimens. Observations through the ventral abdominal wall of young males easily reveal the longer, more coiled testes of *D. tolteca* as compared to the more club-shaped structures in *D. algonquin*. Dark pigmentation of older specimens sometimes makes the distinction of living individuals difficult or impossible, and the shrunken abdomens of poorly fed individuals may add to the difficulty. In general, however, the criterion was found to be of value in doubtful cases.

A NEW SPECIES OF THE D. AFFINIS SUBGROUP

In August of 1964 one of us (D. D. M.) collected Drosophilas in the vicinity of the Rocky Mountain Biological Laboratory (Gothic, Gunnison National Forest), Colorado, and specimens obtained there included three male individuals which, while resembling members of the *affinis* subgroup species, seemed very distinct from any heretofore described. Although similar in size, color and density of rows of acrostical hairs to *D. athabasca*, which was common in these collections, they differed in having sex combs that were distinctly larger than those of *D. athabasca* (six to eight teeth per comb) and in lacking a sex comb tooth on the second foretarsal segment. Further examination revealed still other differences, not only from *D. atha-*

TABLE. 7.—Numbers of gyres in testes of *D. algonquin* and *D. tolteca* males (the right and left testes were not distinguished)

Laboratory strains:	n	Number of gyres				
		1-1½	1½-2	2-2½	2½-3	3-3½
D. algonquin						
Hallock, Minnesota	30	3	27			
Humboldt, Nebraska	30	1	27	2		
D. tolteca						
Chapulhuacán, Mexico	30			5	23	2
Coróico, Bolivia	30			1	19	10

TABLE 8.—*D. algonquin* and *D. tolteca* males raised at 18C. Mean values (eyepiece micrometer units) of testis length (l), width (w) and the ratios of these (right and left testes were not distinguished). Limits of dimensions are given in parentheses beneath the corresponding means

Laboratory strains:	n	l	w	l/w
D. algonquin				
Hallock, Minnesota	30	30.40	6.13	4.96
		(24-38)	(5-7)	(4.00- 6.60)
Humboldt, Nebraska	30	30.86	6.76	4.56
		(22-39)	(6-9)	(3.76- 6.29)
D. tolteca				
Chapulhuacán, Mexico	30	52.32	4.63	11.29
		(48-58)	(4-5)	(9.60-13.75)
Coróico, Bolivia	30	51.1	4.33	11.80
		(39-64)	(3.5-5)	(9.00-17.14)

basca but from other known members of the subgroup. Unfortunately, although all females of the *affinis* subgroup collected at the time were isolated so they might, if so disposed, breed and establish laboratory cultures, none gave rise to a stock of the new type. It is, nevertheless, our conclusion that these males represent a new species of the *affinis* subgroup, and the following description is thus presented. The species is named in honor of Dr. Edward Novitski of the University of Oregon.

Drosophila novitskii, sp. nov.

♂. Frons dull dark brown, nearly ½ width of head. Antennae dark brown; arista with seven branches. Face dark brown, carina small. Cheek dark brown, 1/4 to 1/3 width of eye. Eyes with dark pile. Ratio of first, second and third orbital bristles 2:1:1. First oral bristle about four times as long as second.

Mesonotum dark brown. About six rows of acrostichal hairs. Sterno-index 0.65. Legs yellowish brown. Sex comb on first segment of foretarsus with six to eight teeth; no sex comb tooth on second foretarsal segment. Sex comb index (f/c) 3.5 to 3.9. Tarsal segment index (f/s) 1.20 to 1.26. Wings clear. Costal index 2.4. 4th vein index 2.1. 4c index 0.97. 5x index 2.2.

Abdomen solid dark brown. Primary clasper combs *lacking*. Testes orange, *ellipsoidal*.

Body length about 2 mm (i.e., medium, e.g., similar to *D. athabasca*).

♀. Unknown (no specimen yet collected and identified).

Distribution. Rocky Mountain Biological Laboratory (Gothic), Gunnison National Forest, Colorado.

