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Wing pattern variation in diurnal butterflies received by experimental research, with special reference to intrapupae injections

(Lepidoptera, Rhopalocera)

by

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Changes in the wing patterns of lepidoptera which take place in mature are the result of outer (environmental) as well as inner factors. The latter are mainly genetical. They form important material for genetical, taxonomic, morphological, zoogeographical and ecological research. Specimens of butterflies with abnormal wing pattern occur with variable frequency, but they are as a rule rare. Especially extreme wing pattern changes take place very rarely under natural conditions. Experimental research showed that wing pattern changes occurring in some butterfly species take place following the action of external stimuli i. e. temperatures between –20 °C and +42 °C (STANDFUSS, 1896), ionising radiation or vapours of such substances as sulphuric ether or chloroform (SCHUMANN, 1925) are the best known methods. In 1936 ZAĆWILICHOWSKI worked out the technique of intrapupal injections.

Quantitative and qualitative changes in the wing pattern of a butterfly which result from intrapupal injections depend on the following factors:

1) kind of chemical substance
2) dose
3) species
4) age of pupa
5) origin of the material investigated.

1) The action of each chemical substance which causes changes in the butterfly wing pattern is very specific. The following compounds cause changes in lepidoptera belonging to such genera as *Vanessa* F. or *Zygaena* F., and are similar to aberrations occurring in nature: phosphoro-wolframic acid, phosphoro-molybdenic acid, blue molybdenum oxides obtained through reduction of molybdenum compounds with ascorbic acid, orange compounds of molybdenum with pyrogallol etc. Other compounds, such as for example, solution of ammonium para-molybdate cause brightening of the orange-red groundcolour of the wings of *Aglais urticae* L. The injection of a colloid suspension of silver preparation (Electrocollargoll) results in complete falling out of the orange-red scales and a slight darkening of the dark pigments in the wing pattern.
RYMAR's experiments (1946–1958) proved the independent action of the individual compounds used in injections. If mixed, the changes obtained indicate the individual action of the substances used.
It is remarkable that many of the chemical substances tested, such as dyes, alkaloids, mineral salts, etc., do not cause modifications. Therefore, the introduction of any substance into

the pupal chemolymph is not necessarily connected with the active reaction of the organism resulting in creation of the wing pattern variations.

2) As far as size of a dose is concerned, there is a relationship between the amount of an active substance and the degree of changes in the wing pattern. As a rule, the changes in the wing pattern increase to the amount of the chemical substance used.

3) The reaction of the individual species to the external stimuli is very specific and diversified. Some species do not react at all to the chemical substances which cause considerable changes in the wing pattern of many other species. Such species as *Pieris brassicae* L., *P. napi* L., *Arctia caja* L., *Phalera bucephala* L., *Hyles euphorbiae* L., are species which are completely resistant. Other species, such as *Sphinx ligustri* L., *Papilio machaon* L., *Zygaena ephialtes* L. and *Z. purpuralis* BRÜNN. respond to the introduced substance only sporadically and with difficulty. It is possible that this phenomenon is caused by the metabolic processes of the individual species.
The difference in response to the same stimulus (either thermic or chemical) in species of the same genus (e.g. *Vanessa* or *Zygaena*) is a separate issue. It may be generally concluded that a given chemical substance causing changes in the pattern of the forewings as well as the hindwings of *Vanessa* and *Papilio* acts only on the forewing of species belonging to such genera as *Plusia* (= *Autograpa* HBN.) or *Zygaena*. Furthermore the substance causing wing pattern changes almost identical with those occurring in nature in species belonging to *Vanessa* or *Zygaena* (RYMAR, 1948; RYMAR & DĄBROWSKI, 1959; DĄBROWSKI, 1963, 1966, 1967) brings about changes not found under natural conditions in such species as *Plusia gamma* L. or *Papilio machaon* L.

4) The age of a pupa, that is the period from the moment of pupation, during which the external stimuli are most effective, is one of the decisive factors in causing wing pattern changes. The length of the period of greatest susceptibility varies with the species. In Nymphalidae it is 2 to 16 hours. During the subsequent period, between 16 and 24 hours after pupation, changes are very slight, while after 24 hours the same chemical substance does not cause any visible changes in the wing pattern.
In the case of *Plusia gamma* L. the greatest changes were observed during the period between 36 and 40 hours after pupation. In the case of specimens belonging to *Zygaena* the period of greatest susceptibility of a pupa begins, as a rule, four hours after pupation, and it is worth noting that *Z. angelicae* O. and *Z. filipendulae* L., injections applied to younger pupae, tend to decrease the size of the spots, while those applied to older pupae tend to diffuse the forewing spots.
The experimental determination of the susceptibility of pupae so far considered as resistant will perhaps allow us eventually to obtain variations in them, too.

