PTERIDINES AND GENE HOMOLOGIES IN THE EYE COLOR MUTANTS OF *DROSOPHILA HYDEI* AND *DROSOPHILA MELANOGASTER*

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Received June 29, 1965

FLUORESCENT pteridine compounds have been identified in Drosophila by HADORN and MITCHELL (1951), and FORREST and MITCHELL (1954a,b, 1955). Variation in the accumulation of the various pteridines from species to species in the genus Drosophila has been discussed by RASMUSSEN and SCOSSIROLLI (1954), RASMUSSEN (1954, 1955), HUBBY and THROCKMORTON (1960), and THROCKMORTON (1962). HUBBY and THROCKMORTON (1960) and THROCKMORTON (1962) have made an extensive survey of pteridine patterns among various Drosophila species. They have demonstrated that the use of these patterns as taxonomic characteristics is justified, and that such biochemical characters can be very helpful both in taxonomic and evolutionary studies. Paper chromatographic and electrophoretic techniques have demonstrated the presence of pteridines in various specific organs including heads, bodies, testes, malpighian tubules, and in larval stages of numerous species of the genus. These authors have shown that differences in pteridine patterns between species occur in various organs of the fly, but with regard to the eyes (heads), they found that the same pteridines were present in the same relative amounts in the wild-type eyes of all species. On the other hand HADORN and MITCHELL (1951) and HADORN (1958) have shown locus specific alterations in pteridine patterns of the eyes for several eye color mutants in both Drosophila and Ephestia (even though the metabolic relationships between the various pteridines themselves, and between the pteridines and the eye pigments, are not well understood). In most eye color mutants, the pteridine pattern is only altered in the eyes, and not in any of the other organs, even though there are some mutants such as rosy^2 (HADORN 1958), and white, white^h, brown, brown^{2b}, maroon-like, and rosy in the present study, in which both the bodies (chiefly due to the testes) and the heads show altered patterns. In the case of rosy^2, HADORN showed that some of the changes were in opposite directions in different organs. Thus, it would appear that the pattern of a given genotype can be modified within the organs of an individual according to organ specific properties. At any rate, pteridines clearly seem to play a role in eye pigmentation and the pigmentation of other organs, but pteridine accumulation and excretion in larval stages indicate that some of them at least function in other capacities as well (HADORN and MITCHELL 1951).

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Since variation in amounts of pteridines in the eyes is neglibible among the wild-type flies in different species of Drosophila, but significant in *melanogaster* between wild type and some of the eye color mutants such as rosy, it seems possible that an extensive survey of pteridine patterns of the eye color mutants might provide a basis for determining the homologies of some of the mutant eye color genes in different species. If each mutant gene altered pteridine metabolism in a different way, leading to a specific pattern for each gene, then it would only be necessary to see which mutants in two different species produced the same alteration in pteridine metabolism in order to establish homologies. However, as the present study shows, the matter is not so simple; in several instances two or more different mutants have the same pteridine pattern, which probably indicates that the alterations in pattern are several steps removed from the level of primary gene action. For example, most of the bright red mutants do not differ noticeably in pteridine pattern from the wild type. This should not be surprising since it is generally considered that the pteridines are involved in the production of red pigment, which is present in the bright red mutants. However, the remaining mutants in which there is no red pigment, or in which it is present in reduced amounts, fall into a small number of discrete patterns, and hence, each mutant does not have a different pattern. Thus pteridine patterns cannot be used as a conclusive means of establishing gene homologies, but they do provide an additional criterion for determining the homologies of eye mutants.

