The work of P. W. Whiting and his students has provided an extensive collection of mutant genotypes of the chalcidoid wasp Mormoniella vitripennis (Walker). Many of the mutations cause eye color changes from the brown wild type to black, bright or
dark red, light peach, or grey-white shades. (Saul and Kayhart, 1956). Since these mutants, and physiological studies by Rohner (1959), suggest that Mormoniella eye pigment, like that of Drosophila, has red and brown components, it has appeared desirable to apply the techniques developed for Drosophila to a study of fluorescent substances in Mormoniella. This paper contains the results of experiments designed to provide a foundation for further research.

**Material and Methods.**

Mormoniella is parasitic on the pupae of various muscoid Diptera, (see Whiting, 1955, for an account of the life cycle). For the present experiments wasps were raised on the Florida blowfly Sarcophaga bullata Parker, at a temperature of 21° C. Eggs, and larval and pupal stages, can be recovered easily by breaking open the puparium of the host and brushing desired specimens from the surface of the host pupa.

Individuals to be used for chromatography were homogenized in small centrifuge tubes, and about 1 ml of 80% methanol or a mixture consisting of 4 parts of n-propanol to 3 parts of 2% aqueous NH₃ was added to the tubes for extraction of fluorescent substances. Following extraction for about 2 hours in darkness at 20° C, the solutions were centrifuged and the supernatant was spotted or streaked on Whatman No. 1 filter paper. This method was found to be superior to crushing the wasps directly on the filter paper, due to the low concentrations of some of the fluorescent substances. Between 5 and 10 insects per spot were sufficient for single-dimension chromatograms; 25 per spot were used for two-dimensional types. Sheets of filter paper 15 cm × 28 cm were used for single-dimension chromatograms; two-dimensional chromatograms were on 23 cm × 28 cm sheets. Drosophila used for comparison with Mormoniella were subjected to the same extraction and chromatographic procedures.

Dissections were made in Holtfreter's solution, and the separate organs were crushed directly on the filter paper. Larvae were washed in 80% methanol for one minute before dissection; digestive tracts and associated structures bulged through holes ripped in the larvae and could be easily pulled free of other tissues.
Chromatograms were of the ascending type, and were developed in a solvent mixture consisting of 4 parts of n-propanol to 3 parts of 2\% aqueous \(\text{NH}_3\) (P-A) or in a mixture of 20 parts of n-butanol: 3 parts of glacial acetic acid: 14 parts of water (B-A). One-dimensional chromatograms were developed for three hours at 25° C; two-dimensional chromatograms for 9-14 hours at 20° C. Wider separation of spots on one-dimensional chromatograms could be obtained, when desired, by re-developing in the same solvent following drying. During this process, as during extraction, the material was kept from intense light. Preliminary experiments conducted in the dark revealed no chromatographic changes resulting from subdued light.

Fluorescent spots were observed under an ultra-violet scanning lamp (principal emission at 3600 Å); absorbing spots were found by use of another lamp with emission at 2537 Å. Measurements of fluorescence were obtained by cutting spots from the chromatograms and inserting each in a system consisting basically of an ultra-violet source, a filter, a photocell, and a galvanometer. Galvanometer readings gave comparative measures of fluorescence as detected by the photocell. This system, described by Hadorn and Kühn (1953), does not record differences in color of fluorescence.

Absorption spectra were read from a Beckman spectrophotometer, using quartz cells and an ultra-violet light source. Strips containing fluorescent substances were cut from one-dimensional chromatograms developed in P-A and were eluted from the strips with water. In some cases the water solutions were re-streaked on filter paper and developed in 20\% acetic acid for further purification, and were then eluted again with water. Absorption readings were made at 10 \(\text{m} \mu\) intervals except near peaks, where intervals were reduced to 1 \(\text{m} \mu\).

