PHENOTYPIC WING PATTERN MODIFICATION BY VERY BRIEF PERIODS OF CHILLING OF PUPATING *ARICIA ARTAXERXES VANDALICA* (LYCAENIDAE). *ARICIA* STUDIES NO. 16

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Since Dorfmeister "in 1845" showed that divergent forms were developed when butterfly pupae were kept at increased or decreased temperature, many scientists have worked on this problem because the effect of temperature might explain some of the aberrant forms which now and then are found in natural populations. Some important papers on temperature-induced forms in Lepidoptera are referred to below that concern the age of the pupa at the time of cooling, the temperature used, and the duration of the treatment.

Merrifield (1893) and Standfuss (1896) independently found that pupae chilled immediately after pupation died or produced crippled but normal-patterned imagines. To obtain altered imagines the pupae were chilled not earlier than 12 hours (h) after pupation, when the sensitive period begins. The chilling must continue for at least 14 days. Süffert (1924), working on *Araschnia levana* L. and *Aglais urticae* L., found a sensitive period some days after pupation; there was no effect with earlier chilling. The cooling period was 10 days. Reinhardt (1969), who definitely solved the problems of *Araschnia*'s seasonal dimorphism, only conducted a few experiments with pupae as young as 0–6 h; the other experiments were from 6–36 h after pupation. Kühn (1926) also found no changes in Vanessinae if cooling took place immediately after pupation. The effect increased from 6–24 h after, but there was no effect after 72 h. The temperature used was low, from –3 to –10°C, for two days. Very exact experiments were conducted by Köhler & Feldotto (1935), who, working on Vanessinae, found the heat-sensitive period to be from 0–48 h after pupation. Every pattern element was found to have its special sensitive period. In these experiments only increased temperature, for short periods, was used. In most of these experiments only Nymphalidae were examined, but Merrifield also investigated Geometridae and Kühn also worked with certain moths.

Krodel (1904) performed experiments with three species of Lycaenidae: *Plebeius* (*Lycaena*) *argus* L., *Lysandra* (*Lycaena*) *coridon* Poda, and *Agrodiaetus* (*Lycaena*) *damon* Schiff. The pupae were never less than 5 h old; in the course of 6 days and nights they were exposed to 12

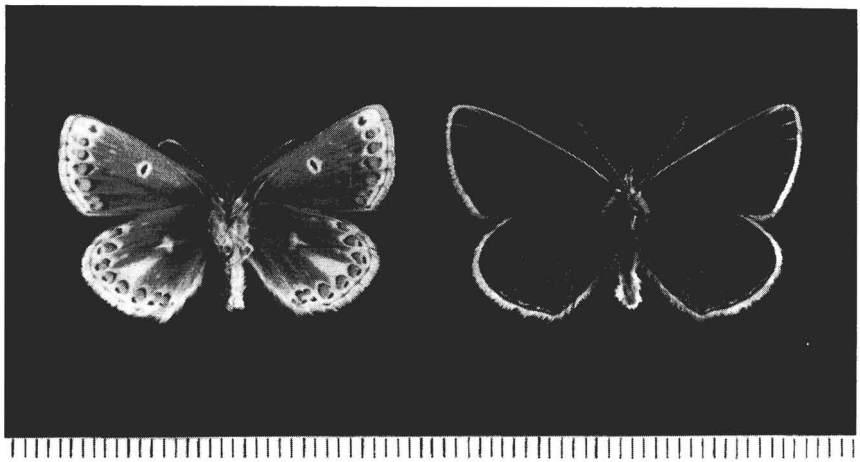

Fig. 1. Wild rare forms of *Aricia artaxerxes vandalica*: (left) ♀, underside, Tversted Strand, Jutland, 16 July 1961, spot value 3—f. "caeca"; (right) ♂, upperside, Tornby Strand, Jutland, 11 July 1960, f. "albicostalis" (= "albivenata").

cold spells each of 6 h duration, during which the temperature went down to –14°C. In this way Krodel produced numerous cold forms, partly corresponding to forms taken in the open, and he was of the opinion that these forms could be explained as ancient characters which for many generations have remained latent, but which reappear under certain circumstances, such as cold treatment. Lorkovic (1938) found obsolescence of eyespots by cooling pupae of *Polyommatus icarus* Rott. Høegh-Guldberg (1971) cooled pupae (1–24 h old) of the same species at +2 to +5°C for 4 weeks and observed many changed imagines. Lorkovic (1943) observed reduction of eyespots by cooling—for 3 weeks —"young" pupae of *Everes argiades* Pall.

