Orientational responses of last (fifth) instar larvae of *Papilio demoleus* to different coloured solutions[a]

Dye solutions[b]	Colour	Spectral region (nm) for maximum light transmission	Larvae turning/moving (%)[g] (mean ± SE) S	B	Orientational preference[h]
Methylene blue	blue	430–450	22 ± 8.6	36 ± 6.0	− 14
Methylene blue + auramin O (4:1, v/v)	bluish-green[c]	440–460	32 ± 3.7	20 ± 5.5	− 12
Methylene blue + auramin O (3:2, v/v)	greenish-blue[d]	470–490	46 ± 8.1	22 ± 2.0	24
Methylene blue + auramin O (5:5, v/v)	green	480–510	46 ± 8.1	14 ± 4.0	32
Methylene blue + auramin O (2:3, v/v	greenish-yellow[e]	490–520	70 ± 6.3	12 ± 2.0	58
Methylene blue + auramin O (1:4, v/v)	yellowish-green[f]	500–530	80 ± 3.2	4 ± 2.5	76
Auramin O	yellow	530–650	92 ± 3.7	4 ± 2.5	88
Auramin O + acridin red (1:1, v/v)	orange	600–650	64 ± 4.0	22 ± 3.7	42
Acridin red	red	620–650	12 ± 3.7	42 ± 2.0	− 30

[a] Each solution was presented in a rectangular glass trough (75 × 20 × 5 mm) on one side of the larvae on the grid. [b] Each dye was used as 0.1% aqueous solution. [c] The colour was more bluish and less greenish. [d] The colour was more greenish and less bluish. [e] The colour was more greenish and less yellowish. [f] The colour was more yellowish and less greenish. [g] The remaining percentages of larvae moved forward. [h] Calculated as the difference (S–B). S, B, percentages of the larvae turning towards the stimulus source (coloured solution) and the opposite blank side respectively.

in a slight repulsion of the larvae by the coloured solutions viz., bluish-green, blue. Even a rise in the wavelength for maximum light transmission above that for the yellow solution resulted in a decrease in the orientational preference to 42% for the orange solution. A further rise caused the larval repulsion by the red solution.

These observations show that *P. demoleus* larvae can discriminate between different colours, some of which attract and others repel the insects. Certain other lepidopterous larvae have also been reported to show differences in their orientational responses to different colours[2,3]. According to Götz[2], a comparison of the responses of *Vanessa io* larvae to white, green, brown and black revealed their maximum attraction to green when the larvae were young and hungry, and to brown or black when they were about to pupate. Hundertmark[3] studied the orientational responses of *Lymantria monacha* larvae to blue, yellow, bluish-green and red colours, each of which was given as a choice against 2–4 other columns of different shades of grey and black. His experiments showed larval discrimination of these 4 colours relative to the shades of grey and black but not relative to one another. Nevertheless, Hundertmark[3] concluded that *L. monacha* larvae could distinguish blue, yellow and bluish-green colours from grey or black, and red was indistinguishable from black. The larvae of *Bombyx mori* were also shown by electrophysiological techniques to distinguish different colours, their spectral sensitivity curves showing one peak in the near UV and another hump or peak in the blue-green region of the spectrum[5]. Thus, these reports give information on the ability of the lepidopterous larvae to distinguish only a few different colours. In this respect, the present work has advanced our knowledge by showing that a lepidopterous larva, e.g., that of *P. demoleus*, cannot only distinguish but also show positive or negative taxis to several different colours ranging from blue to red in the visible region of the light spectrum.

Voltage variation in *Lilium longiflorum* pistils induced by pollination

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Summary. Voltage variations in *Lilium longiflorum* pistils arose 120 min after pollination with pollen of the same flower. No action potential was registered if pollen of *Petunia hybrida* was placed on the stigma. Apparently the voltage variations were evoked by the germination of the pollen grains, and/or the penetration of the style by the pollen tubes.

After pollination, the ovaries of many plants show metabolic changes before the pollen tube reaches the ovules[1–3]. Apparently, the ovaries receive an early signal containing information about the germination of the pollen or the interaction between the pollen tubes and the transmitting tissue in the style. The study reported here indicates that pollination induces an electrophysiological signal.