5) The origin of the material is also quite important, especially in the case of those species which are characterised by considerable geographical as well as individual variation. This applies to species belonging to *Zygaena*, particularly *Z. carniolica* SCOP. Specimens originating from populations situated far away from each other and belonging to different subspecies show individual variation with respect to wing pattern changes brought about by pupal injections (DĄBROWSKI, 1977, 1982). This conclusion is somewhat similar to the one drawn by

BURGEFF (1956), who worked on pupae of such subspecies as *Z. carniolica rocci* BGFF., *Z. carniolica modesta* BGFF. and *Z. carniolica onobrychis* D. & S.

The main difficulty in this kind of experimental work is the high mortality of the material investigated, particularly in the case of high temperature when the mortality of pupae is as much as 90%. Also in the case of gaseous chemical substances the mortality is very high (SCHUMANN, 1925). Pupal mortality depends not only on the toxicity of a given substance, but also on the concentration of the solution used. To exceed the allowable concentration of the toxic preparations results in denaturation of the pupal haemolymph.
It may be generally assumed that the mortality of pupae increases with the size of the dose. This also applies to the number of specimens emerging with defectively developed wings. However the mortality may be reduced below 50% by working out appropriate methods and by maintaining sterile conditions. This makes the method of intrapupal injections superior to other methods.

The following are the results of injections applied to pupae of *P. machaon* L. 68 specimens showing diverse changes in the wing pattern (cf. colour plate XIII, figs. 1–8) emerged from 135 treated pupae (for terminology cf. figs. 1, 2). The changes assumed two basic trends:

1) Intensification of the wing pattern with a tendency towards melanism.
2) Reduction of some wing pattern elements.

The latter was sporadic, with a ratio of 1:25. In some specimens changes in hindwing colours took place. The greatest reductions occurred in a specimen which had no marginal bands and lacked a dark band on the forewing. The dark band on the hindwing is narrowed with partially reduced pigmentation.
Several specimens represent intermediate examples with a tendency towards melanism. They are characterized by complete disappearance of the oval and crescent cream-coloured spots in the background, which became absorbed by the black pigment of the wide, dark band on the forewings and hindwings. The band on the hindwings retained its fluorescent bluish colour, but the brown-red spot turned black. A similar specimen was collected in the vicinity of Kraków in 1947 The greatest melanistic changes occurred in a specimen shown on colour plate XIII, fig. 8). The dark band on the forewings is diffused towards the basal band. The cream-coloured spots of the background have disappeared leaving only traces. The dark band, diffused towards the base of the wing, invaded the median cell area, absorbing its last spot. The hind band retained only traces of blue colouration, while the brown-red spot has been greatly reduced. Another very interesting specimen has a considerable part of the forewing groundcolour absorbed by the black pigment of the dark band. The dark band of the hindwings has been covered by the blue scales, and the elongated crescent-shaped spots may be seen beneath them. The brown-red spot has also been covered by blue colouration. The edges of the wide dark band on the hindwings of another specimen have become rusty-brown, while the middle part of the band has been covered with violet scales. The pattern changes of the underside of the wings correspond to those of the upperside in all specimens. The basal band (B) and dividing, median and cubital veins of *P. machaon* L. did not change after injections. The basal band did not change at all while the veins were only covered up with the black pigment of a wide, dark band. Also the discoidal bands (D1 and D2) were quite

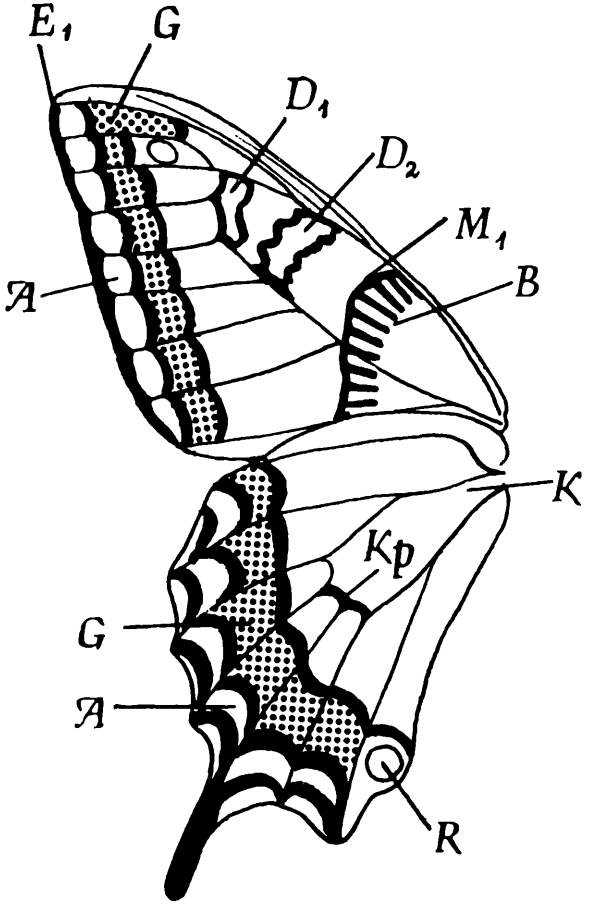

Fig. 1: pattern elements in *Papilio machaon*, underside.
E – marginal band; D – discoidal band; B – basal band; M – median band; K – median cell; Kp – end spot of median cell; G – dark band; R – brown-red spot; A – background spots (oval, crescent).