**Inventory of Substances.**

Figure I shows a sample two-dimensional ascending chromatogram prepared from 25 Mormoniella males. Extraction was in P-A; P-A was used for the first dimension (14 hours at 20° C) and B-A was used for the second dimension (8 hours at 20° C). On
the right side of the figure is represented a one-dimensional chromatogram prepared from 10 *Drosophila* males and developed in P-A with the *Mormoniella* chromatogram. The following is a summary of observed characteristics of spots derived from the *Mormoniella* (see also table 1).

**Figure I.**
Sample chromatograms of fluorescent substances in adult male wild type *Mormoniella* and *Drosophila*. Left column: single dimension in P-A (*Mormoniella*). Center: P-A followed by B-A (*Mormoniella*). Right column: single dimension in P-A (*Drosophila melanogaster*). Shaded areas absorb ultra-violet light. Initial spots contained extracts from 25 *Mormoniella* and 10 *Drosophila*, respectively. D = Drosopterins; X = Xanthopterin; I = Isoxanthopterin; S = Sepiapterin; upper B = HB¹ and HB².
la, lb. (Ocher) On one-dimensional P-A chromatograms these appear as a single spot, yellow in visible light and fluorescing yellow in ultra-violet light. In B-A two spots appear; one (la) yellow in visible light and fluorescing yellow, the other (lb) fluorescing light red. Rf values are in table 1.

IIa, IIb. (Pink) On one-dimensional P-A chromatograms these are poorly separated from la and lb; in B-A following P-A they separate into two spots fluorescing light red. Rf values are in table 1.

III. This appears as a deep blue fluorescence, similar to that of isoxanthopterin. Rf values (table 1) also resemble those of isoxanthopterin spots from Drosophila controls (see figure 1). Absorption curves in 0.1N HCl show a peak at 281 m\(\mu\) and a plateau from 325-340 m\(\mu\); in 0.1 NaOH a peak appears at 255 m\(\mu\) and a plateau from 330-340 m\(\mu\). These curves resemble those published for isoxanthopterin (Visconti et al., 1955); apparently Mormoniella belongs among the arthropods containing this pteridine.

IV. This appears as a very faint blue-green fluorescence; on many chromatograms it does not appear at all. Its color and Rf values (table 1) suggest those of the xanthopterin spots from Drosophila controls (see figure 1), but attempts to obtain it in quantities sufficient for absorption spectra have not yet been successful.

V. (Flesh Pink) This highly striking substance, yellow-pink in fluorescent color, does not appear in Drosophila. Rf values are shown in table 1. Absorption curves, following elution from P-A chromatograms, re-streaking, and development with 20% acetic acid, showed sharp peaks at 257 and 353 m\(\mu\) in 0.1N HCl and at 265 and 365 m\(\mu\) in 0.1N NaOH. Flesh Pink (FP) appears in the larval intestine, but not in chromatographically detectable amounts in other larval organs; it is egested with the feces at pupation. It subsequently reappears in pupal tissues.

VI. This spot, probably composed of more than one substance, fluoresces light blue. Rf values in P-A and B-A (table 1) and in 20% acetic acid (0.70), as well as its fluorescent color, resemble those of 2-amino-4-hydroxypteridine from Drosophila controls (see figure 1). Absorption curves, however, show only two peaks: one at 245 m\(\mu\) in 0.1N HCl and one at 253 m\(\mu\) in 0.1N NaOH.
Since a compound having these peaks and low absorption above 300 m\(\mu\) is not likely to fluoresce strongly under a 3600 A lamp, these data are tentatively interpreted to mean that spot VI contains at least 2-amino-4-hydroxypteridine in low concentration and an unknown substance, in high concentration, which does not fluoresce but has the same Rf values as the pteridine in P-A and 20% acetic acid. Absorption curves for 2-amino-4-hydroxypteridine have been published by Visconti et al. (1955) and by Mitchell and Forrest (1955).

