MORPHOLOGICAL DIFFERENCES AND PROBLEMS OF SPECIATION IN DROSOPHILA *

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Received August 25, 1947

INTRODUCTION

The usefulness of Drosophila in the development of genetic theory and as cytological material is known to all biologists, but it may not yet be obvious to all that the genus Drosophila offers highly favorable material also for the study of speciation. In the early days of Drosophila taxonomy separation of the species of the genus was accomplished by comparison of gross morphological traits. This method missed the interesting "borderline cases" between race and species but it was satisfying in that there was little difficulty involved in making out labels for individual dead specimens. If, however, the scientist becomes interested in the genetics, physiology, and environmental variations among the living members of the genus more and more refined methods are needed for the separation of the species within it. This is particularly true in Drosophila because here speciation is frequently accompanied by relatively little differentiation in morphological characters. The tendency to consider small differences, such as relative sizes of parts of the insect as useful indications of species differentiation, even to the point of separation of species on statistical differences, is no doubt exasperating to the museum man. Nevertheless, this is inevitable if we wish the species names to reflect the biological facts.

Data are presented in this article concerning small morphological differences between some of the "borderline cases" in the *obscura* group of Drosophila. They are of interest in that they show the kind and magnitude of some of the differences between groups of flies which have recently arrived at, or crossed, the threshold of species formation.

The authors wish to acknowledge the kindness of Professor Dobzhansky in furnishing all the stocks and many valuable suggestions.

MATERIALS AND METHODS

Studies such as this one depend upon consistent methods and well controlled environmental conditions for meaningful answers. Large environmental fluctuations could cause greater variability in the quantitative characters studied than the genetic differences which interest us. About one-quarter of the data reported here were collected during 1941 and the rest during 1946–47. The same methods were used in both instances.

The flies which were measured were the offspring of single pair matings. Ten fertilized females of each geographic strain were placed singly in "creamers" and allowed to deposit eggs for two days. Each female was then moved to a fresh "creamer" and allowed to deposit eggs for another two days and so on. Fifty females and fifty males of each geographic strain which developed from these eggs were isolated upon emergence from the pupa cases and allowed to "harden" on fresh food in creamers for three days and then measured. The wings of the females and the wings and fore-legs of the males were mounted in clarite on glass slides, and coverslips applied. After drying, the

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* Research accomplished at Harvard University and supported by a grant from the Milton Fund.

slides were projected on squared paper, a tracing made of the projection and the squares counted. The area of the wing in square millimeters was then obtained by conversion. The wing length was obtained by measuring the maximum length of the tracing on the squared paper followed by the application of a conversion factor. The teeth in the sex-combs were counted under the low power magnification (150 ×) of a compound microscope. These methods are those developed by the senior author for culturing and measuring flies to be used for flight studies as reported by Reed, Williams and Chadwick (1942).

All experimental animals were raised in a room having a constant temperature of 20.0 ± 0.5° C. Temperature control is of importance in all work concerned with wing size and the number of teeth in the sex-combs. Probably more important and more difficult to regulate is the quantity and quality of food material available to each larva.

SEPARATION OF SPECIES IN THE PSEUDOOBSCURA GROUP BY MEANS OF MORPHOLOGICAL CHARACTERS

One of the most interesting groups of "borderline species" known is composed of *D. miranda*, *D. pseudoobscura* (formerly Race A), *D. persimilis* (formerly Race B), and *D. subobscura*. All four species are very similar in most external characteristics. An attempt to classify individual dead specimens as to their species would be hazardous. Yet each of the four populations of flies is a true species according to the dynamic definition proposed by Dobzhansky which states that speciation has occurred when the stage of evolution has been reached "at which the once actually or potentially interbreeding array of forms becomes segregated in two or more separate arrays which are physiologically incapable of interbreeding." This definition may not have universal application but it does very well for the genus Drosophila.