Previous criteria of homology between mutant genes in different species of Drosophila, provided that hybridization was not possible, were primarily that the phenotypes were similar, and that the linkage groups to which each mutant gene belonged displayed similar arrays of mutant types (STURTEVANT and NOVITSKI 1941; SPENCER 1949). In some cases linkage relationships between mutants within a linkage group have also been helpful in establishing homologies, but very often these relationships have been distortded by the many paracentric inversions that have occurred during the evolution of the genus. It is also helpful if genes have obvious pleiotropic effects, but again usually they do not. Transplants of imaginal discs in the case of eye color mutants can provide helpful information regarding the autonomy of a mutant (BEADLE and EPHRUSSI 1936). In some cases the similarity of mutant types within a multiple allelic series in each of two species indicates homology. Surprisingly perhaps, the best existing criterion of gene homology, i.e., the similarity of primary gene products in two species, has not been useful in Drosophila because the knowledge of the relationship between particular genes and specific proteins has not advanced far enough, except for the case of xanthine dehydrogenase in the mutants maroon-like and rosy (FORREST, HANLEY and LAGOWSKI 1961; GLASSMAN and MITCHELL 1959). To repeat then, it has been possible to employ pteridine patterns of the eyes as an additional criterion of homology, and by so doing, to strengthen the case for some previously suggested homologies between *melanogaster* and *hydei* eye mutants, to weaken the case for others, and to suggest some homologies that had not previously been suspected.

METHODS AND MATERIALS

For the grouping and comparison of mutants, 40 *melanogaster* and ten *hydei* strains were used (Table 1). The Ostwald color ratings in Table 1 were obtained by matching (under a dissecting scope, using an ordinary 100 watt bulb) freshly decapitated heads, to plasticized color chips from an Ostwald Color Harmony Manual (Container Corporation of America, Chicago 3, Illinois). It was not always possible to get an exact match between the eye and the chip, but they could be matched closely enough to make a meaningful arrangement of mutants possible. (Fully pigmented males four or more days old were used here, as almost completely throughout this study.) The number in the color rating symbol in Table 1 indicates a particular hue. These range in this study from a buff yellow (4), through bright orange (6), dull orange (6½), red (7), red violet (7½), to a dark red violet (8). The first letter following the number indicates the extent to which the particular hue designated by the number has been lightened by the addition of white, and the second letter indicates the extent to which it has been darkened by the addition of black. According to this system, then, the letters designating successively lighter chips resulting from the addition of white are: p (no lightening of the basic hue), n, i, l, g, e, c, and a (very light). Only three mutants, p^p, w^a, and w^h, matched chips where any lightening of the basic hue was involved. The rest of the mutants all matched chips that involved only darkening of the basic hue by the addition of black. The letters designating successively darker chips are: a (no darkening), c, e, g, i, l, and n (very dark). Thus a bright orange mutant such as karmoisin (6 pc) will match a chip that represents a slight darkening of a bright orange hue. If this orange is darkened a little more (6 pe), it matches the "yellow-browns," light, lightoid, and maroon-like. Further darkening of this hue causes it to match garnet[2] (6 pg), carmine and prune[2] (6 pi), and finally sepia (6 pn), which is the darkest brown known in *melanogaster*. Except that the basic hue differs in each group, the same explanation holds throughout the table. By means of this system of number and letters, it is possible to some extent to compare and contrast eye colors without actually seeing them.

Generally, our chromatographic techniques follow those of HUBBY and THROCKMORTON (1960). The flies were raised on a standard cornmeal-agar medium, at 24±1° C. The heads of male flies that had aged four or more days were used as samples. Their weight was relatively standardized by using 4 mg of whole flies. (Flies were added singly to the pan of an electrobalance until a weight of 4 mg was exceeded by less than the last fly added.) The flies were decapitated with a sharp razor, and the heads squashed with a glass rod in a spot on Whatman No. 1 filter paper. Bodies, when used, were dipped in ethyl alcohol, transferred to boiling water for 3 to 5 minutes, dried on filter paper briefly, transferred to the chromatographic paper, and squashed. Samples were placed approximately ⅜ inch apart across the neck of the paper (see below). The papers were dried, and then, since equilibration time affects the separation of specific compounds, equilibrated in the dark in the presence of 10% aqueous ammonia for the 8 hours necessary to separate the HB fraction of VISCONTINI *et al.* (1955) into biopterin and 2-amino-4-hydroxypteridine, and cause sepiapterin to move beyond the region where isoxanthopterin and xanthopterin are located. Following equilibration, the paper was developed in the dark for 24 hours in a propanol-ammonia solvent (*n*-propanol 8 parts: distilled water 3 parts: ammonium hydroxide 1 part v/v/v).

