

PHENOTYPIC PLASTICITY IN TEMPERATE AND SUBARCTIC
NYMPHALIS ANTIOPA (NYMPHALIDAE): EVIDENCE FOR
ADAPTIVE CANALIZATION

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ABSTRACT. *Nymphalis antiopa hyperborea* from Fairbanks, Alaska failed to produce the aberrant phenotype "hygiaea" when subjected to cold-shock treatment which induces this phenotype in lowland California *antiopa*. This result is consistent with the hypothesis that canalization of the adult phenotype is an adaptive process.

The mourning cloak, *Nymphalis antiopa* L., has an enormous geographic range; it shows parallel N-S clines in the Palaearctic and Nearctic, but overall its phenotype is extraordinarily stable for a species which is not a regular migrant. Although it is double- or even triple-brooded southward, seasonal variation also seems to be non-existent. Still, the phenotype of *N. antiopa*, like its close relatives, is very susceptible to modification by temperature shock applied to the pupa; many of the aberrations thus induced are very drastic, and some duplicate very rare wild-collected examples. This general picture of extreme geographic and seasonal stability coupled with extreme lability under experimental regimes applies to several species variously placed in the genera *Nymphalis*, *Aglais*, *Inachis*, *Vanessa*, and *Cynthia*. It was the object of much research between 1860 and 1940. Discussions of *antiopa* appear in Standfuss (1896) and Fischer (1907); the subject is reviewed analytically by Goldschmidt (1938). This last author, in 1935, coined the term "phenocopy" to describe mutant-like aberrations inducible by the imposition of stress during development. He explicitly recognized the similarity of such phenomena in *Drosophila* and in the Nymphalini.

In my laboratory we have been duplicating the classical European experiments, using Californian stock of common nymphalines as available. The exact procedures used by Fischer, Standfuss, Merrifield, and the other early writers are often incompletely described, so that differences in responses obtained from Californian and European stocks are difficult to evaluate. For the most part, the classical phenomena have been reproduced qualitatively and sometimes quantitatively. Comparisons among different Californian stocks are more easily controlled.

What—if any—is the evolutionary significance of these recurrent, aberrant phenotypes? This was a lively controversy in the heady, highly theoretical, intellectual climate of the late nineteenth century. A number of early writers considered them atavistic (reversional) to

an ancestral type, and some temperature-induced phenotypes of *N. antiopa* and *N. (=Inachis) io* L., the two species which depart most from the usual pattern in the genus, indeed betray "ancestral" elements. Shapiro (1976, 1979), following Standfuss & Merrifield (1894), attempted to relate the shock phenotypes of nymphalines to the seasonal polyphenisms of pierines and of the nymphalid *Araschnia levana* L.: the environmental sensitivity of the phenotype is viewed as an adaptive property of the genome ("canalization," Waddington, 1957) and a product of selection. The present paper reports an experimental test of this idea.

Experimental Protocol and Results

A named subspecies of *N. antiopa*, *hyperborea* Seitz, occurs in subarctic North America. It is characterized by small size, usually heavy dark markings in the yellow border, and a redder chestnut ground-color. This subspecies is potentially subject in nature to cold shocks similar to those which produce shock phenotypes in Californian *antiopa* in the laboratory, although its seasonality minimizes the risk. I have found no records of aberrations taken in Alaska or the Yukon, while they do occur in California; this could easily be an artifact of the relative amount of collecting done in these areas. If, however, canalization were adaptive, one might expect that the phenotype of *hyperborea* would be better buffered against modification by low temperatures than that of Californian *antiopa*.

In 1979 six colonies of fourth-instar *antiopa* larvae, each uniform in age and presumed to originate from a single egg mass, were collected and used for cold-shock experiments. Four of these originated at sea level at Fairfield, California (38°15'N, 122°03'W), collected in May; the other two were from Fairbanks, Alaska (64°51'N, 147°43'W), collected in late June. All were taken from local *Salix* spp. and reared to pupation on *Ulmus procera* L. at 25°C in continuous light. Experimental animals were refrigerated 8 h after pupation and held for 2 weeks at 2°C before being returned to 25°C. Controls remained at 25°C throughout. The results appear in Table 1.