There is extensive evidence all of which indicates that the first three of the above species do not form hybrids in nature even though their geographic ranges overlap in the western United States. The fourth species, *D. subobscura*, is European. A fifth species (or group of them), *D. obscura*, is also European and was not available for this study. Complete reproductive isolation between the four species seems to have been attained and gene interchange between them can be of little importance in nature. These considerations are in themselves enough to establish the four arrays of flies as distinct species according to the definition which we have accepted. Acceptance of this definition seems justified if systematic categories are to express the biological value of the populations concerned and not merely the degree of morphological distinctiveness.

We come now to the question of whether or not there are morphological differences between the four species which rest on differences in their genotypes.

TABLE 1. *Morphological differences between some of the species in the pseudoobscura group*

All flies grown at 20° C. Wing measurements from females only, while the counts of the sex-comb teeth are from their brothers. Standard errors are given for all measurements.

1. Species	2. No. of strains	3. Wing area in mm.^{2}	4. Wing length in mm.	5. Reed's wing index no.	6. No. of teeth in ♂ sex-combs	
					Proximal	Distal
D. miranda	3	3.31 ± 0.02	3.31 ± 0.02	117.5	8.10 ± 0.08	5.92 ± 0.07
D. persimilis	10	2.75 ± 0.03	2.97 ± 0.01	71.9	6.30 ± 0,08	5.12 ± 0.09
D. pseudoobscura	14	2.50 ± 0.03	2.84 ± 0.02	57.3	6.90 ± 0.11	5.68 ± 0.08
D. subobscura	1	2.53 ± 0.03	2.83 ± 0.02	57.4	10.39 ± 0.24	10.00 ± 0.17

The answer is certainly in the affirmative. In order to find the answer the environmental conditions must be held quite constant for all the populations being compared, but that is not an unreasonable stipulation.

In table 1 may be found data on the morphological differences between the four species of the *D. pseudoobscura* group which are under consideration. The fifth column in the table gives what Dobzhansky and Epling (1944) have called "Reed's wing index." This index is calculated by multiplying the area of the wing in square millimeters by the length in millimeters cubed. This particular index was used because it had a relationship to the mechanics of flight as pointed out in Reed, Williams and Chadwick (1942) and gave values which were more useful than wing areas or lengths taken separately. A value combining both the area and the length of the wing adds significance beyond that provided by either measurement alone because two strains of flies may overlap in their wing area measurements but not in their measurements of wing length, or vice versa. If wing area and wing length were perfectly correlated, the index would be useless, but as they are not perfectly correlated, the index provides something in addition to the magnification of the small differences.

Further significance is added to the distinction between *D. miranda* and the other three species by observing the count of the teeth in the sex-combs of the males. Following a consideration of the wing index and the number of teeth in the sex-combs there should be no difficulty in classifying the progeny of individual females captured in nature as to whether or not the strain is *D. miranda.*

The problem of distinguishing *D. subobscura* from the other three species is of academic interest only, because there is no overlapping of its European range with that of the three American species. There is no significant difference between the wing index of *D. pseudoobscura* and *D. subobscura* as may be seen in table 1. The large number of teeth in the sex-combs of *D. subobscura* are a morphological character which in itself would be sufficient to determine whether the progeny of a female belong to that species or not.

It is established that if the flies are raised under uniform conditions, progeny of *D. miranda* and *D. subobscura* can be distinguished from *D. pseudoobscura* and *D. persimilis* progeny on purely morphological characters. Can these characters be used to distinguish the more closely allied pair of species, *D. pseudoobscura* and *D. persimilis* from each other? Table 1 shows that while the differences in wing measurements and sex-comb teeth counts are small, they are, none-the-less, highly significant. For example, the difference between the wing areas of *D. persimilis* and *D. pseudoobscura* is only 0.25 ± 0.042 square millimeters, but this difference is 5.9 times its standard error, and is therefore highly significant.

Finally, then, we may say that all four species considered here can be separated on purely morphological characters provided an adequate number of flies, all of which have been raised under the same conditions, are examined. The differences between the four species are highly significant statistically though not of great magnitude. They are differences in degree which are real and rest upon a genetic basis.

