

Seasonal change in pupation behaviour and pupal mortality in a swallowtail butterfly

C. Stefanescu

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Abstract

Seasonal change in pupation behaviour and pupal mortality in a swallowtail butterfly.— Phenotypic plasticity in pupal colour has evolved to render cryptic pupae. Apart from characteristics of the pupation site, the photoperiod experienced by larvae is important in determining pupal colour, long and short photophases eliciting the formation of green and brown pupae, respectively. This seasonal polyphenism is often correlated with developmental pathway, green pupae developing directly and brown pupae entering into diapause. From 1996 to 2000, immature stages of *Iphiclides podalirius* were monitored on natural hostplants in NE Spain. Larvae were followed to the pupation site and pupal colour, characteristics of the pupation site and the fate of pupae were recorded. Before August, pupae were non-diapausing green while in early August they were dimorphic, after which, they were brown and overwintered. As theory predicts, differences in pupation sites in successive generations were found in relation to pupal colour. Green pupae occurred on the hostplants and brown pupae were found among the leaf litter. Mortality ranged from 14.3 to 100%. Bird predation was the major mortality factor for green pupae and was also important for brown pupae. Results suggest that preference for pupation sites in the litter in diapausing broods evolved to avoid strong bird predation on the hostplants. Preference for sites above ground level in summer generations may have evolved in response to both non-visual (small mammals) and visual (avian) predators.

Key words: Lepidoptera, Swallowtail butterflies, *Iphiclides podalirius*, Pupal colour, Pupation behaviour, Pupal mortality.

Resumen

Cambios estacionales en el comportamiento de pupación y en la mortalidad de las pupas en un papiliónido.— La plasticidad fenotípica en el color de las pupas es el resultado de un proceso evolutivo que permite a las pupas ser crípticas. Además de las características del lugar de pupación, el color de la pupa viene determinado por el fotoperíodo que experimenta la larva. Los fotoperíodos largos y cortos favorecen, respectivamente, la formación de pupas verdes y marrones. Este polifenismo estacional frecuentemente se correlaciona con el tipo de desarrollo, de manera que las pupas verdes siguen un desarrollo directo mientras que las marrones entran en diapausa. Entre 1996 y 2000, se controlaron los estadios inmaduros de *Iphiclides podalirius* sobre sus plantas nutricias naturales en una localidad del noreste de España. Se siguió a las larvas hasta su lugar de pupación, y se anotaron las características de dichos lugares y el destino final de las pupas. Antes de agosto, todas las pupas observadas fueron verdes y de desarrollo directo; a principios de agosto, las pupas fueron dimórficas; posteriormente, todas fueron marrones y entraron en diapausa. Tal como predice la teoría, las diferencias entre generaciones sucesivas respecto los lugares de pupación se relacionaron con el color de las pupas. Las pupas verdes se localizaron sobre la planta nutricia, mientras que las marrones se localizaron entre la hojarasca. La mortalidad osciló entre el 14,3 y el 100%. La predación por parte de aves insectívoras fue el principal factor de mortalidad para las pupas verdes y también fue importante para las pupas marrones. Los resultados sugieren que la preferencia por la hojarasca como lugar de pupación en las generaciones que entran en diapausa ha evolucionado para reducir la gran mortalidad que tendría lugar sobre la planta nutricia. En las generaciones de verano, la preferencia por lugares alejados del suelo podría ser el resultado de una respuesta evolutiva tanto frente a los depredadores no visuales (micromamíferos) como visuales (aves).

Palabras clave: Lepidoptera, Papilionidos, *Iphiclides podalirius*, Color pupal, Comportamiento pupal, Mortalidad pupal.