For better separation of the pteridines, the papers used were in the shape and size shown in Figure 1 (HARRISON, HAYES and CHUA 1963; CHUA 1963). 24 hours development was needed for the solvent to spread out to the sides as well as move down the paper, compared to the 12 hours used by HUBBY and THROCKMORTON (1960) for a rectangular paper. Figure 1 shows the location of the pteridines as they appear in the chromatograms of heads. The xanthopterin-like spot (Figure 1) is very similar in appearance to the xanthopterin spot but has not otherwise been identified. The xanthropterin-like spot was not, in our work, confused with isoxantopterin, which does sometimes appear in the same region as the xanthopterin-like spot, but which has a much deeper violet appearance. The isoxanthopterin spot was not used in the present study because it is present only in small quantity in heads and usually does not separate from the xanthopterin well

TABLE 1

Strains for which pteridine accumulations were determined

Eye mutant	Stock	Ostwald color rating	Comments
D. melanogaster			
w^a (white apricot)	Muller-5	4 ic	
w^h (white honey)	w^h	4 ne	
bw (brown)	*bw*	5 pi	
kar (karmoisin)	*kar cu*	6 pc	
lt (light)	*lt stw*^{3}	6 pe	These 3 are very similar, with *lt* and *ma-l* being virtually indistinguishable.
ltd (lightoid)	*ltd*	6 pe	
ma-l (maroon-like)	*ma-l*	6 pe	
p^p (pink peach)	p^p	6 ne	
g^2 (garnet^2)	g^2	6 pg	
cm (carmine)	*cm*	6 pi	*cm* is slightly browner than pn^2.
pn^2 (prune^2)	pn^2	6 pi	
Hn^{r3} (Henna recessive^3 = *sed*, sepiaoid)	Hn^{r3} *sr*	6 pl	
se (sepia)	*se*	6 pn	
cn (cinnabar)	*cn sp*	6½ pc	The first 4 are virtually indistinguishable, except that *v* has less pile; *st* is just slightly darker than these 4, and *cd* is slightly darker than *st*.
cn/cn^2	cn/cn^2 *cy D*	6½ pc	
v (vermilion)	*v*	6½ pc	
kar^2 (karmoisin^2)	kar^2	6½ pc	
st (scarlet)	*st*	6½ pc	
cd (cardinal)	*cd*	6½ pc	
bri (bright)	*bri*	6½ pe	These 2 appear to differ only in texture.
wild type	Oregon R	6½ pe	
bw^{2b} (brown^{2b})	bw^{2b}	6½ pg	These 4 are virtually indistinguishable; *ras* is slightly more red than ras^2.
ras (raspberry)	*ras dy*	6½ pg	
ras^2 (raspberry^2)	*y* ct^6 ras^2 *f*	6½ pg	
pr (purple)	*pr*	6½ pg	
bw^D (brown dominant)	bw^D	6½ pi	These 3 are very similar, but bw^D is slightly more red than *ry* which is slightly more red than ry^2.
ry (rosy)	*ry*	6½ pi	
ry^2 (rosy^2)	ry^2	6½ pi	
ca (claret)	*ca*	6½ pl	Of these 3, *ca* is slightly lighter than *pd*, which is slightly lighter than *car*.
pd (purploid)	*pd*	6½ pl	
car (carnation)	*car*	6½ pl	
mah (mahogany)	*mah*	7 pg	Virtually indistinguishable, but *mah* has a thicker pile.
pn (prune)	*y pn*	7 pg	
dke (dark eye)	*dke*	7 pi	These 3 are very similar except for texture.
Hn^r (Henna recessive)	*jv* Hn^r *h*	7 pi	
bo (bordeaux)	*bo*	7 pi	
w^{cf} (white coffee)	w^{cf}	7 pl	
rb (ruby)	*rb*	7½ pg	
w^{bl} (white blood)	w^{bl}	7½ pi	
w (white)	*w*	....	
D. hydei			
v (vermilion)		6 pc	Virtually indistinguishable.
or (orange)		6 pc	
cn (cinnabar)		6 pc	
cn^t (cinnabar tangerine)		6 pe	
st (scarlet)		6 pg	
ch (cherry)		6½ pi	
wild type		6½ pl	
52		7 pg	
g (garnet)		7 pi	Close to w^{bl} in *melanogaster*.
se (sepia)		8 pn	

