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# Unusual demonstration of autosomal dominant inheritance of the black coloration of one of America's swallowtails: $F_2$ broods of the hybrid *Papilio polyxenes asterius* with *Papilio machaon gorganus* (Papilionidae)

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**Summary.** From two *inter se* crossings of $F_1$ hybrids between the black American *Papilio polyxenes asterius* Cramer, 1782 (Papilionidae) and the yellow *P. machaon* Linnaeus, 1758 from Europe, 62 $F_2$ offspring were obtained, 79% being black and closely resembling *P. polyxenes asterius* and 21% yellow and almost identical with the European phenotypes of *P. machaon*. These percentages are consistent with the 3:1 ratio expected according to Mendel's law for the inheritance of an autosomal dominant trait. Earlier evidence of such inheritance was based on backcrossing only of black $F_1$ hybrids of the black American and the yellow European *P. machaon* strain with European *P. machaon*. One of the yellow $F_2$ offspring was backcrossed with a specimen of *P. machaon sphyrus* Hübner, 1823, generating 32 healthy and exclusively yellow *P. machaon*-like butterflies, by this confirming Mendel's law. Possible causes of breeding healthy $F_2$ generations are discussed. Keeping down pathogenic germs when rearing the broods appears to be the most likely explanation of our success.

**Zusammenfassung.** Aus zwei Kreuzungen der $F_1$-Hybriden zwischen schwarzen nordamerikanischen *Papilio polyxenes asterius* Cramer, 1782 und gelben *P. machaon* Linnaeus, 1758 aus Europa untereinander resultierten 62 Individuen einer $F_2$-Generation, von denen 79% schwarz gefärbt waren und *P. polyxenes asterius* sehr ähnelten, während 21% gelb gefärbt und nahezu mit europäischen Phänotypen von *P. machaon* identisch waren. Diese Prozentsätze sind konsistent mit einem bei autosomaler Vererbung eines dominanten Allels gemäß der Mendelschen Regeln erwarteten 3:1-Verhältnis der Phänotypen. Frühere Hinweise auf einen solchen Erbgang basierten ausschließlich auf Rückkreuzungen schwarzer $F_1$-Hybride des schwarzen amerikanischen und des gelben europäischen *P. machaon*-Stammes mit europäischen *P. machaon*. Einer der gelben $F_2$-Hybride wurde mit einem Falter von *P. machaon sphyrus* Hübner, 1823 rückgekreuzt; diese Nachkommenschaft bestand aus 32 gesunden, ausschließlich gelb gefärbten Individuen (Phänotyp wie *P. machaon*), womit die Vererbung der Flügelgrundfarbe nach Mendelschen Regeln wiederum bestätigt wird. Mögliche Gründe für die erfolgreiche Aufzucht lebensfähiger $F_2$-Generationen werden diskutiert. Die Unterdrückung der

Ausbreitung von Pathogenen während der Zucht erscheint als die wahrscheinlichste Erklärung für den beobachteten Erfolg.

**Résumé.** À partir de croisements entre hybrides $F_1$, issus d'accouplements entre la forme noire américaine *Papilio polyxenes asterius* Cramer, 1782 (Papilionidae) et la forme jaune européenne de *P. machaon* Linnaeus, 1758, une génération $F_2$ de 62 individus a été obtenue dont 79% sont noirs et ressemblent à *P. polyxenes asterius* et 21% jaunes et presque identiques au phénotype européen de *P. machaon*. Ces pourcentages concordent avec le rapport 3:1 attendu des lois de Mendel pour la transmission autosomique d'un caractère dominant. Les mises en évidence précédentes reposaient sur des rétrocroisements entre hybrides $F_1$ issus de la forme noire américaine et d'une souche européenne de *P. machaon* avec *P. machaon* d'Europe. Un exemplaire jaune de la génération $F_2$ fut croisé avec un exemplaire de *P. machaon sphyrus* Hübner, 1823, donnant 32 papillons vigoureux, exclusivement jaunes, du type *P. machaon*, confirmant ainsi les lois de Mendel. Les causes possibles de l'élevage convenable de générations $F_2$ sont discutées. L'absence de germes pathogènes lors des élevages apparaît la principale explication de notre succès.

Key words: Lepidoptera, *Papilio*, hybrids, crossing, coloration, inheritance, Europe, America.