Another genus of Lycaenidae is *Aricia*, and the following deals entirely with *A. artaxerxes* F. and *A. agestis* Schiff. These are two closely related species widely distributed in Europe. In both species, but especially in some *A. artaxerxes* populations, aberrant forms occur quite often. Two rare forms are depicted in Fig. 1, to be compared with the normal appearance, Figs. 2A & D. It is shown in Fig. 2D that the blackish upperside has marginal lunules most distinct on the hindwings, and without the white nervures at the tip of forewings which are seen in Fig. 1. The normal underside, Fig. 2A, has a complex system of eyespots when compared with the aberrant form in Fig. 1. This eyespot pattern varies gradually from that extreme; the degree can be expressed as "spot value," which registers the number of spots and centres on one forewing and hindwing, including all pupillations plus the sum of their centres. Maximum is 42:

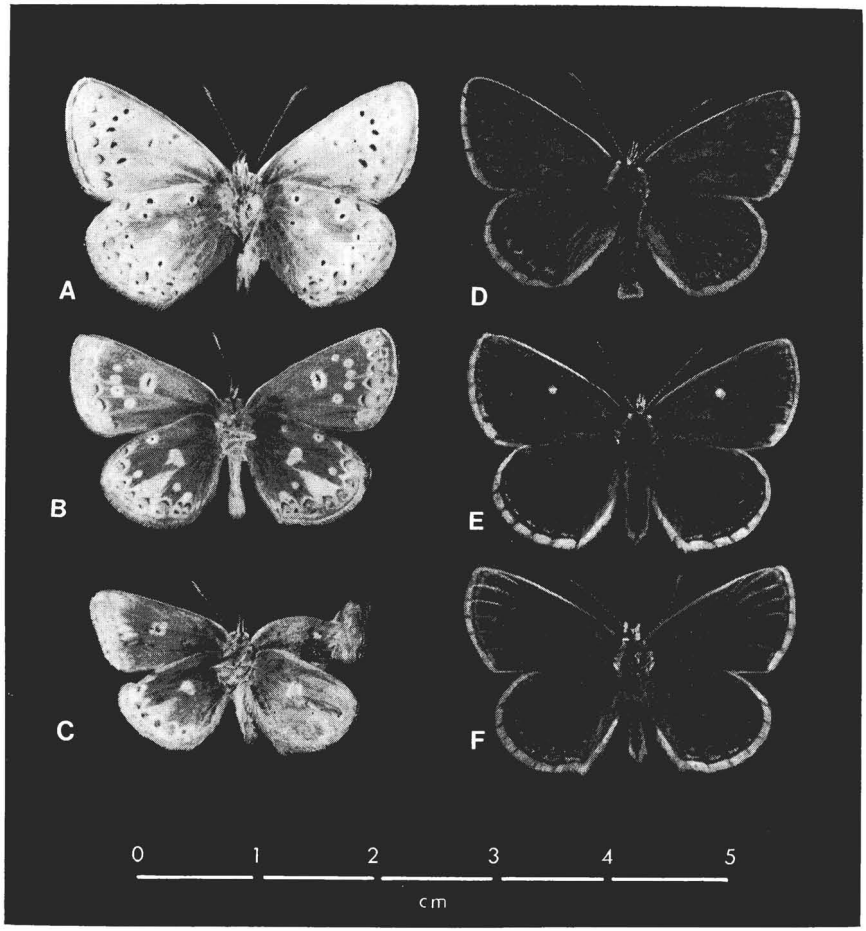

Fig. 2. Wild average forms and chilled specimens of *Aricia artaxerxes vandalica*. Undersides: (A) ♂ from nature (61,60), Tornby Strand, Jutland, 4 June 1961, spot value 25, normal form; (B) Pl ♀ (73,61), ex Tornby Strand, pupa 1 min, chilled 9 h, spot value 12; (C) Pl ♀ (73,125), ex Tornby Strand, pupa 15 min, chilled 2 "nights," spot value 3, f. "caeca." Uppersides: (D) ♂ from nature (66,53), Tversted Strand, Jutland, 6 July 1966, no white elements, normal form; (E) Pl ♀ (73,59), ex Tornby Strand, pupa 1 min, chilled 2 "nights," f. "snelleni," f. "panalbisignata"; (F) Pl ♀ (73,62), ex Tornby Strand, pupa 1 min, chilled 9 h, f. "albicostalis" (= "albivenata").

1 + 1, 7 + 7 (counting the lowest double discal spot as 1); hindwing 4 + 4, 1 + 1, 8 + 8 (the second discal spot from below is counted as 1 even if double). It is very rare to find a spot number exceeding 38. The minimum is 3—only the two discoidal spots remain, the hind one without

pupil (f. "panobsoleta" (="caeca") + f. "carteri"). In *A. artaxerxes vandalica* the normal spot value varies from 26 to 33 with nearly equal representation of each value and very few individuals with lower or higher counts.