Table 1

<table>
<thead>
<tr>
<th>Substance</th>
<th>Visible Color</th>
<th>Fluorescent Color</th>
<th>Where Present</th>
<th>Approximate Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P - A</td>
</tr>
<tr>
<td>I a</td>
<td>yellow</td>
<td>yellow</td>
<td>Heads with colored eyes</td>
<td>0.06</td>
</tr>
<tr>
<td>I b</td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>II a</td>
<td>—</td>
<td>red</td>
<td>same as I a, b</td>
<td>0.07</td>
</tr>
<tr>
<td>II b</td>
<td>—</td>
<td>deep blue</td>
<td>prepupae-adult</td>
<td>0.41</td>
</tr>
<tr>
<td>III</td>
<td>—</td>
<td>blue-green</td>
<td>prepupae-adult</td>
<td>0.15</td>
</tr>
<tr>
<td>IV</td>
<td>—</td>
<td>flesh-pink</td>
<td>larvae feces pupal + adult tissue</td>
<td>0.31</td>
</tr>
<tr>
<td>V</td>
<td>—</td>
<td>light due</td>
<td>prepupae-adult</td>
<td>0.38</td>
</tr>
<tr>
<td>VII</td>
<td>—</td>
<td>blue-green</td>
<td>same as V</td>
<td>0.50</td>
</tr>
<tr>
<td>VIII</td>
<td>—</td>
<td>blue-green</td>
<td>same as V</td>
<td>0.73</td>
</tr>
</tbody>
</table>

VII (PIB-1) and VIII (PIB-u). These spots fluoresce blue-green, but only after exposure to ultra-violet light; after such exposure they no longer move in P-A or B-A. Rf values are given in table 1. Absorption peaks in acid and alkali have not yet been obtained. These substances are not found in Drosophila. Like FP
they appear in and are egested from the larval digestive system, and reappear in the pupal body. Also like FP, they have not been detected chromatographically in larval tissues. V, VII, and VIII can be collected in quantity by soaking host remains in P-A after the wasps have pupated and been removed. They do not appear in fresh, unparasitized host pupae, but V can be detected in trace amounts on chromatograms prepared from old, dried, unparasitized hosts soaked for 24 hours in P-A. They are not present in fresh hosts stung by *Mormoniella* the eggs of which were removed before hatching.

In addition to the fluorescing spots, an ultra-violet absorbing spot with an Rf of about 0.20 in P-A and absorption peaks at 285 m$\mu$ in 0.1N HCl and 295 m$\mu$ in 0.1N NaOH, was found. These properties suggest that the spot may contain uric acid.

Finally, data accumulated by Mr. Dennis Barrett indicate that adult *Mormoniella* contain xanthine dehydrogenase. Wasps were homogenized in 0.1M TRIS buffer at pH 8.4, pteridines were absorbed from the homogenate with charcoal, and the homogenate was incubated at 21° C with 2-amino-4-hydroxypteridine and Methyl Blue. Aliquots were chromatogrammed at several time intervals; examination revealed increasing amounts of isoxanthopterin formed, coincident with decreased amounts of 2-amino-4-hydroxypteridine. The specific activity, calculated from a single set of data, was 15$\mu$ moles/hr. gm. prot. N.

**Characteristics During Development.**

The low concentrations of several of the fluorescing compounds have made reliable estimates of changes in concentration difficult. Some variations can be expressed in a semi-quantitative manner, however. For this comparison 2, 4, and 9 day old larvae were used, together with prepupae (about 12 days old at 21° C), white-eyed pupae (13 days old), red-eyed pupae with no dark body pigment (14 days old), pupae with dark thoraces but light abdomens (15 days old), completely darkened pupae (16 days old), and young adults (about 18 days old). Comparable stages can be reached in fewer days at higher temperatures; at 28° C prepupae form in about 6 days and adults eclose in about 11 days.
No fluorescing substances could be detected chromatographically in larval tissues that remained after the digestive tract had been removed. The presence of V, VII, and VIII in the digestive tract has been described in the previous section; the amounts of these increased with increasing larval age, and were detectable in larvae two days after eggs were laid. Due to the extreme fragility of the wall of the digestive tract, it has not been possible to wash it free of the contents of the lumen; the question of where these larval fluorescing substances are formed therefore remains unanswered. The dissection leaves some glands, such
as the salivaries, firmly attached to the outer walls of the digestive tract; again, attempts to remove these resulted in destruction of the walls and contamination of all structures by the large amount of fecal material.