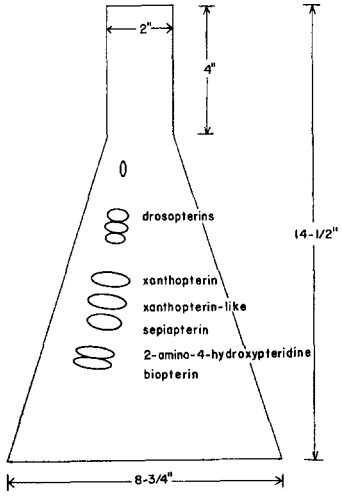

FIGURE 1.—Diagram showing size and shape of chromatograms and locations of the pteridine spots.

enough to permit consistent detection. Even so, it is clear that detectable amounts of isoxanthopterin are present in the heads of both *melanogaster* and *hydei*.

The cylindrical chromatographic jar was 45 cm deep × 25 cm in diameter. A wick (a piece of paper hanging from a glass rod absorbing the aqueous NH_3 from below) was used for more thorough saturation of the atmosphere and to compensate for the fact that the apparatus required that the entire lid be taken off in order to place the solvent in the trough.

After development, the papers were allowed to dry thoroughly in the dark. The fluorescent compounds were detected with either of two Minerallight ultraviolet lamps (principal emissions 256 and 366 mμ). Quantitative estimates were made visually and grouped into four categories: ++++, large; +++, moderate; ++, small; and (+), trace amounts. The fluorescing compounds were identified by comparing the chromatograms with the Rf values and diagrams published by THROCKMORTON (1962). The identification of sepiapterin, biopterin, and 2-amino-4-hydroxypteridine was further confirmed by running samples of the mutants sepia and maroon-like in which characteristic alterations of these three compounds occur. Also, Dr. JACK HUBBY (University of Chicago) kindly verified some of these identifications, and Dr. HUGH FORREST (University of Texas) kindly provided pure samples of xanthopterin, 2-amino-4-hydroxypteridine, isoxanthopterin, 2-amino-4-hydroxy-6-carboxypteridine and isosepiapterin which were run for comparison and still further confirmation.

RESULTS

The pteridine patterns for the heads of the various mutants are given in Table 2. Although THROCKMORTON (1962) lists minor, but consistent, quantitative differences between the wild-type heads of *melanogaster* and *hydei*, we found no detectable difference in numerous direct comparisons with heads of both species run on the same chromatogram. The wild types have, therefore, been placed in pattern I.

Pattern II mutants have a pattern that is very similar to the wild type. Under the present concept that the wild-type eye color in Drosophila results from a mixture of bright red pigment, due to pteridines, and brown pigment synthesized

TABLE 2

Groupings of mutants in D. melanogaster *and* D. hydei *according to pteridine pattern of heads*