Although the California colonies differed markedly among themselves, all gave at least one individual of the named aberration "hygiaea" (alive or dead) in the chilled groups. No hygiaea (Fig. 1) appeared among the Alaskan animals or in the California controls. We have never reared an hygiaea from an unchilled pupa, but we have obtained this striking aberration in 12 of the 13 California broods we have subjected to this treatment since 1973.

Temperature shock sufficient to induce aberrations usually kills a substantial number of the animals. To compensate for this, the ex-

TABLE 1. Results of temperature-shock experiments with *Nymphalis antiopa* from two localities (see text).

Brood, source	Normal live	Normal dead	Hygiaea live	Hygiaea dead	Unscorable ¹ dead	Totals ¹
Calif. no. 1 exptl.	30	1	1	0	12	44
control	15	2	0	0	0	17
						61
Calif. no. 2 exptl.	31	9	10	6	10	66
control	10	0	0	0	0	10
						76
Calif. no. 3 exptl.	25	5	5	8	12	55
control	8	0	0	0	1	9
						64
Calif. no. 4 exptl.	17	8	0	1	6	32
control	10	0	0	0	0	10
						42
Total California exptl.	103	23	16	15	40	197
control	43	2	0	0	1	46
				Total Calif.		243
Alaska no. 1 exptl.	29	3	0	0	11	43
control	10	0	0	0	0	10
						53
Alaska no. 2 exptl.	15	6	0	0	4	25
control	6	0	0	0	1	7
						32
Total Alaska exptl.	44	9	0	0	15	68
control	16	0	0	0	1	17
				Total Alaska		85
Total animals raised: 328						

¹ Excluding individuals parasitized by Tachinidae.

perimental part of each colony was always much larger than the control. Most deaths occur after pigment has been laid down in the wings, so the phenotype can be scored. In any given brood the most extreme individuals generally die as pharate adults, or, if they eclose, are crippled, or unable to feed due to improper fusion of the proboscis. Exactly the same phenomena occur with heat-shock phenocopies in *Drosophila* (Mitchell & Lipps, 1978). There is still a residue of unscorable dead, always much higher in the chilled than the unchilled groups. Since these might have altered the phenotypic ratios had they developed further, a 2×2 contingency table was prepared for total scorable vs. unscorable in the two sets of colonies; the California and Alaska ratios were almost identical ($\chi^2 \sim 0.07$, $P > .750$), permitting

