A COMPARISON OF THE DIFFUSIBLE SUBSTANCES CONCERNED WITH EYE COLOR DEVELOPMENT IN DROSOPHILA, EPHESTIA AND HABROBRACON*

By G. W. BEADLE, R. L. ANDERSON AND JANE MAXWELL

STANFORD UNIVERSITY, JOHNSON C. SMITH UNIVERSITY, DANA HALL SCHOOL AND THE MARINE BIOLOGICAL LABORATORY

Communicated December 22, 1937

Diffusible substances have been postulated as being concerned in eye color development in insects belonging to three orders, Ephestia[1] and Bombyx[2] (Lepidoptera), Drosophila[3] (Diptera) and Habrobracon[4] (Hymenoptera). It is known that the substances concerned in Ephestia and Drosophila are not species-specific.[5,6] It is a matter of some interest to determine the relation to one another of the hormones or hormone-like substances in these insects. For example, is the "A-hormone" of Ephestia identical with either "v^+ substance" or cn^+ substance of Drosophila?[7] In connection with this example, Ephrussi[8] has given a comparative summary of the information concerning the A-hormone of Ephestia and the diffusible substances of Drosophila. In the present communication tests of Ephestia for substances active in Drosophila are reported; other experiments planned to show the relation between substances present in Drosophila and those in Habrobracon are described.

Material and Methods.—In the tests of Ephestia, wild type larvae, regularly used as hosts in culturing the parasitic wasp Habrobracon, were used. In making tests in Drosophila, vermilion brown (*v bw*) animals were used as a test for v^+ substance and cinnabar brown (*cn bw*) animals as a test for cn^+ substance. Wild type Drosophila pupae were used as a source of v^+ and cn^+ substances. Cinnabar pupae were similarly used as a source of v^+ substance alone. In tests of Habrobracon, six eye color types were used: wild, orange (*o*), ivory (o^i), red (*rd*), cantaloup (*c*) and white (*wh*). We are grateful to Doctor P. W. Whiting, who kindly placed these stocks of Habrobracon, as well as the material of Ephestia, at our disposal.

In all cases in which Habrobracon was used as a source of extracts (table 1) these extracts were made by heating larvae and pupae, taken shortly after cocoon formation, to 100°C. for a half minute or so, crushing and then removing the fluid fraction by centrifuging through a glass wool and asbestos filter.[9] Such extracts were tested by injection into Drosophila test larvae in amounts of approximately 0.5 to 1.0 cubic mm. per larva. In certain tests, designated in table 1, a feeding technique was used.[10] From 25 to 75 larvae and pupae (used shortly after spinning of cocoons) were heated to 100°C. for about 40 seconds, crushed and fed to 10 to 12 test larvae of Drosophila some 60 to 72 hours after egg laying (25°C.). The extract

of cinnabar Drosophila pupae was prepared in essentially the same way as described above for Habrobracon. The extracts of wild type pupae injected into ivory Habrobracon were prepared in a more elaborate way by Doctor Edward L. Tatum, to whom we are grateful for making them available.