## Introduction

The black coloration of some American swallowtail butterflies (*P. polyxenes asterius* Cramer, 1782, *P. brevicauda* Saunders, 1869, *P. 'nitra'* Edwards, 1883 — the latter is now considered as a genetically integrated morph within *P. zelicaon* Lucas, 1852, cf. Sperling, 1987) was repeatedly shown to be dominant over the yellow of others (*P. machaon* Linnaeus, 1758 and *P. zelicaon*), due to one single gene dominating over yellow (Clarke & Sheppard, 1955; Fisher, 1977; Blanchard & Descimon, 1988). Evidence for this was the 1:1 segregation of the $F_2$ generations of backcrosses between any random individual of an $F_1$ hybrid of the black/yellow cross with a genuine yellow individual. Confirmation was provided by the 3:1 ratio of black:yellow after sib matings of black individuals after one or more backcrosses with a yellow one. But sib matings of the $F_1$ of swallowtail hybrids remained unsuccessful as in the early fifties (Clarke *et al.*, 1953–1955; Aubert *et al.*, 1997); only after at least one backcross were fertile sib matings obtained (Clarke & Sheppard, 1955; Aubert *et al.*, 1997). In contrast to these results, the findings presented here demonstrate Mendelian autosomal dominance of black coloration by means of $F_1 \times F_1$ *inter se* matings. Possible explanations for our success will be discussed.

## Material and methods

*Pupae*. Most of the non-hybrid pupae were obtained commercially, those of *P. machaon gorganus* Fruhstorfer, 1922 and *P. machaon sphyrus* Hübner, 1823 from caterpillars collected in the Czech Republic and Sicily, respectively. Only a few specimens of *P. machaon gorganus* came from caterpillars collected in Switzerland. Pupae of *P. polyxenes asterius* came from Brandon province, Manitoba, Canada.

*Hand-pairing and oviposition.* The technique of hand-pairing, consisting of holding the male and female butterflies together, in order that their genitalia interlock and insemination can take place (Clarke, 1952; Clarke & Sheppard, 1956), was applied by only one of us (EAL), with the following modifications: first, the butterflies were held with their wings downwards instead of upwards, which enhances the view of the genitalia; second, no rubbing after mutual approximation of the genitalia was applied. Under optimum conditions — healthy well-fed butterflies, a temperature of 20°C or higher, sunshine in the room where experiments took place, early afternoon (13:00–14:30) — the copulation usually took place within one minute and lasted 30–120 min. Before and after copulation, the butterflies were kept in round gauze-covered cages 65 cm wide and 85 cm high. In the event of successful mating eggs would be laid after an interval of 24 hours; for oviposition the females were placed in small cages and at the start of incoming daylight host plants were positioned in direct sunshine. The number of eggs deposited on the first day after mating easily reached 50, the total number being about 250.

*Rearing the larvae.* In view of the excellent pupae material obtained from outdoor caterpillar collection, eggs were not disinfected. Larvae, host plants and other material were treated only after thorough hand washing. $L_1$–$L_3$ were kept indoors, each larva on a separate twig of fennel seedlings (*Foeniculum vulgare*), preferably not more than 5 specimens per plant. Older caterpillars were either placed separately on one large fennel twig kept in water indoors or transported outdoors onto a 10 $m^2$ large bed of full-grown fennel covered with fine gauze for protection against predators. Contact between caterpillars was minimal due to the size of the bed and the plants. Signs of sickness were not observed and loss of caterpillars was negligible.

## Results

In 1996 and 1997, hand-paired mating of the *P. machaon gorganus* male with the *P. polyxenes asterius* female and *vice versa* was obtained repeatedly, but not all females displayed oviposition of fertile eggs. Table 1 shows the results of two such crosses. The $F_1$ hybrid imagines all resembled *P. polyxenes asterius* with a normal sex ratio. $F_1$ *inter se* matings resulted in abundant oviposition by several females. Development and hatching of the $L_1$ larvae did not proceed normally however: in 1996, embryogenesis of the more than 100 eggs of one female stopped in about 70% of cases after they had turned brown, and of the remaining eggs which turned black only hatched 1:3, which means that only 10% came through. In 1997, the rate of successful hatching of the $F_2$ generation was substantially higher, with normal pupation of most caterpillars. The $F_2$ imagines eclosed after a few weeks, slightly more than $^3/_4$ of them again being practically indistinguishable from *P. polyxenes asterius*, those remaining closely resembling *P. machaon gorganus* (Table 2). The sex ratio was not different from 1:1. These figures are consistent with Mendelian-type autosomal dominant inheritance of the black coloration of *P. polyxenes asterius*. In a final experiment, one of the *machaon*-like males of the $F_2$ imagines was successfully mated with a female of *P. machaon sphyrus* (Eller, 1936).

