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Difference of Cuticular Surface between Diapause and Non-Diapause Pupae of *Pieris rapae crucivora* (Lepidoptera:Pieridae)[1]

Adult development of *Pieris rapae* is triggered by the neurosecretion from the brain-corpus cardiacum system at the larval-pupal ecdysis in non-diapause individuals (KONO, 1973a). The white colour of the wing scales of developed adult becomes observable through the cuticle 4 days after pupation when incubated at 20°C (KONO, 1970). If the neurosecretion does not occur at the pupal ecdysis, pupae stop their adult development by the 2nd day after pupation and enter diapause (KONO, 1971). Therefore, the distinction between diapuase and non-diapause pupae used to be made by noting whether the white colour appeared in the wing pads within 4 days after pupation or not. However, the author's experience has been that the surface of pupal cuticle is somehow glossier in diapuase pupae than in non-diapause ones. In the present paper, the surface of pupal cuticle was observed by a scanning electron microscope in order to recognize the difference in the cuticular surface between diapause and non-diapause pupae.

MATERIALS AND METHODS

When the larvae of *Pieris rapae* are reared under 10L-14D photoperiod at 20°C, all the resulting pupae diapause, while under 10L-2D-2L-10D photoperiod at 20°C, all insects proceed to adult development. Therefore, insects reared under 10L-14D cycle and 10L-2D-2L-10D cycle are regarded as diapause and non-diapause pupae, respectively.

A square of cuticle (about 4×4 mm) was cut off from the first day pupal wing pad of both diapause and non-diapause pupae. It was coated with layers of evaporated carbone and gold, and then observed by a JSM-U3 scanning electron microscope.

Negative replica of the cuticular surface was made using a 10% gelatine solution as the casting materials. By this method, the replica could be made without killing the pupa. This was also observed through light and electron microscopes.

RESULTS AND DISCUSSION

Both diapause and non-diapause pupae had the same ripple shaped wrinkles on the cuticle. At low magnification, the surface of the cuticle of a non-diapause pupa appeared to be jagged (Fig. 1a), while that of the diapause one was smooth in appearance (Fig. 1c). Higher magnified electron micrograph (Fig. 1b) showed that the roughness on the surface of the non-diapause pupae was due to the many needle like projections along the wrinkles.

When negative replicas were observed by a light microscope, light interferences occurred along the wrinkles in the non-diapause pupae, but not in the diapause pupae. However, it was not easier to distinguish the non-diapause from the diapause by light microscopic observations of the negative replica than with the naked eye.

Scanning electron micrograph of the replica of the non-diapause pupae showed many indentations which were obviously the negative replia cations of small projections as in Fig. 1d.

These observations suggest the capability of distinction between diapause and non-diapause at early pupal stage by observing the cuticular surfaces.

In *Pieris rapae*, larval instar duration, pupal weight, and diurnal behaviours of the old larvae were different between diapause and non-diapause individuals (KONO, 1970; KONO, unpublished data).

Also in *Pieris brassicae*, pupal weight, glycogen and protein contents in fat body, and haemolymph protein of larvae were different between both individuals (CLARET, 1968). These differences were suggested to be influenced by the different endocrine activities entrained by photoperiods

