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IMMUNOGENETIC STUDIES OF THE WHITE LOCUS ALLELES w^+, w, w^a AND w^h OF DROSOPHILA MELANOGASTER

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ABSTRACT

Immunodiffusion analysis (Ouchterlony) of 4 white-locus alleles w^+, w, w^a, w^h in co-isogenic inbred Oregon-R backgrounds indicated only one precipitate line difference that appears to support a portion of Fuscaldo's analysis. Immunoelectrophoresis with the same material did not allow confirmation of that difference. We suspect that the electrophoretic separation may have eliminated some necessary interaction. We find no clear-cut antigenic differences between those co-isogenic lines differing by white-locus substitutions.

The white locus of Drosophila melanogaster is located on the X chromosome and has been mapped into at least five recombinational sites (Lewis 1952; Judd 1964). Fuscaldo and Fox 1961, Fuscaldo 1961 and 1962, Fuscaldo and McCarron 1965 and 1967, and Fuscaldo, McCarron and Dempsey 1966, did immunogenetic studies on this locus using the Ouchterlony immunodiffusion test. According to Fuscaldo, McCarron and Dempsey 1966, and Fuscaldo and McCarron 1967, antigens prepared from mutants at the two proximal sites (4 and 5) and the wild types exhibit the same specificity as the distal sites (1, 2, and 3), as evidenced by continuity of the precipitation line in agar, but are displaced. This corresponds to the function-spatial subdivision of the white region reported by Green (1959) at the phenotypic level and Ghosh and Forrest (1967) at the biochemical level.

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After preparing inbred and co-isogenic stocks of a series of white-locus alleles, it seemed appropriate to attempt to repeat portions of the work of Fuscaldo and McCarron (1965, 1967) as a test of the nature of the alleles used and the co-isogenicity of the stocks.

MATERIALS AND METHODS

In this study the Ouchterlony technique approximately as used by Fuscaldo and McCarron was also supplemented by the immunoelectrophoretic process to further test the variety of antigen-antibody relationships in the system, specifically, among co-isogenic stocks based on an Oregon-R inbred line and including the alleles w^+, w, w^a, w^h. Oregon R wild type flies had been inbred by sib pair mating for 72 generations before use in this experiment: w, w^a, w^h flies had been made isogenic with the inbred Oregon R (ORI) background by backcrossing to the inbred Oregon R wild type stock for 51, 50, and 50 generations respectively. The whole genic complement should be ORI for all four stocks, with the exception of the white locus substitutions.

Flies of both sexes were collected and starved for 12 hours before lyophilization to eliminate food in their digestive system. After lyophilization, the flies were transferred to a glass tissue homogenizer which was immersed in a beaker of ice, ground for 10 minutes in 0.85% NaCl, buffered at pH 7.4 with 0.005M phosphate to make a 2% (w/v) homogenate. The entire homogenate was used to immunize rabbits. The homogenate was centrifuged at 1000g for 15 minutes and the supernate used as antigen reacting against the antisera.

Six rabbits were immunized intraperitoneally on days 1, 3, 5, 12, 19, 33, 80 and bled from the ear on day 87. The freshly drawn blood stood 1 to 2 hours at about 25°C and 12 to 24 hours at about 4°C to allow clot formation and contraction. After being twice centrifuged at 1000g for 30 minutes, the clear antisera were stored at -15°C.

Ouchterlony immunodiffusion tests were accomplished with 1% purified Difco Noble agar in a phosphate buffer saline (pH 7.4) and 0.01% merthiolate. Antigen and antiserum, at 0.1 ml each, were allowed to diffuse from each well for 24 hours.

For immunoelectrophoretic tests, to remove possible impurities including sulfur which is responsible for the electroendosmotic effect, Special Noble Agar was washed in 6 changes of cold distilled water during 3 days, then washed in 95% ethanol, filtered and air dried. The 8 x 10 cm-plates were washed in boiling detergent solution, rinsed in distilled water, then in 95% ethanol, air dried, painted with a thin layer of 0.2% purified agar and heated at 100°C for 5 minutes. Purified agar (1.5%) in barbital buffer (pH 8.2) was poured on the plates to a 1.3 mm layer. The antigens were prepared from homogenized flies in 0.85% NaCl, buffered at pH 8.2 with Na-barbital and HCl for a 2%(w/v) homogenate. The Ouchterlony test antisera plus

two others were used. Separation of 8 µl samples of antigen proceeded for one hour at a constant current which offered a field strength of 5v/cm. Later, the antiserum troughs were cut on the plates and filled with specific antiserum (0.1 ml). After 24 hours incubation at 24°C, the plates underwent fixing and staining in Buffalo black-acetic acid (NBR Biological stain, Allied Chemical). The plates consistently revealed 14-15 precipitation arcs between antigen well and antiserum trough.