	Pteridine pattern																							
	I	II		III		IV		V		VI		VII	VIII		IX		X		XI		XII	
D. melanogaster	wild type	*bo*	7 pi	*ca*	6½ pl	*p^p*	6 ne	*ras*	6½ pg	*ma-l*	6 pe	*se*	6 pn	*bw*	5 pi	*w^h*	4 ne	*w^a*	4 ic	*w^cf*	7 pl	*w^bl*	7½ pi
		bri	6½ pe	*car*	6½ pl	*pn*	7 pg	*ras^4*	6½ pg	*ry*	6½ pi			*bw^D*	6½ pi								
		cd	6½ pc	*cm*	6 pi	*pn^2*	6 pi			*ry^2*	6½ pi			*bw^2b*	6½ pg								
		cn	6½ pc	*dke*	7 pi	*pr*	6½ pg			*sed*	6 pl			*w*									
		cn/cn^2	6½ pc	*g^2*	6 pg																		
		Hn^r	7 pi	*lt*	6 pe																		
		kar	6 pc	*ltd*	6 pe																		
		kar^2	6½ pc	*pd*	6½ pl																		
		mah	7 pg	*rb*	7½ pg																		
		st	6½ pc																				
		v	6½ pc																				
D. hydei	wild type	*cn*	6 pc			52	7 pg	*ch*	6½ pi			*se*	8 pn	*w*									
		cn^t	6 pe																				
		g	7 pi																				
		or	6 pc																				
		st	6 pg																				
		v	6 pc																				
pteridines																							
Drosopterins	++++	++++		++		(+)		(+)		++													
Xanthopterin	+++	++		(+)		(+)		(+)		++		++++						(+)		(+)		(+)	
Xanthopterin-like	++	++										++								(+)		(+)	
Sepiapterin	++	++		++		(+)				+++		++++								(+)		(+)	
2-amino-4-hydroxypteridine	+++	++		++		++		(+)		++++		++++				++		+++		+++		++++	
Biopterin	+++	++		++		++		(+)		++++		++++										+++	

++++ large amount. +++ moderate amount. ++ small amount. (+) trace.

TABLE 3

Comparison of pteridine pattern with chromosome element for various mutants in D. melanogaster *and* D. hydei

Chromosome element	Species	Pteridine pattern																					
		II		III		IV		V		VI		VII	VIII		IX		X		XI		XII	
A	*melanogaster* X	*bo*	7 pi	*car*	6½ pl	*pn*	7 pg	*ras*	6½ pg	*ma-l*	6 pe		*w*		*w^h*	4 ne	*w^a*	4 ic	*w^cf*	7 pl	*w^bl*	7½ pi
				g^2	6 pg																		
		v	6½ pc	*cm*	6 pi	*pn^2*	6 pi	*ras^2*	6½ pg														
				rb	7½ pg																		
	hydei X	*g*	7 pi					*ch*	6½ pi				*w*										
		v	6 pc																				
B	*melanogaster* IIL	*bri*	6½ pe	*lt*	6 pe	*pr*	6½ pg						*bw*	5 pi									
													bw^D	6½ pi									
													bw^2b	6½ pg									
	hydei IV																						
C	*melanogaster* IIR	*cn/cn^2*	6½ pc	*dke*	7 pi																		
		cn	6½ pc	*ltd*	6 pe																		
				pd	6½ pl																		
	hydei III	*cn*	6 pc																				
		cn^t	6 pe																				
		or	6 pc																				
D	*melanogaster* IIIL	*st*	6½ pc									*se*	6 pn										
		Hn^r	7 pi																				
	hydei V											*se*	8 pn										
E	*melanogaster* IIIR	*cd*	6½ pc																				
		kar	6 pc	*ca*	6½ pl	*p^p*	6 ne			*ry*	6½ pi												
		kar^2	6½ pc							*ry^2*	6½ pi												
		mah	7 pg							*sed*	6 pl												
	hydei II	*st*	6 pg			52	7 pg																

from tryptophan, it is not surprising that in the mutants in which the red pigment is present, the pteridine pattern is virtually unchanged. The only differences are in the slightly reduced amounts of xanthopetrin, biopterin, and 2-amino-4-hydroxypteridine, and these differences are not always easy to detect. In addition, however, it is important to note that bordeaux, henna recessive, and mahogany in *melanogaster*, and garnet in *hydei* produce pattern II. These are dark reddish-brown mutants. Since the pteridines are intact in these mutants, it is reasonable to assume that the red eye-pigments are also unchanged. If this is true, then the dark color must result from an enchanced production of brown pigment, or from the presence of novel pigments that are not ordinarily found in Drosophila eyes, or both.