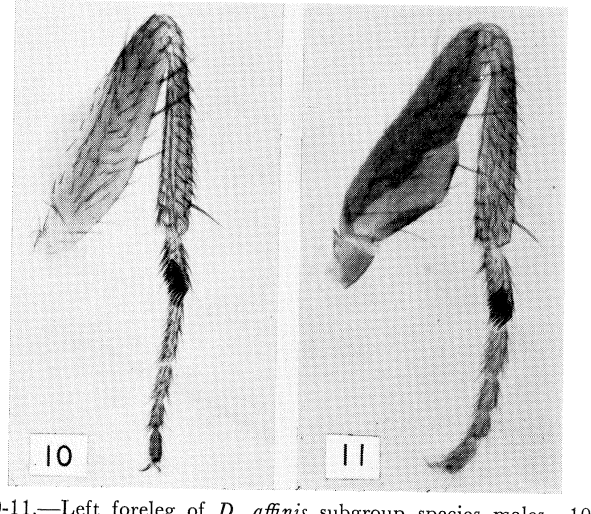
The preceding description includes certain distinctive and surprising features. An obvious one concerns the foretarsi of the males. Although number of sex comb teeth puts *D. novitskii* in the ranges of *D. algonquin* and *D. tolteca*, the relative length of the first and second tarsal segments of a foreleg is quite different in this new species. In *D. novitskii* the tarsal segment index ranged from 1.20 to 1.26, whereas Table 5 shows that we found this index to range from 0.725 to 0.933 in *D. algonquin* and from 0.701 to 0.920 in *D. tolteca*. The relatively long first tarsal segment of *D. novitskii* contrasts strikingly with the relatively short one of the other two species, as is shown in Figures 10 and 11, which illustrate forelegs of male *D. algonquin* and *D. novitskii*, respectively. Elsewhere in the *D. affinis* subgroup only *D. dobzhanskii*, with its very large sex comb (about 18 teeth), appears to have a large sex comb on a long first tarsal segment (Patterson, 1943).

Two features of the male reproductive system of *D. novitskii* are especially surprising and deserve additional attention whenever new specimens become available. First, the primary claspers of the male genitalia appear to *lack entirely* an organized comb, unlike any other member of the *D. affinis* subgroup so far observed. Second, the testes of the three *D. novitskii* specimens were distinctly short and ellipsoidal rather than spiral, resembling *D. pseudoobscura* testes more than those of any of the other *D. affinis* subgroup species.

RESUMÉ AND DISCUSSION

As anticipated, certain description and key characteristics of *D. affinis* subgroup species transgressed or were otherwise at variance with earlier accounts. Numbers of sex comb teeth varied outside stated limits, and such variation could conceivably cause difficulty in species identification. In particular, this might sometimes be important for the two pairs of widely sympatric species *D. affinis* and *algonquin*, and *D. azteca* and *tolteca* (Tables 1 and 2). Absolute length of sex comb teeth did not completely separate *D. affinis* from *D. athabasca, azteca* and *narragansett* (Table 3), and comparisons of sex comb tooth length with diameter of tarsal segment did not provide a satisfactory basis of separation of *D. affinis* from *azteca* (as it had not for *D. athabasca*, Miller, 1955). Some caution in species identification on the basis of number of sex comb teeth and size of sex comb teeth would seem to be in order. Regarding the single sex comb tooth of the second foretarsal segment, not only is *D. helvetica* aberrant in having two such teeth and *D. tolteca* in having none, but both duplication and absence of this tooth is sometimes encountered in the North American species *D. affinis, algonquin, athabasca,* and *azteca*. Especially significant is the relatively common lack of this tooth in *D. algonquin*, which often resembles *D. tolteca* both in number of sex comb teeth and in absence of second tarsal segment tooth.

The sex comb index, now defined as length of first tarsal segment divided by length of longest sex comb tooth, appears likely to be very useful for separating males of *D. affinis* from those of *D. athabasca, azteca,* and *narragansett* (*affinis* having values of this index less than 3.00, the others having values greater than 3.00). Likewise, the tarsal



Figs. 10-11.—Left foreleg of *D. affinis* subgroup species males. 10.—*D. algonquin* (Duluth, Minnesota). 11.—*D. novitskii* (Gothic, Colorado).

segment index, or length of first tarsal segment divided by length of second, separates males of *D. affinis, algonquin* and *tolteca,* which have values less than 1.00, from *D. athabasca, azteca* and *narragansett,* with values greater than 1.00 (though *D. affinis* does sometimes have a tarsal segment index value close to unity). Especially likely to be useful would be a separation of *D. azteca* from *tolteca* on this basis. Environmental (temperature) and genetic (strain) factors influence variation of this ratio, and further experience in attempting to separate wild males on this basis would seem very desirable. Additional useful criteria of separation now appear to be the number of primary clasper comb teeth for *D. athabasca* (generally eight or more) and *azteca* (generally six or fewer) and the shape of the testes for *D. algonquin* (short, thick, little coiled) and *tolteca* (long, slender, much coiled).