Table 1. Results of backcrosses of $F_1$ imagines of the hybrid *P. machaon gorganus* ♂ × *P. polyxenes asterius* ♀ with a *P. machaon gorganus* ♂ and ♀, respectively.

| | *P. polyxenes asterius*-like (black) | | *P. machaon-like* (yellow) | |
|---|---|---|---|---|
| | ♂ | ♀ | ♂ | ♀ |
| m ♂ × (m/a) ♀ | 2 | 1 | 2 | 3 |
| (m/a) ♂ × m ♀ | 5 | 7 | 1 | 5 |
| Total number | 15* | | 11* | |
| Percentage | 58 | | 42 | |

m – *P. machaon gorganus*; a – *P. polyxenes asterius*

* The deviation from the hypothetical expectation of 13:13 is so small that the probability of its occurring by chance is > 0.50 (chi-square, degrees of freedom = 1).

Table 2. Results of $F_1$ *inter se* matings of the hybrid *P. machaon gorganus* ♂ with *P. polyxenes asterius* ♀

| | *P. polyxenes asterius*-like (black) | | *P. machaon*-like (yellow) | |
|---|---|---|---|---|
| | ♂ | ♀ | ♂ | ♀ |
| 1996 | 1 | 5 | 2 | 0 |
| 1997 | 23 | 20 | 3 | 8 |
| Total number | 49* | | 13* | |
| Percentage | 79 | | 21 | |

* The deviation from the hypothetical 46.5:15.5 is so small that the probability of its occurring by chance is > 0.50 (chi-square, degrees of freedom = 1).

In spite of the low vitality of the female, sufficient progeny were obtained to confirm Mendelian-type inheritance, all 32 imagines being yellow with a sex ratio of 1:1.3.

## Discussion

The first report on spontaneous mating in captivity of a *P. machaon* male with a *P. polyxenes asterius* female appeared at the beginning of this century (Floersheim, 1910). The author, a well-known entomologist, observed the copulation in his butterfly house on July 29, 1908. He pointed out: "The day was a gloriously fine one, and the pairing, which took place about mid-day, lasted about four hours". The resulting hybrid imagines all closely resembled *P. polyxenes asterius*. The backcross with a *P. machaon* unfortunately ended in drowning of the female. More than 40 years later, after the introduction of hand-pairing (Clarke, 1952), the hybrid was obtained many times (Clarke & Knudson, 1953; Clarke & Sheppard, 1953), leading within a couple of years to the conclusion that black coloration of some of the American *P. machaon*-group species is determined by a single gene which is dominant over yellow (Clarke & Sheppard, 1955). This conclusion was, however, not based on straightforward *inter se* crossing of the $F_1$ hybrids, since the latter remained unsuccessful. It was deduced from the results of backcrossings which resulted in a black:yellow ratio of 1:1. More recently, this Mendelian-type dominance of black coloration was confirmed by backcrossing over four generations black $F_1$ females

with wild *P. machaon* males, which yielded imagines with a sex ratio opposite to that predicted by Haldane's Rule (Blanchard & Descimon, 1988; Sperling, 1993). This back-cross evidence of autosomal dominance of black over the yellow coloration was also shown by us (Table 1). Numbers were too small, however, to assess male:female ratios. But, contrary to other authors, we were also able to deduce the Mendelian-type autosomal dominant inheritance of black from offspring of *inter se* crossings of $F_1 \times F_1$ hybrids (Table 2). To corroborate the results, one of the yellow $F_2$ males was backcrossed with a *P. machaon sphyrus* female, yielding 32 healthy, exclusively yellow imagines.

We believe that our results of *inter se* crossing are indeed largely due to particularly healthy broods from outstandingly viable pupae as well as careful breeding under strictly aseptic conditions, handling the caterpillars as explained under *Methods*. Infection with viruses is a well-known complication of swallowtail rearing and is probably enhanced by inbreeding. Such an infection killed the whole $F_2$ brood in the laboratory of a Japanese researcher (Ae, 1964) and is obviously also well known to British scientists (Clarke & Larsen, 1986). This increased susceptibility to infection quite certainly explains the failure to obtain viable offspring of $F_1 \times F_1$ hybrid crossings of *P. machaon* with *P. hospiton* instead of *P. polyxenes asterius* (Clarke & Larsen, 1986; Aubert *et al.*, 1997). The French researchers also suspect another infectious agent, i.e., *Bacillus thuringiensis*, which is contained in insecticides thereby threatening caterpillar rearing.