Pattern III differs from the previous patterns by having a markedly reduced amount of drosopterin and xanthopterin, and no xanthopterin-like compound at all. In group IV the drosopterin and sepiapterin which were present in group III are reduced to trace amounts, and in group V none of the pteridines are present in more than trace amounts. In group VI the amount of drosopterin is reduced and the xanthopterin-like compound is absent. However, sepiapterin, biopterin, and 2-amino-4-hydroxypteridine are present in increased amounts. In group VII the drosopterins are completely absent. There is a normal amount of the xanthopterin-like compound, and all of the other compounds are present in increased amounts. In group VIII, represented by white in both species, and the brown alleles in *melanogaster*, there is no trace of any of the compounds.

The remaining groups are represented by alleles of white. In group IX, white^{h}, which has a small amount of pigment, contains a moderate amount of biopterin but no others. In group X, white^{a}, which is a bit darker than white^{h}, contains a larger amount of biopterin than white^{h}, and in addition, contains a trace of xanthopterin. In group XI, white^{cf}, which is darkly pigmented, contains still other pteridines with traces of the xanthopterin-like compound, sepiapterin, and a small but distinct amount of the drosopterins added. In group XII, white^{bl}, which is the most darkly pigmented allele of white that was tested, contained no drosopterins, but traces of xanthopterin, the xanthopterin-like compound, and sepiapterin, and a large amount of biopterin and 2-amino-4- hydroxypteridine.

In Table 3 various tested mutants are compared both with respect to their pteridine pattern and to the chromosome element on which they are located. It appears that in the course of evolution in this genus, the chromosome elements have remained relatively intact (STURTEVANT and NOVITSKI 1941; SPENCER 1949). There have been multitudinous inversions within elements but very few exchanges other than the centric fusions between elements. Consequently, homologous genes in different species will usually be in the same chromosome element, and thus homologous chromosomes will contain similar arrays of mutant genes. Based on the presence of similar arrays of mutant genes, the homologies of the chromosome elements in the two species (SPENCER 1949) are as shown in Table 3. The X chromosomes in both are homologous, IIL in *melanogaster* is homologous to IV in *hydei*, IIR to III in *hydei*, IIIL to V in *hydei*, and IIIR to II in *hydei*.

With regard to the sex-linked mutants, four observations can be made. (1) The

suspected homology of the vermilion genes in the two species is further substantiated by the fact that they both have the same pteridine pattern. (2) The gene garnet in *hydei* was formerly considered to be homologous to garnet in *melanogaster*, but another dark eye in *melanogaster*, bordeaux, produces the same pteridine pattern as garnet in *hydei*, whereas an allele of garnet in *melanogaster*, garnet^2, produces a different pattern. It was not possible to obtain the *melanogaster* garnet for this study, but according to the description in BRIDGES and BREHME (1944), garnet is similar in appearance to garnet^2, although somewhat darker. Judging from the other multiple alleles in *melanogaster* in the present study, such as karmoisen and karmoisen^2, prune and prune^2, raspberry and raspberry^2, rosy and rosy^2, and brown, brown^D, and brown^{2b}, it seems likely that garnet and garnet^2 would have the same pattern despite the fact that each of the four white alleles produces a different pattern. In the latter series the phenotypic differences between alleles is considerable. Consequently, it appears more likely that bordeaux in *melanogaster*, rather than garnet, is homologous to garnet in *hydei*. (3) SPENCER (1949) suggested that on the basis of similarity in phenotype, cherry in *hydei* might be homologous to ruby, garnet, carmine, carnation, or raspberry. With the demonstration that cherry, raspberry, and raspberry^2 produce the same pattern, it seems likely that it is these two loci that are homologous. (4) The homology of white in the two species, though never in doubt because of their striking similarity, is also further confirmed by the complete absence of pteridines in the eyes of white mutants in both species.

For element B, no eye-color mutants have been reported in *hydei*. The three mutants in this element in *melanogaster* are very close to the centromere, and could have been shifted from IIR to IIL by a small pericentric inversion. Although pericentric inversions appear to have survived only rarely in Drosophila, presumably because of aneuploidy resulting from crossing over within the inversion in inversion heterozygotes, it is theoretically possible that small pericentric inversions could persist if they were so small that crossing over rarely or never occurred within the inverted region in a heterozygote.