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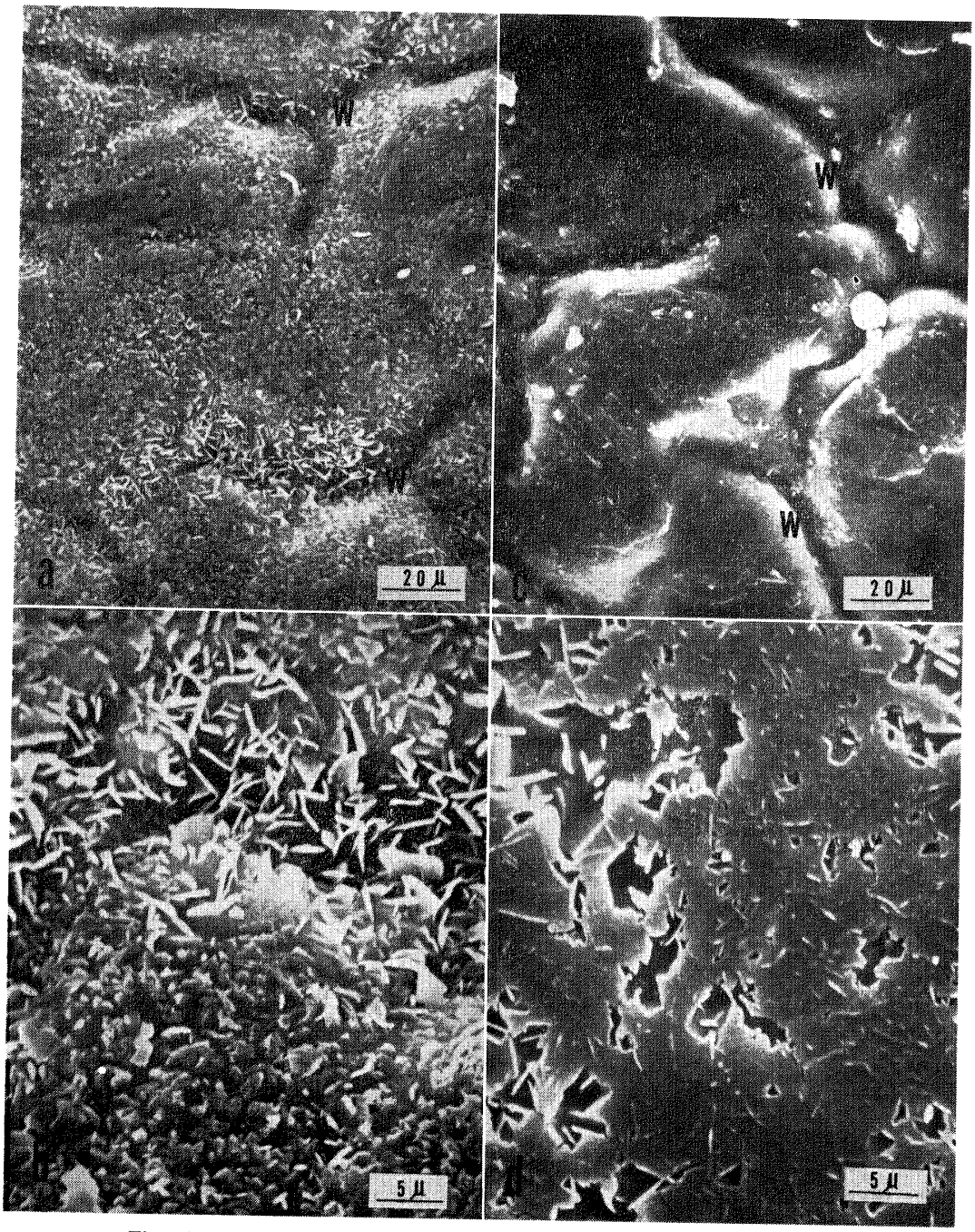

Fig. 1. a: Scanning electron micrograph of the cuticular surface of a non-diapause pupal wing pad. b: Enlargement of a. c: Scanning electron micrograph of the cuticular surface of a diapause pupal wing pad. d: Scanning electron micrograph of the negative replica of a non-diapause cuticular surface. w, wrinkle.

(Claret, 1968; Kono, 1973b). In fact, fine structures of neurosecretory IV cells in the brain showed different appearances between the larvae under long day and the larvae under short day periods (Kono, 1973a). Therefore, pupal cuticle construction may also be regulated by the neurosecretory activity in the brain.

There have been few reports on morphological

differences between diapause and non-diapause pupae except in the rice leaf miner, *Agromyza orizae* (KUWAYAMA and NISHIJIMA, 1947). But differences of cuticle construction between diapause and non-diapause pupae must be found in many insect species because the particular construction of cuticle was thought to be necessary for suppressing water loss during the long duration of diapause.

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