TABLE 1

MODIFICATIONS OF THE EYE COLOR OF DROSOPHILA BY DIFFUSIBLE SUBSTANCES FROM HABROBRACON AND THE RECIPROCAL

SOURCE OF SUBSTANCES	RECIPIENT	METHOD OF TREATMENT	NUMBER OF SEPARATE TESTS	NUMBER OF TEST ANIMALS POSITIVE	NEGATIVE	STRENGTH OF EFFECT
Wild Hab.	*v bw* Dros.	Extract injected	3	13	1	1.1
Same	Same	Fed	2	16	0	1.8
Wild Hab.	*cn bw* Dros.	Extract injected	3	14	1	1.0
Same	Same	Fed	3	23	0	2.0
Orange Hab.	*v bw* Dros.	Extract injected	2	9	0	1.6
Same	Same	Fed	2	17	0	1.9
Orange Hab.	*cn bw* Dros.	Extract injected	2	0	9	0.0
Same	Same	Fed	2	0	16	0.0
Ivory Hab.	*v bw* Dros.	Extract injected	1	14	0	2.3
Same	Same	Fed	2	18	0	2.5
Ivory Hab.	*cn bw* Dros.	Extract injected	1	0	16	0.0
Same	Same	Fed	2	0	18	0.0
Red Hab.	*v bw* Dros.	Extract injected	1	8	0	3.0
Same	Same	Fed	1	11	0	2.2
Red Hab.	*cn bw* Dros.	Extract injected	1	7*	0	0.1
Same	Same	Fed	1	10	0	2.1
White Hab.	*v bw* Dros.	Extract injected	1	5	0	1.0
Same	Same	Fed	2	16	0	2.0
White Hab.	*cn bw* Dros.	Extract injected	1	7*	0	0.2
Same	Same	Fed	2	9*	13*	0.2
Cantaloup Hab.	*v bw* Dros.	Fed	1	8	0	3.1
Cantaloup Hab.	*cn bw* Dros.	Fed	1	9	0	2.8
Wild Dros.	Ivory Hab.	Purified extract injected	2	32	2	brown
Cinnabar Dros.	Ivory Hab.	Extract injected	1	0	7	none
Cinnabar Dros.	*v bw* Dros.	Extract injected	1	12	0	1.0
(Control for above experiment, same extract used)						
Ringer's solution	Ivory Hab.	Injected (operation control)	1	0	6	none

* Classification as "positive" or "negative" not certain.

Strengths of tests are recorded according to an arbitrary numerical scale, 0 representing no modification and 5 the maximum change possible. It

should be emphasized that the numerical values obtained in different tests are not strictly comparable; to be so, tests must be run in parallel and the test animals directly cross-compared.

Tests of Ephestia.—One apparent difference between the A-hormone of Ephestia and either v^+ or cn^+ substance of Drosophila is the fact that A-hormone is produced and released by gonad transplants[1] whereas neither v^+ nor cn^+ substance appears to be released by such transplants in Drosophila.[11] Extracts were made of larval testes of Ephestia by heating the excised testes in Ringer's solution. Such extracts gave a positive test for both v^+ substance (21 test animals, all positive, average color value 1.5) and cn^+ substance (13 test animals, all positive, average color value 1.7). Similar tests of larval testes of Drosophila are negative for both substances (15 and 10 individuals in tests for v^+ and cn^+ substances). Transplanted testes of wild type Drosophila are likewise without effect on the eye color of either vermilion brown or cinnabar brown hosts (11 and 10 individuals with well developed implants in the respective two tests). It is thus clear that extracts of Ephestia testes contain substances similar in effects to those found in Drosophila. These facts, in connection with the general similarity in effects of A-hormone and the Drosophila substances and the similarities in solubility properties,[9,12,13] suggest that the A-hormone might well be identical with either v^+ or cn^+ substance. Appropriate tests of the red eyed mutant of Ephestia, not available to the authors, should show with which, if either, of the Drosophila substances the A-hormone might be identical.

Tests Involving Habrobracon.—It has been shown that in certain types of double nucleus mosaics in Habrobracon the eye color characters orange and ivory (differentiated by the genes o and o^i, members of an allelic series) are non-autonomous.[3] There appears to be a diffusion of some substance from orange-plus (o^+) to orange (o) or ivory (o^i) tissue such that orange or ivory eye tissue is modified in the direction of wild type (black). If this assumed substance is identical with one or the other of the eye color substances known in Drosophila, it should be possible to demonstrate the presence of the substance in wild type Habrobracon by tests made on Drosophila. It should likewise be possible to demonstrate that the substance concerned is either absent or reduced in amount in orange or ivory Habrobracon.

Such tests have been made following the methods outlined in a previous section of this paper. The results are summarized in table 1. Extracts of wild type wasps are positive for both substances. Both orange and ivory wasps gave extracts positive for v^+ substance but negative for cn^+ substance. These results suggest that the substance concerned in the differentiation of orange and ivory from wild type is similar to, or identical with, cn^+ substance of Drosophila. Reciprocal tests, extracts of Drosophila

pupae injected into Habrobracon larvae or pupae, show that wild type Drosophila pupae contain a substance capable of modifying ivory eye color toward wild type. Furthermore, an extract of cinnabar pupae, known from previous experience[9] to contain v^+ substance but not cn^+ substance, was without apparent effect on the eye color of ivory Habrobracon pupae. A parallel test of the same extract of cinnabar pupae was made in vermilion brown larvae to determine whether v^+ substance was actually present in the particular extract used in Habrobracon; the test was positive.