The Consistency of the Species Differences

Let us examine the consistency of the results on wing area and wing length obtained by Reed, Williams and Chadwick (1942) and the more recent data obtained by us. The average area of the wings of five strains of *D. pseudoobscura* as shown in the earlier paper was 2.44 square millimeters compared with an average of 2.53 square millimeters for nine recently measured strains of the same species. For *D. persimilis* the areas were 2.73 and 2.76 square millimeters respectively. The wing lengths for *D. pseudoobscura* were 2.83 millimeters in the earlier paper and 2.84 in this one. Finally, wing lengths of *D. persimilis* were 2.98 millimeters and 2.96 millimeters respectively.

The above differences are small and except for the difference in areas between the five strains of *D. pseudoobscura* given in the first study and the nine in this study there is no suspicion of any true difference, either genetic or environmental, between the results recorded in the first paper and those just completed. The difference in wing area of nine hundredths of a square millimeter between the two samples of *D. pseudoobscura* is statistically significant but not of sufficient magnitude to affect the general finding of a highly significant difference in wing area between *D. pseudoobscura* and *D. persimilis*. Considering the fact that none of the five strains making up the average in the first study was the same as any one of the nine strains used in the present study, it is clear that our results obtained during the two studies are reliable and can be reproduced under our experimental conditions.

Let us see how our results compare with those of Mather and Dobzhansky (1939) which were obtained under remarkably different environmental conditions. They had no wing measurements that are strictly comparable to ours but provided abundant counts of the teeth in the sex-combs. The culture technique of Mather and Dobzhansky was quite different from ours in that as many as 20 females were allowed to oviposit in a half-pint milk bottle while we used a single female in each creamer. Consequently each larva in our experiment might be expected to have more available area of food surface and to produce a larger fly with more teeth in the sex-comb. This seems to have been the case as our results, shown in tables 2 and 3, are a little higher in frequency of teeth than Mather and Dobzhansky might have obtained. Bearing this in mind, our results fit into the picture very well indeed and give additional support to their finding that lowering the temperature in-

TABLE 2. *Frequencies of teeth in the proximal and distal sex-combs of* D. pseudoobscura *at different temperatures and in different laboratories*

Author	No. of strains	Temperature	No. of teeth in sex-comb	
			Proximal	Distal
Mather and Dobzhansky ('39)	4	17.5° C.	6.95 ± 0.17	5.57 ± 0.15
Reed and Reed (table 4 of this paper)	14	20.0° C.	6.90 ± 0.11	5.68 ± 0.08
Mather and Dobzhansky ('39)	11	24.5° C.	6.46 ± 0.09	5.32 ± 0.07
Dobzhansky ('35)	5	24.5° C.	6.57 ± 0.05	5.22 ± 0.05

TABLE 3. *Frequencies of teeth in the proximal and distal sex-combs of* D. persimilis *at different temperatures and in different laboratories*

Author	No. of strains	Temperature	No. of teeth in sex-comb	
			Proximal	Distal
Mather and Dobzhansky ('39)	4	17.5° C.	5.81 ± 0.16	5.16 ± 0.13
Reed and Reed (table 5 of this paper)	10	20.0° C.	6.30 ± 0.08	5.12 ± 0.09
Mather and Dobzhansky ('39)	12	24.5° C.	5.55 ± 0.12	4.73 ± 0.12
Dobzhansky ('35)	3	24.5° C.	5.77 ± 0.06	4.81 ± 0.06

creases the number of teeth in both the proximal and distal combs.

The important observation which comes from a comparison of the data in tables 2 and 3 is that even with different culture methods and over a wide range of temperatures no value for the teeth in *D. persimilis* ever gets as high as the lowest value for *D. pseudoobscura* even when as few as three geographic strains (total, 88 flies) were used. Consequently, if about five strains are used, a clear cut difference between the two species would be expected under any likely culture conditions and temperature. There is little doubt then, that the differences between the species in regard to the frequency of teeth in the sex-combs are genetic.