Jarvis and Høegh-Guldberg have conducted numerous experiments by rearing large series of butterflies from eggs of both species. Young pupae have been cooled for various periods, but pupae less than 1 h old have been avoided because they are very fragile. Jarvis (1959) demonstrated that four conditions had to be fulfilled if changes are to be obtained: (1) temperature must be constant, (2) it must be +1 to +3°C, (3) the cooling period must be 20 days or more, (4) the age of the pupa must be 1–2 h (or up to 24 h), but most extreme results are obtained in the younger pupae.

Høegh-Guldberg (1968), after examining large numbers of bred sibs, confirmed this. He also had the same results at +2 to +5°C, and he added a fifth condition: that a positive result also depended on a particular genotype in the individual and in the population. Jarvis also suggested that extreme forms, such as f. "caeca" (Fig. 1, left) found in the wild, might be due to accidental cooling on cold nights during a critical phase of pupation. This was a tempting theory, but in experiments such a phase of short duration had never produced these forms. Since pupation in nature in these species takes place in the early summer, where a long-lasting, very low and constant temperature is not found, it seemed impossible to explain aberrant wild forms as cold forms. Later, other experiments by Høegh-Guldberg & Jarvis (1969), with conditions which might occasionally be found in nature, also produced no changes in the imaginal pattern. These experiments were with prepupae (24 h before pupation) and pupae (2–12 h after pupation), cooled at various temperatures for shorter periods. Thus, still no possibility existed for explaining natural aberrant forms as the result of cold.

But a solution to the problem was found by starting cooling just around the time of pupation. At that time the sensitivity is so high that only a short cooling sometimes suffices to create changes in the imaginal pattern (Fig. 3). First the author (Høegh-Guldberg, 1974a) had to establish the timetable for pupation of *Aricia* by careful observation of prepupae; thus it is possible to judge when the shedding of the larval skin will occur.

EXPERIMENTAL PROCEDURE

One hundred and thirty-four specimens of *Aricia artaxerxes vandalica* were reared in single vials indoors from eggs laid by two females from Tornby Strand and Skallerup Klit found on 17 June 1973. Thirty-three