stable, and only in four cases they have joined each other, forming a single dark spot. The greatest lability on the other hand was expressed by the wide dark bands on the hindwings as well as on the forewings.

The scientific importance of wing pattern varieties obtained by the method discussed may be summarized as follows:

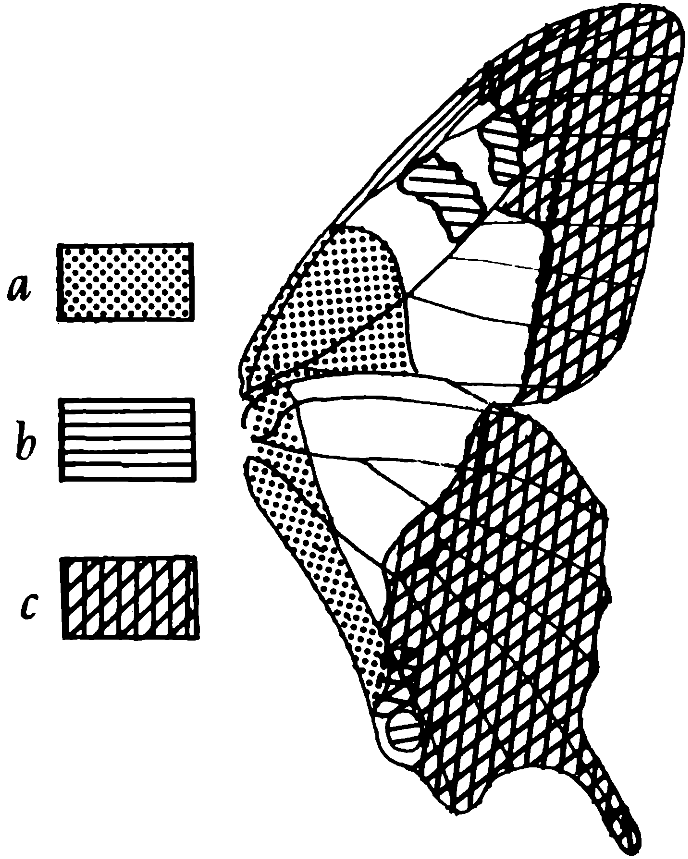

Fig. 2: changes in the pattern of *Papilio machaon*, underside.
a – area of no change; b – area of small change; c – area of great change.

a) In genetics – as an indicator of susceptibility towards the external stimuli modifying inherited characteristics. Also as comparative material in the investigations on the heredity of some wing pattern characteristics (DRYJA, 1959), and in the separation of the fixed characteristics from modifications occurring in nature through external stimuli.

b) In zoogeography – in research of the natural range of some subspecies and local forms, as a supplementary indicator of the direction of the variation, characteristic to a given unit.

Thanks to the possibility of obtaining forms which are very similar to some aberrations occurring under natural conditions it is possible to get a wide range of characteristic changes without long term collections in a given biotope.

c) In morphology – in research on phylogenesis of the wing pattern initiated by SCHWANWITSCH in 1923, and later developed by the same author (SCHWANWITSCH, 1956).

d) In physiology – in research on the action of chemical agents which are biologically active, and on the mechanism of development of wing pattern modifications.

In conclusion, the supplementary character of the method under discussion should be stressed. It is necessary to interpret the experimental results separately for each species.

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Explanation of colour plate XIII (p. 691):

Figs. 1–8: Wing pattern changes in *Papilio machaon* L. induced by intrapupal injection of 2% H_2WO_4

1	2
3	4
5	6
7	8

Fig. 1: Podgórki-Tyniec, V.1974, e.o., leg. W. DOBRANSKI.
Fig. 2: Podgórki-Tyniec, V.1973, e.o., leg. W. DOBRANSKI.
Fig. 3: Podgórki-Tyniec, V.1974, e.o., leg. W. DOBRANSKI.
Fig. 4: Podgórki-Tyniec, IV.1972, e.o., leg. W. DOBRANSKI.
Fig. 5: Podgórki-Tyniec, IV.1973, e.o., leg. W. DOBRANSKI.
Fig. 6: Podgórki-Tyniec, IV.1972, e.o., leg. W. DOBRANSKI.
Fig. 7: Podgórki-Tyniec, IV.1974, e.o., leg. W. DOBRANSKI.
Fig. 8: Podgórki-Tyniec, V.1974, e.o., leg. W. DOBRANSKI.

