produce melanin has been successfully grown in vitro.

The present culture (RPMI No. 1846) was derived from a spontaneous melanotic tumor which had been carried for 41 animal passages. It was transplanted into several noninbred hamsters in our laboratory in June 1961. On 2 February 1962 biopsy material from these transplants was minced, treated with trypsin, and cultured. The suspension of cells and cell clumps was cultured under perforated cellophane sheets in T-15 flasks. Successful growth was attained in only one flask, which contained RPMI medium No. 651 supplemented with 1 percent fetal calf serum. The original outgrowth was subcultured 33 days later and at 6-day intervals thereafter. The subcultures have been grown in a variety of media supplemented with 20 percent calf serum. All of the subcultures contained some cells with pigment granules. Cells of transfer generations 4, 5, and 7 produced coal-black tumors when reinjected back into hamsters. Cultures containing lactalbumin hydrolysate in the media became very black and the cells were heavily pigmented. These cultured cells with heavier pigmentation did not grow as fast as those in other media.

The chromosome constitution of the melanotic melanoma is shown in Table 1. The melanoma was examined on 2 April and 18 April 1962. On both days the modal chromosome number was found to be 68, with a wide distribution around the mode (range 42 to 73). This contrasts with the sharp mode of 44 chromosomes counted in cultured normal cells of the golden hamster (*8*). In addition, the mode of 68 chromosomes in the melanotic melanoma differs from that described for an amelanotic tumor and a pituitary carcinoma of the golden hamster (*8*).

Karyotyping was performed on 22 plates from cells with a chromosome number ranging from 64 to 71. No unusual marker chromosomes were observed. In all of the plates, 3 or 4 X chromosomes were easily identifiable, and 43 to 49 chromosomes belonged to groups A, B, and C (normal 30 or 31), 9 or 10 to group D (normal 8), 3 to 5 to group E (normal 2), and 3 to 5 to group F (normal 2). It appears that the hyperploidy of this particular cell line is due to an excessive number of chromosomes in all of the groups described for normal cells of the golden hamster (*8*).

This melanotic cell line derived from a malignant melanoma of the golden hamster has continued to produce melanin in vitro. Whether or not it will continue to produce pigment permanently cannot be determined at this time. Nevertheless, this cell line will produce melanin for a prolonged period in vitro and, therefore, should provide a unique opportunity for studying melanin synthesis (*9*).

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## Factors Causing Seasonal Forms in Ascia monuste (Lepidoptera)

*Abstract.* Light effects will produce the seasonal forms of *Ascia monuste* L. but they are not yet proved to be the only causative agent. The long-day form is melanic; the short-day form is white. Only females exhibit the effect. The capacity to form melanic females is most frequent in the Florida population and is probably genetically determined.

Increased recognition is being given to photoperiod as a factor inducing diapause and determining environmental forms in insects (*1*). Experiments with the European butterfly *Araschnia levana* L., for example, are interpreted as indicating that day length is responsible for the striking difference between the spring and summer forms. (*2*).

Before selecting one lepidopteron for experiment, five species with suspected seasonal forms were raised under the two conditions of 16 hours light alternating with 8 hours dark (condition 1, 16L/8D) and 8 hours light alternating with 16 hours dark (condition 2, 8L/16D) in each 24-hour period. The five were *Precis zonalis* Felder, *Precis lavinia* Cr., *Phyciodes phaon* Edw. (Nymphalidae), *Eurema daira* Latreille, and *Ascia monuste* L. (Pieridae). The last was chosen for a further experiment since it gave the clearest phenotypic difference and was easiest to breed. It can be raised from egg to adult in less than a month on young cabbage or other Cruciferae (*3*).

The dimorphism of the Great Southern White butterfly, *Ascia monuste* L., has been attributed to environment (*4*). This species is unusual in that the female alone displays a conspicuous dimorphism. One form is white with narrow black borders on the wings, and the other, form *phileta*, is more or less completely suffused with melanic pigment. Intermediates occur.

Five white females of *Ascia* were collected in December 1960, at the Florida State Board of Health Entomological Research Center at Vero Beach. Eggs were obtained by enclosure of females in lamp chimneys over the food plant *Batis maritima* L. under electric lights. Larvae were raised on young cauliflower plants, since *Batis* is restricted to the coastal salt marshes far from the Archbold Station where the rearing was carried out.

The two light regimes were used. Room temperature was maintained at 80°F. The experiment was performed in the temperature-light control room at the Archbold Biological Station in Lake Placid, Florida. Since only one room was available, a special light-tight box, 13 by 49 inches by 17 inches high (inside dimensions), was constructed for 8L/16D conditions and placed in the chamber. It was equipped with a single fluorescent tube (General Electric F40T124500), baffles, and a fan to give a steady air exchange without admitting light. The room was lighted by a bank of fluorescent tubes containing 15 cool white General Electric F96T12CW and five daylight General Electric F96T12D fluorescent tubes.

Eggs from each female were kept separate and distributed seven to a rearing container. The containers were arranged in pairs housing progeny from the same parent. One of each pair was subjected to 8 hours of light and 16 hours of dark, and the other was subjected to 16 hours of light and 8 hours of dark. Eight pairs ($8 \times 14 = 112$ ova, or 56 under each light condition) were used. Offspring from each of the five females were used in at least one of the pairs.