Although the proof cannot be considered to be complete, the above facts do provide a reasonable basis for postulating that the substance deficient in orange or ivory wasps is the same as that deficient in cinnabar flies.

Three additional eye color types of Habrobracon have been tested for both v^+ and cn^+ substances. Red and cantaloup give tests for both substances as strong or stronger than wild type (table 1). Assuming that the indicated differences in the amount of substances are significant, it is suggested that the relation between pigment formation and utilization of diffusible substances found by Ephrussi and Chevais[14] in Drosophila might account for them. Extracts of white Habrobracon larvae and pupae give positive tests for v^+ substances but are very low in activity in tests for cn^+ substance. In fact, cn^+ substance tests of white wasps are so weak that it is only by the most careful comparison with control test animals that a modification can be detected. On the basis of these tests alone, one would hesitate to differentiate between white and ivory. However, it is known from studies of mosaics that the white character is autonomous in development and in addition that white tissue in such mosaics is capable of producing and releasing a substance that modifies orange eye tissue in the same apparent manner as does cn^+ substance.[4]

Discussion.—The host-parasite relations between Ephestia and Habrobracon are of interest in relation to the eye color substances. Ephestia larvae contain both v^+ and cn^+ substances. It is known that both of these substances can be effectively administered to Drosophila by feeding.[10] Furthermore, it has been shown that cn^+ substance (from Drosophila) will produce a modification in genetically ivory wasps when injected into larvae. Why does an orange or ivory Habrobracon larva, feeding on an Ephestia host, not obtain sufficient cn^+ substance to modify its eye color; in other words, how, under these conditions, can there be an orange or ivory character in Habrobracon? Several explanations are possible; for example, it is possible that cn^+ substance is not present in the blood of paralyzed Ephestia larvae or that it is not effective when taken in with food material as it is in Drosophila. Another possibility, and one that we consider more probable, is as follows: It has been postulated that both v^+ and cn^+ substances are inactivated in air by enzymic oxidation.[9] If this is correct, the enzyme system concerned is present in Drosophila larvae and pupae.

It is probable, then, that this system is likewise present in Ephestia larvae. The parasitic Habrobracon larvae, according to this view, removes both cn^+ substance and the enzyme system from the host and it is at least possible that under these conditions the cn^+ substance obtained is inactivated in the intestine of the wasp larva.

Among more than twenty-five eye color characters studied in *Drosophila melanogaster*, differentiated from wild type by as many non-allelic genes, only one is both non-autonomous and differentiated from wild type by a marked deficiency (or absence) of cn^+ substance.[15] Among the known eye color characters in Habrobracon, only the orange series is similarly non-autonomous and characterized by a deficiency or absence of a diffusible substance that gives the same reaction in Drosophila as does cn^+ substance. We are therefore tempted to suggest that the cn^+ and o^+ genes are homologous and that their mutant alleles represent parallel mutations. Similar arguments, though having a less substantial experimental basis, would suggest that the a^+ (A) gene in Ephestia is homologous with either the v^+ or the cn^+ gene in Drosophila. A more nearly adequate knowledge than we now have of the chemical processes involved in eye color development in these insects should provide a more satisfactory basis for these and similar inferences.

NOTE: After the manuscript of the present paper was submitted for publication, a paper appeared (E. Becker and E. Plagge, *Naturwiss.*, **25**, 809 (1937)) in which it is shown that the red eyed mutant of Ephestia (*a*) is deficient in cn^+ substance (tested in Drosophila) and that wild type and cinnabar Drosophila contain a substance that modifies the red eyed mutant of Ephestia toward wild type. Although the direct test of the *a* mutant of Ephestia for v^+ substance remains to be made, it appears probable from the evidence now available that the a^+ (A) gene in Ephestia and the v^+ gene in Drosophila are homologous and that the recessive alleles (*a* and *v*) of these genes represent parallel mutations.

* That part of this work carried out at Stanford University was aided by funds granted by the Rockefeller Foundation.

[1] E. Caspari, *Arch. Entw.-mech.*, **130**, 353–381 (1933); A. Kühn, E. Caspari and E. Plagge, *Nachr. Ges. Wiss. Göttingen*, **2**, 1–29 (1935).