Substances III-VIII could be detected chromatographically in non-digestive tissues from the prepupal stage through adulthood. The complex including I and II first appeared in white-eyed pupae. Galvanometric measurements of (I and II), III, and IV appear in figure II; it can be seen that all increase through pupal stages and reach maximum concentrations in adults. The other substances do not increase significantly with increasing age of the pupae.

**Localization in Specific Organs and Tissues.**

Again, quantitative determinations are difficult due to the low concentrations of some of the substances. In table 2, classification is on the basis of whether the substance is chromatographically undetectable (—), present in trace concentrations, (+), or present in higher concentrations (++).

The table shows that I and II are in the head only, perhaps due to a relation to the red component of the eye pigments. III is in all body regions, although only in trace amounts in the thorax. IV and VI can be detected chromatographically only in the abdomens, and in trace amounts there. V, VII, and VIII can be detected in the abdomens, but not in heads and thoraces. No fluorescing substances were found in testes or ovaries (ten per spot), but traces of all except I and II are in the fine, fragile Malpighian tubes.

**Pattern in Mutants (freshly hatched imagos).**

Although quantitative data are not complete, qualitative studies on 9 red eyed mutants and 4 mutants with eye colors approaching white show no spots not present in wild type. They also show no great accumulations of substances, nor do they lack any spots present in wild type. In all of these mutants the concentration of V, VII, and VIII is somewhat higher than in wild type.
It is noteworthy that the concentration of the I-II complex is not greatly lowered.

In black eyed mutants, presumably lacking at least some of the red component of the wild type pigment, the concentrations of all the fluorescent substances are somewhat lower than in wild type. In one mutant, black, (Saul and Kayhart, 1956), I and II are lacking and III and VI accumulate; larval patterns are unchanged.

**Table 2**

*Amounts of fluorescent substances in adult Mormoniella, as detected chromatographically.*

<table>
<thead>
<tr>
<th>Substance</th>
<th>Head</th>
<th>Thorax</th>
<th>Abdomen</th>
<th>Testes</th>
<th>Ovaries</th>
<th>Malpighian Tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>I a, b</td>
<td>+++</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>II a, b</td>
<td>+++</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>III</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>IV</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>V</td>
<td>—</td>
<td>—</td>
<td>++</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>VI</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>VII</td>
<td>—</td>
<td>—</td>
<td>++</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>VIII</td>
<td>—</td>
<td>—</td>
<td>++</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
</tbody>
</table>

**Summary.**

Paper chromatography of extracts from various developmental stages of *Mormoniella* has revealed 8 major fluorescent spots and a large ultra-violet absorbing spot. Fluorescent colors, Rf values, and absorption peaks are given; comparisons with known pteridines in *Drosophila* suggest that *Mormoniella* contains isoxanthopterin, xanthopterin, 2-amino-4-hydroxypteridine, and uric acid; the three remaining spots are not comparable with any known in *Drosophila*. 
Three substances can be detected in intestinal contents of larvae and in non-intestinal tissue of prepupae, pupae, and adults, but not in larval stages. The other substances appear in young pupae; most increase in quantity through eclosion. With the exception of two classes of substances which are localized in the heads, no major accumulations of fluorescent materials were found in any organs studied. No major sex differences in types or quantities of the substances were found.

Results of preliminary surveys of mutant types show that only one mutant, black, differs greatly from wild type. This lacks two substances normally found in wild type heads and accumulates what may be isoxanthopterin and 2-amino-4-hydroxypteridine.

REFERENCES


MARKIERUNG VON ROTWILD 281


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