In element C, there are bright eye-color mutants at two loci in *hydei* (cinnabar and orange), but only one in *melanogaster* (cinnabar). The possibility that the locus corresponding to one of these has been shifted to IIL in *melanogaster* by an inversion is questionable because a second such bright orange mutant is also absent on that arm. SPENCER (1949) tentatively suggested that orange might be homologous to light or lightoid, even though the phenotypes were very different. This explanation becomes even more tenuous in light of our observation that these suggested homologs have different pteridine patterns as well. A more plausible, though still questionable, explanation would be that orange and bright are homologous. The similarity between orange and bright is greater than the similarity between orange and any of the other second chromosome mutants in *melanogaster*. Furthermore, they both have the same pteridine pattern, and as suggested above, bright might have been shifted to the left arm of the second chromosome by an inversion. Another distinct possibility is that the evolutionary integrity of the elements is not as great as has been believed, and that a translo-

cation is involved. Also in element C there exist no *hydei* counterparts to the brown alleles in *melanogaster*. (A brown mutant in *hydei* which SPENCER (1949) considered to be homologous to brown in *melanogaster* has been lost.)

In element D, the accepted homology between the sepias is further strengthened by their sharing the same pteridine pattern. In this element, scarlet and henna recessive in *melanogaster* are the only other mutants present. As must be true for other loci, the absence of homologous mutants in *hydei* in this element is probably due to a failure to discover them, since far fewer mutants are known in *hydei*. A shift of the scarlet locus from IIIR to IIIL, in *melanogaster*, by a small pericentric inversion is a possibility. The proximity of scarlet to the centromere is consistent with this possibility, in which case the homologous locus in *hydei* would be in element E, or chromosome II, where several other bright orange mutants, in addition to scarlet, have been reported (SPENCER 1949).

In element E, *hydei* scarlet, as Table 3 indicates, is in the same accumulation group with cardinal and karmoisen in *melanogaster*. On the ground that scarlet is slightly darker than the rest of the bright red mutants in *hydei*, and cardinal is slightly darker than the rest of the bright red mutants in *melanogaster*, it might plausibly have been suggested that scarlet and cardinal are homologous. However, SPENCER (1949) suggested that another *hydei* mutant, cardinal, was probably homologous to cardinal in *melanogaster*; (cardinal in *hydei* may well have been even darker than scarlet, but the point remains moot since this mutant has been lost). The results with the one other *hydei* mutant in element E are surprising. The eye color in No. 52, though phenotypically nearly identical to mahogany in *melanogaster* in the same element, has a very different pteridine pattern. Furthermore, it did not produce the same pattern as any of the phenotypically similar *melanogaster* mutants, in this same element, with which it might have been considered homologous on the basis of phenotypic comparison. Rather, it produces the same pattern as pink peach, which has a very light phenotype. Thus, it seems that No. 52 is homologous neither to mahogany nor to any of the other similar but lighter *melanogaster* mutants in element E; it is homologous to pink peach. Also, there is no comparable mutant on IIIL that might have been shifted from IIIR to IIIL by a small inversion. For the present, no name corresponding to the supposed homolog in *melanogaster* has been given to the eye color in stock No. 52 because of the considerable difference in phenotype.

The results of the chromatograms of the bodies of the various mutants have not been given in detail, because none of the differences in pattern were helpful in determining homologies. Furthermore, whereas white and white^{h} in *melanogaster* differed both quantitatively and qualitatively from wild type, white in *hydei* does not, or at most differs slightly quantitatively. This may well indicate that no eye mutants in *hydei* have pteridine alterations in the bodies of the flies, and preclude using chromatograms of bodies in this species for comparative purposes. Moreover, in *melanogaster*, not only white and white^{h}, but brown, brown^{D}, brown^{2b}, maroon-like, and rosy have alterations in the accumulation pattern. These differences, although not useful in the present study, may be useful in helping to establish homologies between *melanogaster* and other species.