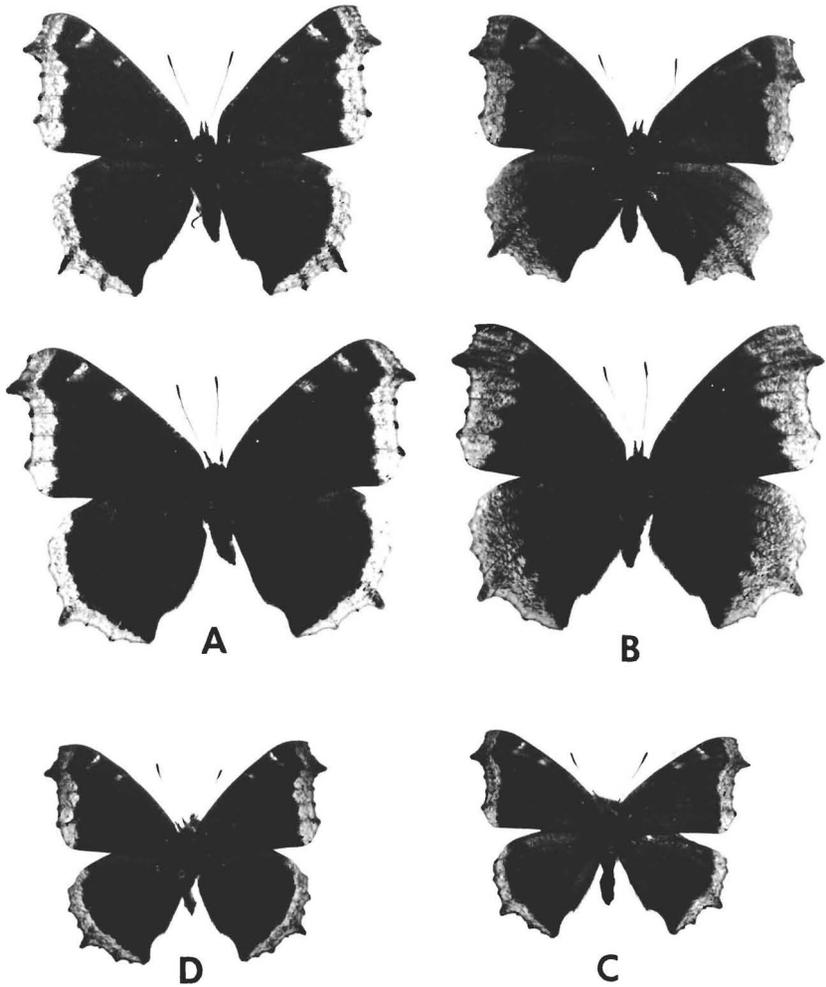


FIG. 1. Normal (A) and "hygiaea" (B) phenotypes of Fairfield, California *Nymphalis antiopa*, with normal chilled (C) and unchilled (D) *N. a. hyperborea* from Fairbanks, Alaska.

us to exclude the unscorables from the analysis. When total normal (alive + dead) vs. total hygiaea (alive + dead) are considered, the difference between California and Alaska is very highly significant ($\chi = 12.28$, $P \leq .005$): based on these samples Alaskan animals *are* better buffered against cold than Californian.

Discussion

This result is less convincing than appears at first glance, for three reasons. (1) The larval environment prior to collection was totally uncontrolled. Although there is no particular reason to think adult phenotype can be determined in this species by influences acting on the young larva, this possibility should be rigorously excluded. (2) The variability among the California colonies suggests that the hygiaea response is genetically variable. The adequacy of 4 California and 2 Alaska colonies as population samples is unknown. If only California broods 1 and 4 had been used in this experiment, the paper would not have been written! (3) Some modification of phenotype did occur in chilled Alaskan animals. The *hyperborea* phenotype is itself phenocopyable in California *antiopa* by temperature treatment (Shapiro, 1976), and chilled *hyperborea* respond by exaggeration of the sub-specific characters, rather than by major changes in the pattern elements (Fig. 1).

The hygiaea phenotype is probably a phenocopy in the rigorous sense. In May 1979 one of more than 140 *antiopa* reared by A. P. Platt under outdoor Maryland conditions displayed this phenotype. The brood was from a female collected by P. Kean in Ann Arundel Co., Maryland (Platt, *pers. comm.*). Shapiro (1976; and unpublished) has found genetically-determined tendencies toward production of the elymi-muelleri-letcheri series of aberrations in *Vanessa annabella* Field in selected lines bred from wild aberrants. These facts underscore the notion that the difference between a simple "mutant" and a temperature-sensitive one is a modifier complex. In the case of nymphaline aberrations, the temperature-sensitive genes may be ubiquitous in many natural populations.

Shapiro's 1976 argument may be condensed as follows: in polyphenic species (pierines, *Araschnia levana*), alternate phenotypes are coupled to environmental factors which induce their expression *in those environments in which they are adaptive*. In species like *N. antiopa*, the normal phenotype is canalized over the entire range of probable environments, leaving plasticity to be expressed only under unnatural laboratory regimes. Since it is normally not expressed in nature, it is safe from selection; only the occasional individual expressing it under ecologically meaningful conditions is selected against.