Another cause of unsuccessful endeavours to obtain $F_1 \times F_1$ adults might be the fact that the other investigators worked with *P. machaon* from southern Europe, while our material came from Switzerland and from northern Europe. The British authors, in their early work in the fifties, used the insular *P. machaon sphyrus* from Malta (Clarke & Sheppard, 1955), the French investigators pupae from Cahors (Lot). Both report the presence of some caterpillars, the French even an $L_4$, all of which died, however. Later endeavours to obtain imagines from $F_1 \times F_1$ crosses in which *P. hospiton* was used instead of *P. polyxenes asterius* (Clarke & Larsen, 1986; Aubert *et al.*, 1997), were equally disappointing. The British authors observed a large variation in the hatching ratio of their

10 broods with low numbers of eggs, averaging only slightly more than 10, clearly pointing to low quality of the commercially obtained material. The hatching ratio varied from 0 to 56%, about 15% on the mean. Only one deformed female imago was obtained from two typical hybrid caterpillars. In comparison to the British, the French researchers obtained about 10 times as many eggs from 12 females. They report the occurrence of brown crowns on the eggs as a sign of embryonic development in about 10% of cases, and about 5% turned black, indicating full development of the caterpillar, none of which hatched, however. Since, in their later work, British researchers used *P. machaon* from Poland — the French did not specify — our supposition that *P. machaon* strains from southern Europe might be responsible for the low hatching percentages and brood failures becomes very unlikely. Equally improbable an explanation offers our modification of the handpairing method, the disappointing results of the French researchers having been obtained with broods from spontaneously copulating butterflies (Aubert *et al.*, 1997).

The above deliberations suggest that the success of obtaining healthy $F_2$ adults from *inter se* crossing of swallowtail butterfly hybrids is largely due to healthy livestock and germ-free handling of the imagines, ova and larvae. This hypothesis is supported by the report of an additional 8 healthy $F_2$ imagines in 1991 by another well-known, highly experienced and exceedingly precise amateur entomologist (Harbich, personal communication 1996).

Lastly, the results of an unintentional cross between a commercially obtained *P. polyxenes asterius* male (pupa provided by Mr John Reichel, Vancouver, CA) with a *P. machaon gorganus* female (Kutscher, 1992) are also interesting in this context. The fairly abundant offspring consisted of black as well as yellow specimens in a proportion of about 1:1, suggesting heterozygosity of the black male from Canada, possibly a natural hybrid specimen from the cross of one of the yellow American swallowtails in the Manitoba region. According to Sperling (1987 and personal communication 1998) Kutscher's specimens are, judged from colour photographs, "very reminiscent of '*kahli*' and '*avinoffi*' which are natural hybrids". As Brandon (Manitoba) was the origin of our *P. polyxenes asterius* pupae material and lies close to the *P. machaon* ×

*P. polyxenes asterius* swarm in central Manitoba, we cannot exclude the possibility that it contains some introgressed *machaon* genes (Sperling & Hansen, 1994). Relevant discussion is far beyond the scope of the present communication, however.

We conclude with the remark made by investigators of swallowtail butterflies of the Americas in the *Do it yourself* chapter of their recent handbook which states that: "Each cross needs its own tenderness that must be discovered empirically" (Tyler *et al.*, 1994).

**New data (addendum)**

In 1999, we repeated our experiments using Japanese *P. machaon hippocrates* Felder & Felder, 1864, kindly supplied by O. Yoshiaki (Tokyo), instead of the European *P. machaon gorganus*; the black American *P. polyxenes asterius* originated from the same area as in the earlier experiments. Hybridization was again easily achieved, resulting in 44 $F_1$ specimens, but there were only 10 females, that emerged only one to four weeks later than the males. This low number and late emergence of females interfered with *inter se* mating, and only two females produced enough eggs for meaningful statistical analysis. Of the 186 eggs deposited by the first one, 37 turned brown macroscopically. Inspection by means of a low-power binocular microscope identified another 20 with signs of embryonic pigmentation. Only one egg turned dark brown, which is usually observed shortly before the hatching of the larva. The latter did not come out, however. A second female produced 143 eggs, none of which displayed any signs of embryogenesis.

These additional observations suggest a larger phylogenetic distance between the American and the Japanese than between the American and the European swallowtail species. They indicate that the insular Maltesian material of *P. machaon sphyrus* used by the British researchers could at least partly have been responsible for the failure to produce viable offspring of the $F_1$ *inter se* crossings.