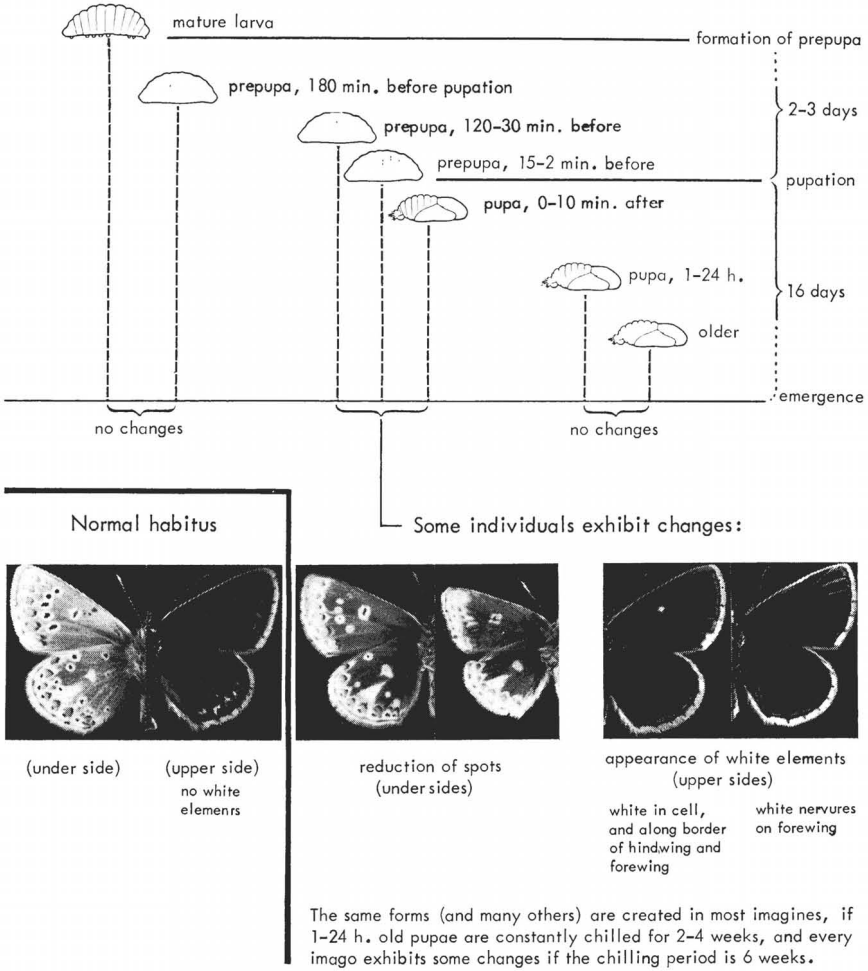

Fig. 3. Effect of chilling *Aricia artaxerxes vandalica* at +2 to +5°C for 1, 2 or 3 "nights" and then returning to room temperature.

individuals served as controls; they were kept at room temperature (+18–20°C) until emergence. Seventy mature larvae and prepupae, whose time for pupation was judged from the appearance, and 31 pupae in the first minutes (min) after pupation were cooled at +2 to +5°C for one, two, or three periods of 9–12 h, the latter two cases with room-temperature periods of 12 h in between. All pupae were kept at room temperature for the remaining 14–16 days of the pupal period. Normally the pupation takes place at any time, so the start of cooling could be at

TABLE 1. The effect on "spot value" of cooling *Aricia artaxerxes vandalica* during critical phases of pupation.

Experimental treatments:			No.	Died	Crippled	No. with low spot value	χ^2	P	
	Room temperature controls		33			3			
	Cooling at +2–5°C								
Stage of development	Age in relation to time of pupation	Cooling periods "nights"							
Mature larva		2	5		1				
		3	8				0.1405	0.5	0.7
Prepupa	–2 days	2	4			1			
	–1 day	2	4				0.1388	0.5	0.7
	–2 to 1.5 h	1	3						
	–3 to 2.0 h	2	2		1				
	–2 h	3	4		1	2	0.2477	0.3	0.5
	–1 to 0.5 h	1	7		2				
		2	11	1	1	4			
		3	15		1	3	1.1758	0.2	0.3
	–15 to 2 min	1	3			1			
		2	3		1	3			
		3	1	1			7.8526		0.01
Pupa	0 to 10 min	1	16			5			
		2	7	1		3			
		3	8			1	3.1662	0.1	0.05

χ^2 is calculated from $n(|ad - bc|) - \frac{1}{2}n)^2/(a + b)(c + d)(a + c)(b + d)$
a = number of controls with normal spot value.
b = number of controls with low spot value.
c = number of experimental animals with normal spot value.
d = number of experimental animals with low spot value.
P is the probability that low spot values occur with the same frequency as in the control group.

any time of the day and night. These conditions are as close as possible to natural conditions occurring in early Danish summer, i.e., one, two, or three cold nights rising to 15–25°C in the daylight hours.