About 24 h after anthesis, a microelectrode was inserted into the pistils of *Lilium longiflorum* cv. Mount Everest (figure 1). Voltage variations were recorded monophasically on a FM tape recorder for about 6 h after microelectrode insertion. Some erratic electrical activity was observed for less than 30 min after microelectrode insertion. Pollinations with incompatible pollen from the same flower were made at various periods after microelectrode insertion. The voltage variation observed was quite distinct with a duration time from 30 to 65 sec and an amplitude of from −2 to −6 mV (figure 2). The

Time of appearance of the voltage variation after pollination at different time intervals after microelectrode insertion

No.	Time of pollination after insertion of the microelectrode (min)	Time of appearance of the voltage variation after pollination (min)
1	30	172
2	30	112
3	30	15
4	31	162
5	144	126
6	164	32
7	183	145
8	106	162
9	150	133

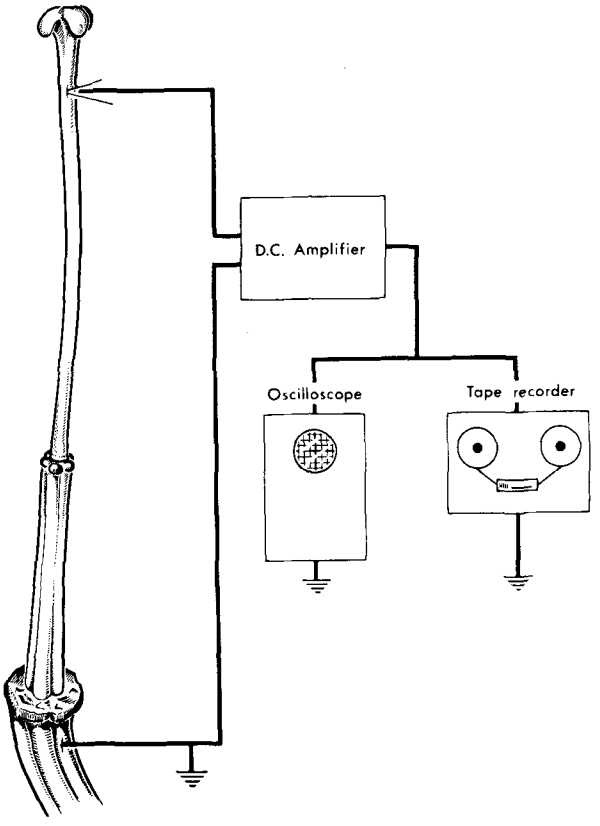

Fig. 1. Diagram of the connections of the measuring devices to the pistil. 1 microelectrode of a DC amplifier was inserted into the style in or in the vicinity of a vein[4]. The tip diameter was less than 2 µm. The pipettes were made of glass and filled with 3 M potassium chloride. Chlorinated silver wires were used as electrodes[5].

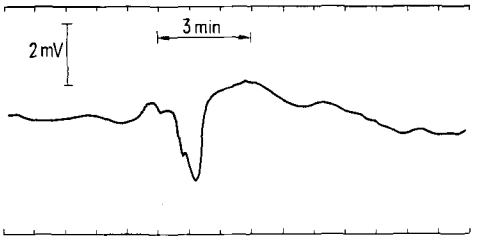

Fig. 2. A typical voltage variation in the pistils of *Lilium longiflorum*.

variation in amplitude was apparently related to the distance between the microelectrode tip and cell(s) transmitting or producing the voltage variation.

The relationship between pollination and the time of appearance of the voltage variation is presented in the table. When the pollinations were made about 30 min after microelectrode insertion, the voltage variation appeared on the average about 120 min after pollination. When the pollinations were considerably delayed relative to microelectrode insertion, the voltage variation appeared on the average about 120 min after pollination. Apparently, the germination and/or tube penetration of the self-incompatible pollen grains are responsible for the voltage variation detected about 120 min after pollination.

To determine if the germination and/or tube penetration of the *L. longiflorum* pollen grains were reponsible, pollen grains of *Petunia hybrida* (clone W166H), which do not germinate on *L. longiflorum* stigmas, were placed on *L. longiflorum* stigmas. No voltage variations were registered within 8 h after pollination indicating that in order to obtain the voltage variation, germination and/or tube penetrations are necessary.

The duration and appearance time of the voltage variation found in this study have not been reported previously. Other workers[6–9] have observed action potentials appearing less than 30 min after physical and chemical shocks. Action potentials measured in the pistils of *Lilium martagnon*[10] and *Zea mays*[11] persist for only a few sec and occur less than 30 min after pollination.

The biological mechanism associated with the voltage variation has not been established. Possibly, a change in membrane permeability, resulting from the pollen-stigma interaction or the penetration of the style by the pollen tube, could be responsible.

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