Clarke, Mittwoch & Traut (1977), in a study that we had overlooked so far, present data that agree remarkably with our own: from 32 matings of $F_1$ imagines, that are hybrids between a *P. machaon* male from Finland and a *P. polyxenes asterius* female from the east of the United States, these authors obtained 79 $F_2$ imagines of which 78% are black and 22% yellow, with a normal

sex ratio in both. Considering our results — 79% and 21% respectively — the close similarity is striking, the deviation from the expected 3:1 ratio in particular. Considering the observation by these authors that many larvae had died from disease, one is tempted to suppose that larvae predestined to develop into black imagines resist influences of nurture easier than those becoming yellow imagines.

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### References

Ae, S. A., 1964. A study of hybrids in the *Papilio machaon* group. — *Bull.Japan ent.Acad.* 1: 1–10.

Aubert, J., Barascud, B., Descimon, H. & Michel, F., 1997. Ecology and genetics of interspecific hybridization in the swallowtails. — *Biol.J.Linn.Soc.* 60: 467–492.

Blanchard, R. & Descimon, H., 1988. Hybridization between two species of swallowtails, meiosis mechanism, and the genesis of gynandromorphs. — *J.Lepid.Soc.* 42: 94–102.

Clarke, C. A., 1952. Hand pairing of *Papilio machaon* in February. — *Entomologist's Rec.J.Var.* 64: 98–100.

Clarke, C. A., & Knudsen, J. P., 1953. A hybrid swallowtail. — *Entomologist's Rec.J.Var.* 65: 76–80.

Clarke, C. A., Mittwoch, U. & Traut, W., 1977. Linkage and cytogenetic studies in the swallowtail butterflies *Papilio polyxenes* Fab. and *Papilio machaon* L. and their hybrids. — *Proc.R.ent.Soc.Lond.* (B) 198: 385–399.

Clarke, C. A. & Sheppard, P. M., 1953. Further observations on hybrid swallowtails. —*Entomologist's Rec.J.Var.* 65 (Suppl.): 1–12.

Clarke, C. A. & Sheppard, P. M., 1955. A preliminary report on the genetics of the *machaon* group of swallowtail butterflies. — *Evolution* 9: 182–201.

Clarke, C. A. & Sheppard, P. M., 1956. Hand-pairing of butterflies. — *Lepid. News* 10: 47–53.

Clarke, C. A. & Larsen, T. B., 1986. Speciation problems in the *Papilio machaon* group of butterflies (Lepidoptera: Papilionidae). — *Syst.Ent.* 11: 175–181.

Eller, K., 1936. Die Rassen von *Papilio machaon* L. — *Abh.bayer.Akad.Wiss., math.-naturwiss. Abt., N. F.* 36: 1–96.

Fisher, M. S., 1977. The taxonomy and identity of *Papilio nitra* W. H. Edwards in Colorado (Papilionidae). — *Bull.Allyn Mus.* 47: 1–7.

Floersheim, C., 1910. Cross-pairing of *Papilio machaon* and *P. polyxenes*. — *Entomologist's Rec.J.Var.* 22: 200–202.

Kutscher, W., 1992. Unfreiwillige spontane Kreuzung von *Papilio machaon gorganus* und *Papilio polyxenes asterius*. — *Nachr.ent.Ver.Apollo, Frankfurt/M., N.F.* 13: 164.

Loeliger, E. A., Karrer, F., 1996. Breeding of an $F_2$ of *Hyles* hybrid *vespertilioides* (Boisduval, 1827) after induction with ecdysone of metamorphosis in diapausal pupae. — *Nota lepid.* 19: 243–260.

Sperling, F. A. H., 1987. Evolution of the *Papilio machaon* species group in Western Canada (Lepidoptera: Papilionidae). — *Quaestiones Entomologicae* 23: 198–315.

Sperling, F. A. H., 1992. Mitochondrial DNA variation and Haldane's rule in the *Papilio glaucus* and *P. troilus* species groups. — *Heredity* 71: 227–233.

Sperling, F. A. H., 1993. Mitochondrial DNA phylogeny of the *Papilio machaon* species group (Lepidoptera, Papilionidae). — *Mem.ent.Soc.Canada* 165: 233–242.

Sperling, F. A. H. & Harrison, R. G., 1994. Mitochondrial DNA variation within and between species in the *Papilio machaon* group of swallowtail butterflies. — *Evolution* 48: 408–419.

Tyler, H. A., Brown, K. S. & Wilson, K. H., 1994. Swallowtail butterflies of the Americas. A study in biological dynamics, ecological diversity, biosystematics, and conservation. — Scientific Publishers, Gainesville, USA. — 376 p.