MORPHOLOGICAL DIFFERENCES BETWEEN GEOGRAPHIC STRAINS OF D. PERSIMILIS AND D. PSEUDOOBSCURA

There is no reasonable doubt about the significance of the differences between *D. pseudoobscura* and *D. persimilis* as shown in tables 1, 2, and 3. The averages shown there are based upon fourteen and ten different strains respectively and therefore have considerable stability. The differences between the averages based on *several* strains are highly significant but it is not true that the difference in a single characteristic between a particular geographic strain of *D. pseudoobscura* and one of *D. persimilis* is always significant. Occasionally a strain of *D. pseudoobscura* will be as large as a small strain of *D. persimilis.* It should be borne in mind that each of our geographic strains is composed of the descendants of a single fertilized female captured in nature. If this female deviated greatly from the mean for her species in size, genetically, it is quite possible for her offspring of later generations which were measured in the laboratory to do likewise. However, this female is not as likely to "overlap" with the other species in characters which are imperfectly correlated with the first character. It will be seen in table 4 that four out of fourteen strains of *D. pseudoobscura* have wings with areas as those of the two smallest strains of *D. persimilis.* With wing length, however, only one of the strains of *D. pseudoobscura* overlaps with *D. persimilis.* As wing area and wing length are not perfectly correlated, "Reed's wing index" becomes useful and it can be seen from table 4 that none of the strains of the two species give index numbers which overlap. If the frequency of the teeth in the sex-combs be considered, there is again overlapping between strains of the two species which is somewhat worse than it was with wing area and considerably worse than with wing length. However, as teeth in the sex-combs are imperfectly correlated with the wing measurements, it is possible to construct an index combining wing measurements and frequency of teeth in the sex-combs. As it is of no theoretical importance which wing measurement is used, the less advantageous one for our argument was chosen; that is wing area. Wing area was multiplied by 100 in order to dispense with two decimal places and then divided by the product of the number of teeth in the proximal comb times the number of teeth in the distal comb. This "new index" is given as the last column of tables 4 and 5. It can be seen that there is no overlapping of the "new index" numbers for the two species. The "new index" is somewhat better than "Reed's wing index" due to the fact that sex-combs and wing areas are not as highly correlated as wing areas and wing lengths.

The morphological data for males are given in table 5. Except for the smaller dimensions they show no fundamental differences from those of the females. Wing sizes of males of *D. persimilis* are larger than those of *D. pseudoobscura* as one would expect from the data for the females. Mather and Dobkhansky (1939) found the wing dimensions of *D. persimilis* females to be greater than those of *D. pseudoobscura.* They found the reverse to be true for males, however, which is not a reasonable answer in the light of our present knowledge. All the evidence from developmental rates, type of habitate, etc., indicates that *D. persimilis* should have the larger wings of the two species and our results show that this is certainly true for males, as well as for females for which there is no conflicting evidence. It is possible that the males behaved differently from the females in the experiments of Mather and Dobzhansky (1939) at 24.5° C. and under their particular environmental conditions, but it is more likely that their results were due to statistical fluctuations in the case of the males, or other causes which are not known to us.

It is clear that the variation between geographic strains is so great that classification of a strain on the basis of *one* character only, say wing area, is dangerous and not to be recommended. The use of an index number involving two or more characters therefore becomes profitable. Were it not for the fortuitous advantage offered by the salivary gland chromosomes, the use of some such index num-

TABLE 4. *Morphological differences between* females *of* D. pseudoobscura *and* D. persimilis. *Frequencies of the teeth in the sex-combs obtained from their brothers. Standard errors are given for all measurements*