DISCUSSION

It is obvious that a number of mutants at different loci produce the same pteridine pattern. It is possible that more precise quantitative methods might subdivide these mutants into a number of unique patterns, but this is unlikely. That the same rather substantial changes in pteridine pattern in the eyes of Drosophila can be brought about by single gene mutations at several loci has certain implications with regard to the use of pteridines in evolutionary studies. Differences in pteridine metabolism between species do not necessarily represent different gene complexes that have been selected in the course of evolution, but could represent fixation of a single gene. Of course, the same could be said for a number of morphological differences as well, so that such an observation does not preclude the use of biochemical characters in taxonomic and evolutionary studies such as those of HUBBY and THROCKMORTON (1960), even in the case of pteridines. It is easy to imagine such a situation arising, with respect to either a biochemical or a morphological character, when a long series of biochemical reactions in a single "pathway" control the expression of a particular character.

Thus far no mutant has been discovered in which the pteridine metabolism has been altered in the testes but not the eyes. In the present study, 17 mutant and two wild-type stocks had pteridine metabolism altered in neither the eyes nor the testes. Of 32 in which the pteridine metabolism was altered in the eyes, it was also altered in the bodies (testes) of seven. The selection of eye mutants in the first place, as subjects for study, creates a bias against finding mutants in which pteridine metabolism is altered in the testes but not the eyes. Nonetheless, such mutants undoubtedly exist because, as HUBBY and THROCKMORTON (1960) have shown, the differences between the various Drosophila species with respect to pteridine metabolism is restricted mostly to the testes.

All of the five alleles tested at the white locus produced different pteridine patterns. The pseudoallelic nature of the white locus is well known, and it has been tempting to explain pseudoallelic loci in terms of a cistron within which crossing over occurs. However, the equivalence of a pseudoallelic locus and a cistron specifying a single polypeptide has not been demonstrated. The fact that the five white alleles showed five different metabolic patterns raises the distinct possibility that five different enzymes are involved. As more is learned about the metabolic relationships among the pteridines, it should be possible to distinguish between the alternatives that several enzymes are produced at the white locus, and that several different changes in a single enzyme give rise to the different patterns. Production of several enzymes at a complex locus might explain complementation of the type observed at the purple locus by NARAYANAN and WIER (1964).

It is also apparent from the present data that the relationship between the pteridines and the eye pigments is complex. For instance, the eye mutants raspberry, raspberry^{2}, purple, and brown^{2b}, all have the same Ostwald color rating (Table 1) and are virtually indistinguishable from each other. However, these mutants produce three different pteridine patterns, whereas a number of different

mutants with distinguishable eye colors all produce the same pattern (Table 2). For example, carnation, carmine, dark eye, garnet^2, light, purploid, and ruby are easily distinguishable, and they all produce pattern III. Thus on one hand, virtually identical phenotypes produce different patterns, and on the other hand, distinguishable phenotypes produce the same pattern. In this regard, the wild-type eye color in *hydei* is much darker than that in *melanogaster* even though there is no difference between them in terms of pteridine content. Mutants in *hydei* are generally, but not always, darker than their homologs in *melanogaster*. It is quite possible that part of the complexity of the relationship between the pteridines and the eye pigments involves alterations in the brown pigments, as well as the red pigments, as a direct result of the alteration in pteridine metabolism. It has been suggested by FORREST (1963) that certain pteridines, though converted to red pigments themselves, actually serve as co-factors for enzymes involved in the synthesis of brown pigment from tryptophan.

SUMMARY

Pteridine patterns were determined for 39 eye color mutants in *Drosophila melanogaster*, and nine in *Drosophila hydei*, by means of paper chromatography. Only chromatograms of the heads (eyes) were used; chromatograms of the bodies in all cases differed from chromatograms of the heads, but were not helpful in establishing homologies.

The majority of mutants in both species did not exhibit unique pteridine patterns, but rather produced one of several discrete patterns. Although there were more patterns represented in *melanogaster*, each pattern in *hydei* corresponded to one of the patterns in *melanogaster*. Thus pteridine patterns could be used as an additional criterion for homology of the eye color genes.

Using this new criterion, several accepted homologies were further confirmed, several tentative homologies were strengthened, several tentative homologies were rejected and replaced with more reasonable alternatives, and one completely new homology was suggested.

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