[2] H. Kikkawa, *Zool. Mag.* (Japan), **49**, 348–353 (1937).

[3] A. H. Sturtevant, *Proc. Sixth Int. Cong. Genet.*, **1**, 304–307 (1932); B. Ephrussi and G. W. Beadle, *Bull. Biol. Fr. Belg.*, **71**, 54–74 (1937); G. W. Beadle and B. Ephrussi, *Genetics*, **22**, 76–86 (1937).

[4] P. W. Whiting, *Biol. Bull.*, **63**, 296–309 (1932); P. W. Whiting and A. R. Whiting, *Jour. Genet.*, **29**, 311–316 (1934).

[5] B. Ephrussi and M. H. Harnly, *C. R. Acad. Sci., Paris*, **203**, 1028 (1936).

[6] E. Plagge, *Nachr. Ges. Wiss. Göttingen*, **2**, 251–256 (1936).

[7] Experiments as yet unpublished suggest that the so-called "ca^+ substance" of Drosophila[15] does not exist as a distinct substance but that what may be called the "claret effect" is one aspect of the action of either v^+ or cn^+ substance.

[8] B. Ephrussi, *Amer. Nat.* (in press).

[9] K. V. Thimann and G. W. Beadle, these PROCEEDINGS, **23**, 143–146 (1937).

[10] G. W. Beadle and L. W. Law, *Proc. Soc. Exptl. Biol. Med.* (in press).

[11] B. Ephrussi and G. W. Beadle, *Bull. Biol. Fr. Belg.*, **69**, 492–502 (1935). Also unpublished results.

[12] Y. Khouvine, B. Ephrussi, and M. H. Harnly, *C. R. Acad. Sci. Paris*, **203**, 1542 (1936).

[13] E. Becker, *Naturwiss.*, **25**, 507 (1937).

[14] B. Ephrussi and S. Chevais, these PROCEEDINGS, **23**, 428–434 (1937).

[15] G. W. Beadle and B. Ephrussi, *Genetics*, **21**, 225–247 (1936).

THE EFFECT OF pH ON THE DEVELOPMENT OF ULTRA-CENTRIFUGED FUCUS EGGS[1]

BY D. M. WHITAKER

SCHOOL OF BIOLOGICAL SCIENCES, STANFORD UNIVERSITY

Communicated December 19, 1937

It has been shown (Whitaker[2]) that the developmental polarity of fertilized eggs of *Fucus furcatus* is determined by centrifuging at 3000 x g. for 15 or 20 minutes. When the stratified eggs are reared in the dark in normal sea water (pH 7.9–8.0), more than 99% form rhizoids on the centrifugal halves of the eggs, and 93–99% do so within 10° of the centrifugal pole.

Beams[3] ultra-centrifuged fertilized eggs of *Fucus serratus* at 150,000 x g. for half an hour and found the polarity to be unaffected by stratification of the visible cell inclusions. It is possible but not probable that the difference lies in the two species of *Fucus*. It also appeared possible that the effects of centrifuging at 3000 x g. might be lost at 150,000 x g. Might some substance or structure be moved at 3000 x g. but be broken down at 150,000 x g.? Thousands of *F. furcatus* eggs were ultra-centrifuged in quartz centrifuge tubes at various forces including 150,000 x g. and 200,000 x g. for various durations, including 5 minutes and half an hour. The eggs were grown in normal sea water (pH 7.8–8.1) in the dark in a constant temperature room at 15°C. in thin cultures to avoid group effect (Whitaker[4]). Invariably 99% formed rhizoids on the centrifugal halves of the eggs, about 90% or more doing so within 10° of the centrifugal pole. The results are therefore essentially the same at 150,000 x g. and at 3000 x g., except that at the higher force 5 minutes of centrifuging is more than adequate to sharply stratify the eggs and determine polarity.

A number of environmental factors affect the determination of polarity in the *Fucus* egg.[2,4] When ultra-centrifuged eggs of *F. furcatus* are illuminated from one side during development, the polarity is affected by the direction of the light as well as by the stratification[5] so that in a population rhizoids are observed in all positions with respect to the stratification. The polarity of ultra-centrifuged eggs is also altered by the group effect, if