Strain ♀ ♀	Wing area in mm.^{2}	Wing length in mm.	Reed's wing index	No. of teeth in ♂ sex-combs		New index
				Proximal	Distal	
Pseudoobscura						
Willapa Bay*	2.31 ± 0.01	2.83 ± 0.01	52.72	6.11 ± 0.08	5.18 ± 0.08	7.3
Perpetua*	2.23 ± 0.02	2.73 ± 0.02	45.72	6.95 ± 0.09	5.87 ± 0.06	5.5
Kaibab*	2.48 ± 0.04	2.84 ± 0.02	56.80	6.72 ± 0.08	5.72 ± 0.07	6.5
Prescott*	2.53 ± 0.03	2.88 ± 0.02	60.44	6.51 ± 0.08	5.54 ± 0.07	7.0
Quezaltenango*	2.63 ± 0.03	2.88 ± 0.02	62.84	6.56 ± 0.07	5.31 ± 0.07	7.6
Piñon Flats 140	2.47 ± 0.01	2.81 ± 0.02	54.81	6.84 ± 0.05	6.10 ± 0.06	5.9
Piñon Flats 6	2.61 ± 0.02	2.94 ± 0.01	66.32	7.31 ± 0.07	5.85 ± 0.07	6.1
Piñon Flats 11	2.42 ± 0.02	2.80 ± 0.01	53.12	6.57 ± 0.08	5.13 ± 0.05	7.2
Piñon Flats 103	2.54 ± 0.01	2.85 ± 0.01	58.80	6.75 ± 0.08	5.66 ± 0.06	6.6
Tuolumne Meadow	2.50 ± 0.01	2.85 ± 0.01	57.88	7.01 ± 0.07	5.62 ± 0.06	6.3
Aspen Valley	2.52 ± 0.02	2.87 ± 0.02	59.57	7.19 ± 0.07	6.07 ± 0.06	5.8
Mather	2.46 ± 0.02	2.73 ± 0.01	50.06	7.77 ± 0.08	5.95 ± 0.05	5.3
Lost Claim	2.67 ± 0.03	2.88 ± 0.02	63.79	7.34 ± 0.09	5.60 ± 0.08	6.5
Jacksonville	2.60 ± 0.02	2.85 ± 0.01	60.19	6.90 ± 0.08	5.92 ± 0.06	6.4
Average	2.50 ± 0.03	2.84 ± 0.02	57.36	6.90 ± 0.11	5.68 ± 0.08	6.4
Range	2.23–2.67	2.73–2.94	45.72–66.32	6.11–7.77	5.13–6.10	5.3–7.6
Persimilis						
Willapa Bay*	2.74 ± 0.02	3.00 ± 0.01	74.00	6.27 ± 0.08	5.63 ± 0.08	7.8
Perpetua*	2.82 ± 0.03	3.00 ± 0.02	76.16	6.58 ± 0.08	5.32 ± 0.05	8.1
Stony Creek*	2.60 ± 0.02	2.98 ± 0.02	71.32	6.20 ± 0.10	4.80 ± 0.09	8.7
Yolla Bolly*	2.75 ± 0.03	2.96 ± 0.02	68.80	5.89 ± 0.08	4.64 ± 0.07	10.1
Hope	2.62 ± 0.03	2.96 ± 0.01	67.94	6.28 ± 0.07	5.00 ± 0.06	8.3
Sequoia	2.78 ± 0.01	2.92 ± 0.01	69.22	6.38 ± 0.06	5.22 ± 0.07	8.3
Tuolumne Meadow	2.97 ± 0.02	2.96 ± 0.01	77.01	6.67 ± 0.07	5.27 ± 0.06	8.4
Porcupine Flat	2.78 ± 0.02	2.94 ± 0.01	70.64	5.91 ± 0.07	5.02 ± 0.05	9.4
Aspen Valley	2.76 ± 0.02	3.04 ± 0.01	77.52	6.20 ± 0.06	5.11 ± 0.05	8.7
Lost Claim	2.68 ± 0.02	2.92 ± 0.01	66.73	6.60 ± 0.08	5.17 ± 0.05	7.9
Average	2.75 ± 0.03	2.97 ± 0.01	71.93	6.30 ± 0.08	5.12 ± 0.09	8.6
Range	2.60–2.97	2.92–3.00	66.73–77.52	5.91–6.60	4.64–5.63	7.8–10.1

* Data for these strains obtained in 1941. Data for the others obtained in 1946–47.