Explanation of colour plate XIX (p. 693):

Figs. 1–8: Wing pattern changes in various species of Rhopalocera and Zygaenidae induced by intrapupal injection of 2% H_2WO_4, unless otherwise stated

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Fig. 1: *Iphiclides podalirius*, Podgórki-Tyniec, VII.1955, e.o., leg. J. S. DĄBROWSKI.
Fig. 2: *Nymphalis antiopa* L., Żegocina ad Bochnia, VIII.1987, e.l., leg. J. S. DĄBROWSKI.
Fig. 3: *Aglais urticae* L., Kraków, VI.1957, e.l., leg. J. RYMAR & J. S. DĄBROWSKI; injection of Molybdan acid 2%.
Fig. 4: *Araschnia levana* L., Olsztyn, VII.1961, e.l., leg. J. S. DĄBROWSKI.
Fig. 5: *Zygaena carniolica* SCOP., Mydlniki ad Kraków, VII.1963, e.l., leg. J. S. DĄBROWSKI.
Fig. 6: *Zygaena carniolica* SCOP., Klonów ad Miechów, VII.1964, e.l., leg. J. S. DĄBROWSKI.
Fig. 7: *Zygaena carniolica* SCOP., Glanów ad Wolbrom, VII.1962, e.l., leg. J. S. DĄBROWSKI.
Fig. 8: *Zygaena angelicae* O., Klucze/Olkasz, VII.1964, e.l., leg. J. S. DĄBROWSKI.

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Colour plate XIII

DĄBROWSKI, J. S. & DOBRAŃSKI, W.: Wing pattern variation in diurnal butterflies received by experimental research, with special reference to intrapupae injections (Lepidoptera, Rhopalocera). – Atalanta **27**: 657–664.

Figs. 1–8: Wing pattern changes in *Papilio machaon* L. induced by intrapupal injection of 2% H_2WO_4
Fig. 1: Podgórki-Tyniec, V.1974, e.o., leg. W. DOBRANSKI.
Fig. 2: Podgórki-Tyniec, V.1973, e.o., leg. W. DOBRANSKI.
Fig. 3: Podgórki-Tyniec, V.1974, e.o., leg. W. DOBRANSKI.
Fig. 4: Podgórki-Tyniec, IV.1972, e.o., leg. W. DOBRANSKI.
Fig. 5: Podgórki-Tyniec, IV.1973, e.o., leg. W. DOBRANSKI.
Fig. 6: Podgórki-Tyniec, IV.1972, e.o., leg. W. DOBRANSKI.
Fig. 7: Podgórki-Tyniec, IV.1974, e.o., leg. W. DOBRANSKI.
Fig. 8: Podgórki-Tyniec, V.1974, e.o., leg. W. DOBRANSKI.

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Colour plate XIII

Colour plate XIV

DĄBROWSKI, J. S. & DOBRAŃSKI, W.: Wing pattern variation in diurnal butterflies received by experimental research, with special reference to intrapupae injections (Lepidoptera, Rhopalocera). – Atalanta **27**: 657–664.

Figs. 1–8: Wing pattern changes in various species of Rhopalocera and Zygaenidae induced by intrapupal injection of 2% H_2WO_4, unless otherwise stated

Fig. 1: *Iphiclides podalirius*, Podgórki-Tyniec, VII.1955, e.o., leg. J. S. DĄBROWSKI.

Fig. 2: *Nymphalis antiopa* L., Żegocina ad Bochnia, VIII.1987, e.l., leg. J. S. DĄBROWSKI.

Fig. 3: *Aglais urticae* L., Kraków, VI.1957, e.l., leg. J. RYMAR & J. S. DĄBROWSKI; injection of Molybdan acid 2%.

Fig. 4: *Araschnia levana* L., Olsztyn, VII.1961, e.l., leg. J. S. DĄBROWSKI.

Fig. 5: *Zygaena carniolica* SCOP., Mydlniki ad Kraków, VII.1963, e.l., leg. J. S. DĄBROWSKI.

Fig. 6: *Zygaena carniolica* SCOP., Klonów ad Miechów, VII.1964, e.l., leg. J. S. DĄBROWSKI.

Fig. 7: *Zygaena carniolica* SCOP., Glanów ad Wolbrom, VII.1962, e.l., leg. J. S. DĄBROWSKI.

Fig. 8: *Zygaena angelicae* O., Klucze/Olkasz, VII.1964, e.l., leg. J. S. DĄBROWSKI.

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Colour plate XIV