To establish the plausibility of this argument, one needs to demonstrate that (1) the seasonal phenotypes of polyphenic species are adaptive in their respective seasons, and (2) the normal (canalized)

phenotype of monophenic species is adaptively superior to the shock phenotypes at all seasons. As usual, the pertinent data are difficult to obtain. The best evidence to date bearing on (1) is Watt's (1968, 1969) work on the thermoregulatory properties of the seasonal phenotypes of *Colias*. Even here, a purist might object that the inference of fitness advantage is unsupported by actual data on reproductive success. There are no direct data bearing on (2). Field tests will only be possible if a selected strain can be produced which reliably presents the aberrant phenotype under normal conditions, thereby circumventing the general weakness and inviability of temperature-shocked animals. The logic of the argument parallels current theory regarding the evolution of diapause and facultative sexuality.

A Multi-Level Approach

Recently Bowden (1979) severely criticized "the extreme selectionist position": "... visible characters appear not to be specially adapted to present local conditions." To some extent Bowden is demolishing a straw man; no one claims omnipotence for selection, and the role of history in limiting the variation available to be selected ("phylogenetic inertia") is generally, if grudgingly, acknowledged. The existence of such a controversy 120 years after Darwin is embarrassing, because rigorous and complete demonstrations of ecological causality in evolution are virtually non-existent. The evidence for adaptiveness is generally indirect. In Bowden's *Pieris napi* L. group, the parallels among seasonal, altitudinal, and latitudinal forms—all tending to be darker in colder or cloudier climates—point to thermoregulatory advantages of pattern. If it is objected that this does not rule out a fortuitous physiological effect of chilling during development—reflecting common ancestry rather than parallel selection—a mirror-image situation exists in South America, where similar melanin and pteridine phenotypes occur seasonally, altitudinally, and latitudinally in the endemic pierine genus *Tatochila*. Here the physiological control of the seasonal polyphenism differs from Holarctic systems, and there is a strong, if indirect, case for its convergent evolution from monophenic ancestors (Shapiro, 1980); convergence implies adaptive value.

Both variation and the *lack* of variation are potentially adaptive. Developmental geneticists working on *Drosophila* have now established that at least some phenocopies are related to temperature-stimulated synthesis of specific new proteins ("heat shock proteins") and cessation of normal protein synthesis in progress for a period after the application of stress (Mitchell, 1966; Tissières, Mitchell & Tracy,

1974; Mitchell & Lipps, 1978). If we assume that the cellular physiology of nymphaline phenocopies is not dissimilar, we have a new, more sophisticated framework in which to ask the old question about adaptiveness. We have a proposed cellular mechanism which could account for both seasonal and shock phenotypes. If they are evolutionarily related, it should account for these phenotypes. We know that in at least one phenocopy ("straw," Seybold, Meltzer & Mitchell, 1975) the same molecular control is at work in both mutant and shock examples. Waddington (1953) and Milkman (1963, 1966) proved the selectability of similar phenomena. The phenotypes and reproductive behavior of the nymphaline *Polygonia c-aureum* L. in Japan are under demonstrated neurohormonal control (Fukuda & Endo 1966; Endo, 1970, 1972). Such hormones may act by turning on and off the same genetic loci affected by temperature shock in our experiments.

Referring to its great stability, Bowden (loc. cit.) says: "How are the wing-markings of *Nymphalis antiopa* L. determined? Normalizing selection is working on ancestral genotypes, probably with no visible response to ecological conditions." Precisely, if the stress is on the work "visible." If the stable phenotype of *N. antiopa* is adaptively important, we would expect physiological adjustments to protect it in varying environments. Both polyphenism and monophenism are epiphenomena of a genetic control system, but that control system itself can evolve (Gould, 1977). Its evolution depends on the fitness of the phenotype it produces, which is what will be selected.

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