TABLE 5. *Morphological differences between* males *of* D. pseudoobscura *and* D. persimilis. *Standard errors are given for all measurements*

Strain ♂♂	Wing area in mm.^{2}	Wing length in mm.	Reed's wing index	No. of teeth in sex-combs		New index
				Proximal	Distal	
Pseudoobscura						
Piñon Flats 140	2.12 ± 0.01	2.69 ± 0.01	41.27	6.84 ± 0.05	6.10 ± 0.06	5.1
Piñon Flats 6	2.19 ± 0.01	2.67 ± 0.01	41.67	7.31 ± 0.07	5.85 ± 0.07	5.1
Piñon Flats 11	2.06 ± 0.01	2.62 ± 0.01	37.04	6.57 ± 0.08	5.13 ± 0.05	6.1
Piñon Flats 103	2.06 ± 0.01	2.62 ± 0.01	37.04	6.75 ± 0.08	5.66 ± 0.06	5.4
Tuolumne Meadow	2.02 ± 0.01	2.56 ± 0.01	33.90	7.01 ± 0.07	5.62 ± 0.06	5.1
Aspen Valley	2.12 ± 0.01	2.58 ± 0.01	36.40	7.19 ± 0.07	6.07 ± 0.06	4.9
Mather	2.06 ± 0.02	2.50 ± 0.01	32.20	7.77 ± 0.08	5.95 ± 0.05	4.5
Lost Claim	2.26 ± 0.02	2.67 ± 0.01	43.01	7.34 ± 0.09	5.60 ± 0.08	5.5
Jacksonville	2.15 ± 0.01	2.56 ± 0.01	36.08	6.90 ± 0.08	5.92 ± 0.06	5.3
Average	2.12 ± 0.02	2.61 ± 0.02	37.62	7.08 ± 0.12	5.77 ± 0.10	5.2
Range	2.02–2.26	2.50–2.69	32.30–43.01	6.57–7.77	5.13–6.10	4.5–6.1
Persimilis						
Hope	2.24 ± 0.01	2.75 ± 0.01	46.59	6.28 ± 0.07	5.00 ± 0.06	7.1
Sequoia	2.23 ± 0.01	2.70 ± 0.01	43.89	6.38 ± 0.06	5.22 ± 0.07	6.7
Tuolumne Meadow	2.51 ± 0.01	2.85 ± 0.01	58.11	6.67 ± 0.07	5.27 ± 0.06	7.1
Porcupine Flat	2.31 ± 0.02	2.75 ± 0.01	48.05	5.91 ± 0.07	5.02 ± 0.05	7.9
Aspen Valley	2.19 ± 0.02	2.69 ± 0.02	42.64	6.20 ± 0.06	5.11 ± 0.05	6.9
Lost Claim	2.24 ± 0.02	2.69 ± 0.01	43.61	6.60 ± 0.08	5.17 ± 0.05	6.6
Average	2.29 ± 0.05	2.74 ± 0.02	47.15	6.34 ± 0.11	5.13 ± 0.04	7.0
Range	2.19–2.51	2.69–2.85	42.64–58.11	5.91–6.67	5.00–5.27	6.6–7.9

ber or similar method for classification of the strains as to their species would be the only morphological method available.

Similar systems of index numbers have been used on various occasions, one of the most interesting studies having been made by Anderson and Whitaker (1934) on the separation of *Uvularia grandiflora* from *Uvularia perfoliata*.

THE CONSISTENCY OF THE STRAIN DIFFERENCES

One of the remarkable aspects of tables 4 and 5 is the uniformity within the samples of animals measured. It will be noticed, for example, that all the standard errors are very small. Consequently the dimensions of many of the "geographic strains" are statistically significantly different from some of the other geographic strains of their own species.

The small standard errors obtained for the samples of single strains indicate that there is little genetic segregation or individual variability within a sample of a strain. It is quite possible, however, that samples studied at different times, even with our fairly constant culture technique, might vary significantly. Such was the case. Table 6 provides a good picture of the variability to be found in one strain. The character studied was the number of teeth in the sex-combs and it can be seen that there was considerable fluctuation in the counts obtained. The maximum difference in counts was five or six times its standard error and was consequently highly significant. The main diference to be observed is the one related to time. The counts in 1941 were lower than those taken in 1946–47. This is the largest and most important varia-

TABLE 6. *Comparison of effects of environmental fluctuations, statistical chance factors, and personal equations on the number of teeth in the sex-combs of a strain of* D. persimilis. *All at 20° C.*