The result was unequivocal. Of the

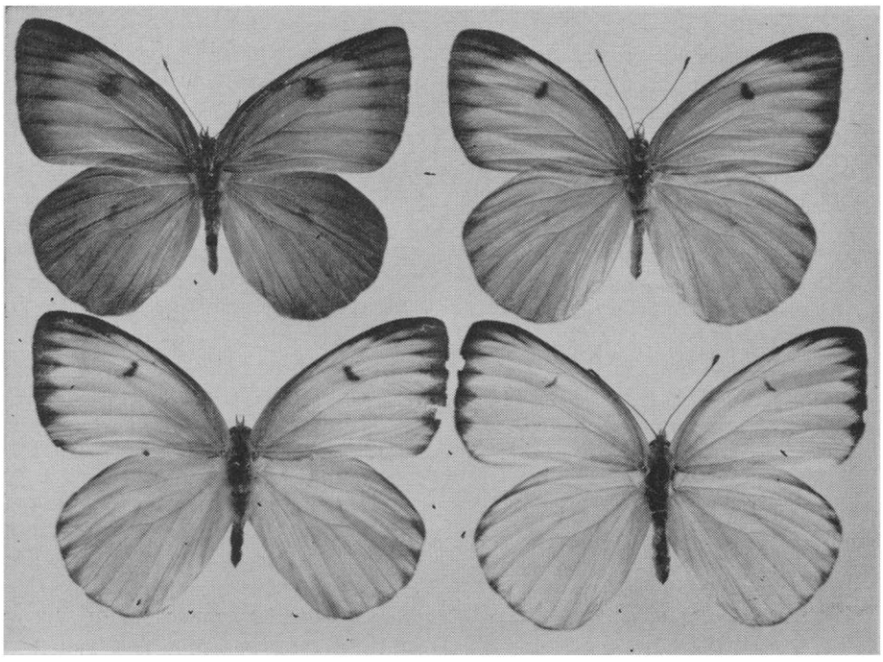

Fig. 1. Variation in pigmentation of female *Ascia monuste*. (Top row) black (16 hours light) and gray (16 hours light). (Bottom row) light gray on left (16 hours light) and white on right (8 hours light).

butterflies that were produced, all 16L/-8D females were black (nine individuals), gray (five), or light gray (three), while all 8L/16D females (16 individuals) were white (Fig. 1). (All males—28 under 16L/8D and 16 under 8L/-16D—were white.) Eight of the nine black females were offspring of female XI and have a violet cast which the remaining female lacks. Segregation for two categories occurred among offspring of two parents. The black, gray, and light gray categories intergrade.

Not all of each seven-egg lot survived to produce pupas. Crowding is apparently not a factor in this experiment, since white females emerged from lots with seven, five, four, and one larvae surviving, while black females emerged from lots with seven, six, and four survivors.

A qualification must be made in evaluating the experiment. The light unit in the 8L/16D box caused the daytime temperature to reach a peak of 87°F even with the fan and system of baffles. A higher temperature was thus associated with the shorter day. In the field, darker females are frequent in the spring, summer, and fall, but some white females are present during these seasons. A sample of 99 females taken at Jupiter Island, Florida, on 30 July 1961 by Thomas Pliske of Amherst College included 56 melanics, 33 intermediates, and 10 whites. White females are the only type found from November to February (*5*). Since long day and high temperature are associated with the same effect under natural conditions as long day and lower temperature in the laboratory, day length seems a more likely cause of the response than temperature.

A single experiment with only two alternative conditions will not establish whether photoperiod, intensity of light, or total amount of light per day is decisive in causing dark and white forms. Therefore, the contribution of each of these factors cannot be separated from the others on the basis of this experiment.

Dark females are not restricted to Florida, but they are more common there at the proper time than in any other area (*6*). South American populations do not have the dusky females. The environmental conditions required for production of the dark form certainly should occur in the Southern as well as the Northern Hemisphere. It is suggested that the South American subspecies lacks the capacity to produce it.

A hypothesis explaining both the experimental results and the occurrence of melanics in Florida is the following. The capacity to produce melanic females in response to the proper environmental stimulus is conferred by a gene or genes. The gene frequency is high in Florida but not at fixation, since a few white females occur at all times. Light is implicated as a factor stimulating the dimorphism, but the experiment does not exclude the possibility that other factors such as starvation, crowding, or condition of food plant may be important, although they are not relevant to the described experiment. Thus, the question may be asked whether the dark pigment is laid down in response to a single factor (an environmental induction) or in response to any factor which influences development sufficiently.

The adaptive significance of the dark and white forms remains obscure. The critical stage in development during which the organism is most sensitive to light effects is not known. However, since the adult wing pigment is laid down in the pupa, this stage is suspect (*7*).

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## Photoautotrophy in Gymnodinium breve Davis

*Abstract.* Pure cultures of the Florida "red-tide" flagellate required light and carbon dioxide for growth. Multiplication in darkness was not supported by any of a number of organic compounds and mixtures. The ecological importance of micronutrients is suggested.

The catastrophic mortalities of marine animals associated with dense populations of the dinoflagellate, *Gymnodinium breve* Davis, have occasioned considerable interest in the biological requirements of this organism (*1*). Sev-