An interesting inverse relationship appears to exist between size of sex comb, in terms of number of teeth, and length of first tarsal segment relative to length of second tarsal segment in most of the *D. affinis* subgroup species. In *D. algonquin* and *tolteca,* the numbers of teeth per sex comb are greater than in the other species studied, and the first tarsal segment is relatively shorter than in the others. In *D. athabasca, azteca* and *narragansett* the sex comb is smaller than and the first tarsal segment relatively longer than in the rest. *D. affinis,* which is intermediate in sex comb size, has a first tarsal segment of intermediate length, with a tarsal segment index close to 1.00. Apparently also consistent with this relationship is the fact that in all these species females, lacking a sex comb entirely, have a relatively long first tarsal segment, with tarsal segment index values always greater than 1.00, though varying from species to species in a manner roughly parallel to the tarsal segment index in males. No satisfactory explanation for this relationship is at hand. One may, however, speculate that either, a) the developments of first tarsal segment and sex comb are somehow antagonistic processes so that an increase of one inhibits the other, or b) there is generally some adaptive value to having a short first tarsal segment if the sex comb is large or a long first tarsal segment of the sex comb is short or lacking. On the other hand, *D. novitskii* presents an interesting deviation from the relationship of size of sex comb and length of first tarsal segment suggested by the other species; here a large sex comb (of size comparable to that of *D. algonquin* or *tolteca*), is borne on a relatively long first tarsal segment (Fig. 11). *D. dobzhanskii,* which we have not seen, appears to be another exception, having an enormous sex comb (about 18 teeth) on a first tarsal segment that looks somewhat longer than the second (Fig. 19E of Patterson, 1943), though here it would be hard to imagine how a sex comb of such size could be accommodated on a segment that was not itself long.

A MODIFIED KEY FOR AMERICAN D. AFFINIS SUBGROUP SPECIES

The following modified and expanded key for the separation of

American *D. affinis* subgroup species is proposed on the basis of characteristics discussed in this paper. It is intended as a substitute for couplets 41 through 45 of the key to the North American species of *Drosophila* of Patterson (1943). It includes two species which we have not seen, *D. dobzhanskii* and *seminole,* for which the description and key characteristics, respectively, of Patterson (1943) have been used.

1a. Sex comb large (usually seven or more teeth), nearly parallel to axis of tarsal segment 2

1b. Sex comb smaller (usually six or fewer teeth), more oblique with respect to tarsal axis 5

2a. First tarsal segment longer than second (tarsal segment index greater than 1.0) 3

2b. First tarsal segment shorter than second (tarsal segment index values 1.0 or less) 4

3a. Sex comb with 6-8 teeth. Colorado. ..... *D. novitskii*

3b. Sex comb with about 18 teeth. Mexico. ..... *D. dobzhanskii*

4a. Second tarsal segment tooth usually present. Testis shorter and only slightly coiled (less than seven times as long as wide, less than two gyres). Range: central and northeastern United States, southeastern Canada. ..... *D. algonquin*

4b. Second tarsal segment tooth absent. Testis more slender and coiled (more than nine times as long as wide, more than three gyres). Range: southern Mexico to Haiti and Bolivia. ..... *D. tolteca*

5a. First tarsal segment generally shorter than (or about the same length as) the second (i.e., tarsal segment index generally 1.0 or less). Sex comb index values less than 3.0. Range: United States east of the Rockies, southeastern Canada. ..... *D. affinis*

5b. First tarsal segment longer than second (i.e., tarsal segment index greater than 1.0). Sex comb index greater than 3.0 6

6a. Front appearing pollinose when viewed laterally 7

6b. Front not appearing strongly pollinose 8

7a. Front not pollinose when viewed from vertex. Mesonotum not uniformly pollinose, with longitudinal stripes. Alabama. ..... *D. seminole*

7b. Front distinctly pollinose (silvery) when viewed obliquely. Mesonotum not striped. Range: United States east of the Rockies. ..... *D. narragansett*

8a. Primary genital clasper comb with eight or more teeth. Mesonotum very inconspicuously striped if at all. Range: Alaska to eastern Canada; Pacific coast south to Oregon; Rocky Mountains south to New Mexico; northern midwest; northeastern United States; Appalachians south to North Carolina and northern Georgia. ..... *D. athabasca*

8b. Primary genital clasper comb usually with six or fewer teeth. Mesonotum sometimes marked with four dark brown longitudinal bands (two inside and two outside dorsocentral lines). Range: Oregon to Costa Rica. ..... *D. azteca*

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