Strain	Sample no.	No. of feet in sample	Date prepared	Av. no. of teeth		Investigator
				Proximal	Distal	
Yolla Bolly	1	55	July '41	5.89 ± 0.08	4.64 ± 0.04	S. C. Reed
Yolla Bolly	2	43	Nov. '41	5.74	4.54	E. Hunt
Yolla Bolly	3	100	Oct. '46	5.96 ± 0.06	4.84 ± 0.04	E. W. Reed
Yolla Bolly	4	100	June '47	6.31 ± 0.05	5.06 ± 0.04	E. W. Reed
Yolla Bolly	4	100		6.27	5.03	S. C. Reed (Recount of June '47 slides)

tion demonstrated by the strain. It is not restricted to this strain, however, as is indicated by an average count of 6.57 teeth in the proximal comb of five strains of *D. pseudoobscura* done in 1941, compared with a count of 7.08 teeth for nine strains of the same species done in 1946–47. The 1941 counts were done by S. C. Reed and the 1946–47 counts by E. W. Reed. A recount by S. C. Reed of slides counted by E. W. Reed shows no significant deviation, as demonstrated in the last two lines of table 6. The personal equation seems to be of no importance. It is doubtful whether the genotype has changed consistently between 1941 and 1946 in these strains, so we are left with the conclusion that the major fluctuations in our data result from uncontrolled changes in the culture technique. The fluctuation in the number of teeth in the sex-combs seems to have been proportionally much greater than in the wing areas and lengths following the change in conditions. We have no knowledge as to what feature of our technique has varied though it seems most likely that there has been some change in the composition or consistency of the food itself.

SUMMARY AND CONCLUSIONS

The four species *D. miranda, D. persimilis, D. pseudoobscura* and *D. subobscura* are true species because their reproductive isolation seems to have become irrevocable. They are excellent illustrations of a characteristic of the genus Drosophila in which speciation is frequently accompanied by relatively little differentiation in morphological characters. Apparently these four species are adapted to their ecological niches with such precision that genotypes causing any extensive changes in morphology would be eliminated by natural selection. Most genes causing large morphological changes apparently have a poor chance of survival, but those causing small changes in morphology may be favored because they would allow the group of closely related species to utilize the ecological range more completely than a single species could.

The three American species show a gradation in wing size which is correlated with climate. The species living in the coldest environment, *D. miranda,* has the largest wings. *D. pseudoobscura* has the smallest wings and inhabits the warmest regions. *D. persimilis* is intermediate, though closer to *D. pseudoobscura,* in both respects. The genotypes of all three species are fluid and individuals which deviate greatly from the mean of their own species in regard to any one characteristic could be misclassified if only one morphological character were considered. If an adequate sample of a species is measured under uniform conditions, there will be little doubt as to which species it is. If more than one morphological character is considered and some sort of index number calculated, there will be much less doubt as to which of the species the array belongs. In this paper two characters were employed in the study of morphological differences between the species. These were wing size and the number of teeth in the sex-combs. With only two morphological characters it was possible to classify each strain as to its species. The absolute differences were small, as expected, but significant. The calculation of an index number including other morphological characteristics in addition to the ones considered here would give even sharper differentiation and would no doubt permit classification of specimens, as well as populations.

The results of this paper show that the four forms of Drosophila, (*D. miranda, D. persimilis, D. pseudoobscura,* and *D. subobscura*), are not only true species on the grounds of reproductive isolation, but that they are genetically different in regard to the morphological characters studied. Therefore, they differ from the so-called "good" morphological species only in degree, that is, in the magnitude of the morphological differences.

It is clear that the difficulties inherent in a system of classification based entirely upon such small morphological differences may be considerable. Consequently the chances of devising some system of classification for the genus Drosophila which will be wholly satisfactory to the museum man are small, if the system is to be based upon genetic divergence protected by reproductive isolation. However, the only system that will give an accurate picture of the evolutionary status of closely related groups of the genus Drosophila will be one which is based upon a decision as to whether gene exchange between the groups is proceeding at a faster rate than gene divergence within the groups, or whether it is likely to do so in the future.

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