RESULTS

In some of the cooled imagines, differences were found partly in the presence of white elements of the upperside and partly in the "spot value." It is seen from Table 1 that in the cooled groups there is a tendency toward low spot values; this tendency is statistically significant at the $P < 0.05$ level just around pupation. Of the individuals with low spot values, the specimen in Fig. 2C is a true f. *caeca* (*panobsoleta*), a very rarely found form in nature. It had been cooled 15 min, before

pupation should have occurred, and when removed from the refrigerator it had not yet pupated; but it did so one hour later and then had a further "night" of cooling. Of the fresh pupae subjected to cold, the specimen with a spot value of 12 emerged from a pupa which was cooled for only 9 hours when it was 1 min old (Fig. 2B).

Only one of the controls had white elements; it belonged to the rather common f. "albisignata" (white along border of hindwing). Among the cooled groups, white elements only occurred if cooling took place just around the time of pupation. The f. "caeca" specimen was of the rare form "albicostalis" (white nervures at the upperside of forewing). A 1-min-old pupa cooled for two "nights" gave a joint ff. "snelleni" (white in upperside cell) + panalbisignata (white along border of both hindwing and forewing) (Fig. 2E), and another 1-min-old pupa cooled for only 9 h produced an f. albicostalis (Fig. 2F).

To summarize, it was found that ca. 12% of the controls show minor and common variations, whereas ca. 32% of the prepupae cooled 1 h or less before pupation and ca. 39% of the cooled fresh pupae show variation (the remaining are within the normal range of variation). In these latter two groups, eight rare forms were found.

It is seen that immediately before and after pupation of *Ariciae*, there is a brief period (from 15 min before to ca. 10 min after) of great sensitivity to cold in some individuals (Table 1).

With these findings, the possibility of explaining the natural forms as partly produced by cold has advanced considerably. Such conditions as are simulated in the experiments easily occur at many sites where *Ariciae* are found. Bitterly cold nights can occur during the early summer months, when pupation takes place. The experiments show that pupation may occur at any hour and may coincide with the formation of a "cold pool" in a hollow. This occurrence corresponds to the experiments with pupae cooled for 9 hours.

Some may argue that the cold will stop the prepupa from pupating; this often is so, but not always. These experiments show that one-third of the prepupae cooled up to 2 h before pupation continued their transformation during cooling. However, whether or not pupation occurs, depends if they receive a cold shock at a critical time.

CONCLUSIONS

The following four conditions are necessary to produce experimental phenocopies of an *Aricia* form: (1) the individual must be genetically sensitive to cold, (2) exposure to cold must commence immediately before or at the time of pupation, (3) the temperature should be +2 to

+5°C, and (4) exposure should last at least 9 h and preferably be repeated once or twice.

If pupae more than 1 h (up to 48–72 h) old are used in experiments, changes also may occur, but the temperature should be constant and of 3 or more weeks duration.

How can it be explained that a short cooling at the moment of pupation produces the same changes as a prolonged cooling at a later stage? Actually, we find a transition from very rare cases, in which the tendency is so strong that aberrant forms such as f. "caeca" are produced even when the insect has been reared at room temperature during its entire life (Høegh-Guldberg, 1968, p. 44). But in most cases cooling is necessary; just around pupation the cooling period can be as short as 9 hours. With the pupa 1–24 h old, the cold treatment must be as least 14 days, preferably 3–4 weeks, if changes are to be obtained; even then some non-reacting individuals still exist, but if the cooling period extends to 6 weeks, every individual will exhibit at least some changes.

Pupae older than 24 h treated with cold exhibit less sensitivity, and the forms produced are different. Pupae more than 72 h old do not react to cold (Høegh-Guldberg, 1974b). Another question that arises is why identically treated pupae from the same brood react differently?

The answer to both questions must lie in variations in sensitivity, probably due to genetical differences.

On the basis of these findings it is reasonable to assume that the occurrence of aberrant forms in other genera of Lycaenidae and other families may be caused in the same manner.

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