RESULTS AND DISCUSSION

There were seven to eight precipitation lines between each antigen and antiserum. The fourth precipitate line of $\underline{w}^a$ was repeatedly shown displaced inward or outward compared to those of $\underline{w}^+$ (ORI), $\underline{w}$, and $\underline{w}^h$ depending upon the antiserum used (Table 1).

TABLE 1.

Characterisics of the fourth precipitate line formed by antigens from different stocks with corresponding antisera.

Antiserum	Peripheral antigens	Normal Precipitate Precipitate	Displaced Precipitate
$\underline{w}$-42-1	$\underline{w}^+$, $\underline{w}$, $\underline{w}^a$, $\underline{w}^h$	$\underline{w}^+$, $\underline{w}$, $\underline{w}^h$	$\underline{w}^a$ (outward)
$\underline{w}^a$-86-3	$\underline{w}^+$, $\underline{w}$, $\underline{w}^a$, $\underline{w}^h$	$\underline{w}^+$, $\underline{w}$, $\underline{w}^h$	$\underline{w}^a$ (inward)
$\underline{w}^h$-74-2	$\underline{w}^+$, $\underline{w}$, $\underline{w}^a$, $\underline{w}^h$	$\underline{w}^+$, $\underline{w}$, $\underline{w}^h$	$\underline{w}^a$ (outward)
ORI-0-19	$\underline{w}^+$, $\underline{w}$, $\underline{w}^a$, $\underline{w}^h$	$\underline{w}^+$, $\underline{w}$, $\underline{w}^h$, $\underline{w}^a$	none

The tests were run twice, each time in duplicate plates. The results from all four experiments were identical.

The displacement appeared to be correlated with the mutant site of the allele used as antigen, as shown in Fig. 1, $\underline{w}^a$ (site 3) caused inward displacement; $\underline{w}^h$, $\underline{w}$ (site 4) the reverse. The latter partially confirmed Fuscaldo and McCarron's work (1965, 1967).

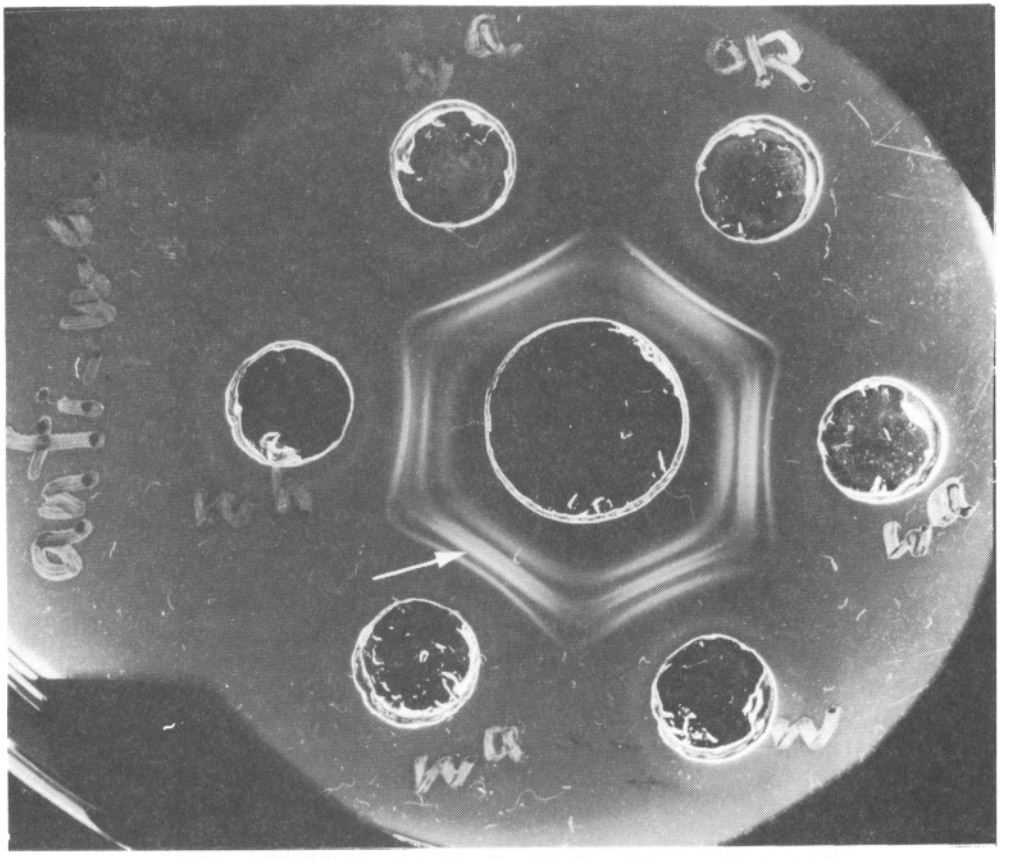

Figure 1. Typical Ouchterlony agar diffusion plate. Central well: antiserum (in this plate, $\underline{w}^a$-86-3). Peripheral wells: antigens as marked. The arrow points out the displaced precipitation line.

In the immunoelectrophoresis plates, each of the six antisera has its own specific precipitation pattern which remained the same

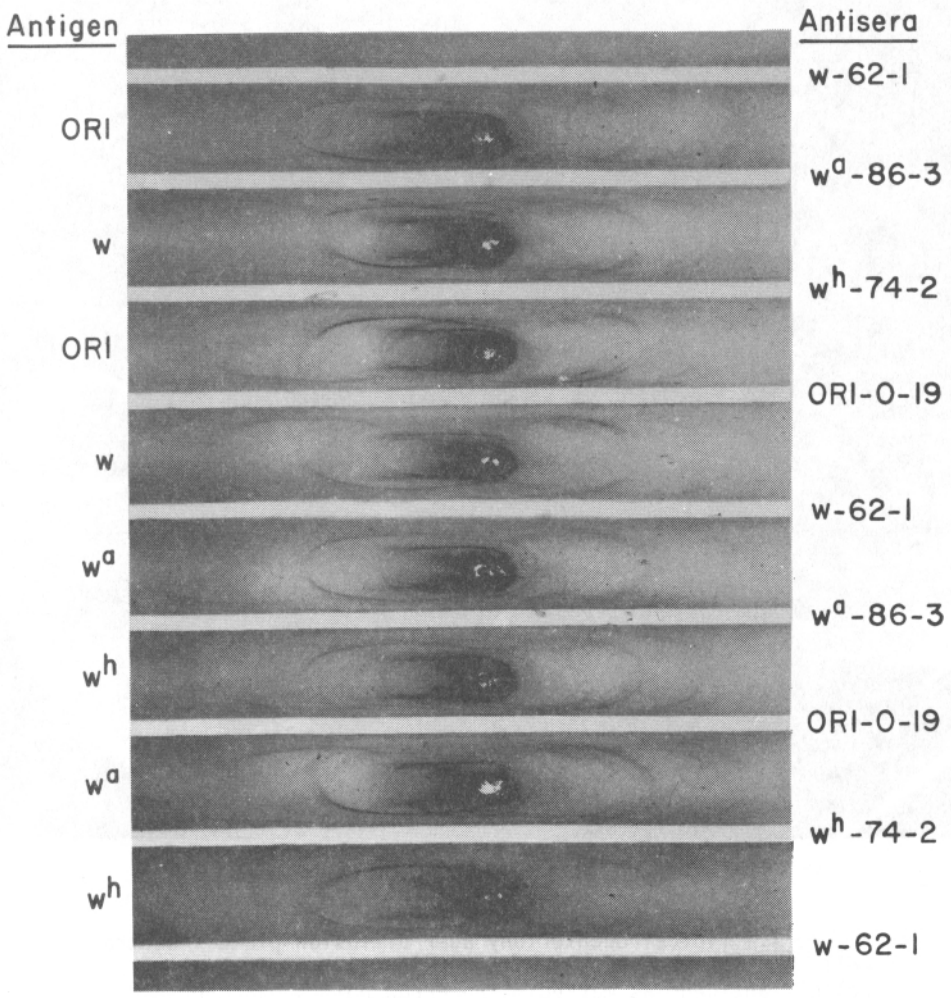

Figure 2. Typical immunoelectrophoresis plate. Antisera in the troughs and antigens in the wells, as marked.

with different antigens (Fig. 2). Two sets of antisera which were immunized against the same antigen behaved differently from each other just as noted with any two of the six antisera immunized from different antigens. This finding might imply that each rabbit became partially immune to only a portion of antigenic material and that none of the six animals produced any antibody that could detect differences among the four lines.

Thus, the immunoelectrophoretic analysis did not appear to demonstrate any qualitative antigenic differences among ORI-$\underline{w}^+$ $\underline{w}$, $\underline{w}^a$, and $\underline{w}^h$ co-isogenic lines. We do not understand the apparent discrepancy between the immunodiffusion and immunoelectrophoretic results. It seems likely that the electrophoretic separation may have removed the possibility of some interaction of components that led to the displacement of the 4th precipitate line of $\underline{w}^a$ in the Ouchterlony plates. Because the only apparent differences between the co-isogenic lines paralleled those predicted from Fuscaldo's results, we conclude that the backcrossing had indeed made the lines similar in genetic background to the limits of the sensitivity of these procedures.

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