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Constantí Stefanescu, Butterfly Monitoring Scheme, Museu de Granollers Ciències Naturals, Francesc Macià, 51, E-08400 Granollers, Spain. E-mail: canliro@teleline.es

Introduction

The pupal stage is critical in the survival of any holometabolous insect, including Lepidoptera (Cornell & Hawkins, 1995), above all because its lack of mobility makes it very vulnerable to predation. To a large extent its survival is based on a "primary anti-predator defence", that is to say, on the avoidance of detection by predators (Brakefield et al., 1992). Therefore, it comes as no surprise that the pupa is the hardest stage of a butterfly's life cycle to locate and few studies have included detailed data on the nature of pupation sites (e.g. West & Hazel, 1996) or on pupal mortality (e.g. White, 1986).

Since the pioneering work of Poulton (1887, 1892), it has repeatedly been shown that pupal colour can vary within swallowtail butterflies. Thus, the pupae of many species are dimorphic, either brown or green, exhibiting phenotypic plasticity or environmentally cued polymorphism for colour (reviewed in Hazel, 1995). Both the colour and texture of the pupation site have been recognised as the most important environmental cues governing polymorphism (Smith, 1978; Hazel & West, 1979). The upshot is that pupae match the background of their resting sites, which, in turn, leads to enhanced survival (e.g. Wiklund, 1975; Sims & Shapiro, 1984; Hazel et al., 1998).

An additional factor, the photoperiod experienced by larvae, can also be important in determining pupal colour (Smith, 1978; Hazel & West, 1979, 1983). Thus, under a short photophase, the larvae of some species produce almost exclusively brown pupae. Because diapause in many insects is also influenced by the larval photoperiod (Leather et al., 1993), a phenotypic correlation between pupal colour and diapause strategy may be a common phenomenon.

This kind of seasonal polyphenism (Shapiro, 1976) seems to apply in the scarce swallowtail butterfly, *Iphiclides podalirius* (L.). Under laboratory conditions, larvae exposed to long day lengths produce green pupae that do not diapause, while those exposed to short day lengths produce brown pupae that overwinter (Wohlfahrt, 1954, 1957; Friedrich, 1986; Tolman & Lewington, 1997). Available data thus suggest that pupae are seasonally monomorphic, with colour being correlated to the developmental pathway.

According to Clarke & Sheppard (1972) and Wiklund (1975), monomorphic pupae have evolved in stable predictable environments where larvae can develop strong preferences for a particular kind of substratum, whereas the reverse is true for polymorphic pupae. A seasonal polyphenism would arise in cases of differences in the pupation sites in successive generations. Hence, a first prediction regarding *I. podalirius* is that diapause and non-diapause pupae should be found at different sites (see also West & Hazel, 1979).

Second, it has been hypothesized that green pupae evolved from monomorphic brown pupae

that suffered from high levels of non-visual predation (mainly by small mammals) at their pupation sites in the leaf-litter. Green coloration would have been selected in the event of pupation site preference changing to sites among green vegetation above ground level which render pupae more cryptic to visual (mainly avian) predators (e.g. West & Hazel, 1982; Hazel, 1995; Hazel et al., 1998). According to this hypothesis, green non-diapausing pupae of *I. podalirius* should be found on green sites above ground level, where avian predators would play a significant role in overall mortality. On the other hand, brown overwintering pupae should occur near the ground, where they would mainly be preyed upon by non-visual predators.

In the present paper, these two predictions are tested, having first confirmed the existence of seasonal polyphenism in *I. podalirius* pupae (and the correlation between pupal colour and developmental pathway) under natural conditions.

Material and methods

Study species

Iphiclides podalirius is a swallowtail butterfly which is widespread in the Palaearctic region (Tolman & Lewington, 1997). It overwinters in the pupal stage and, in the Iberian peninsula, is usually multivoltine. Hostplants are shrubs and trees of the Rosaceae family, mainly blackthorn *Prunus spinosa*, fruit trees of the genus *Prunus*, and hawthorn *Crataegus monogyna*. Eggs are laid singly on the underside of leaves.

Caterpillars are sedentary and live on a silk cushion spun onto the surface of the leaf selected as a resting site. They pass through five instars with a total developmental time of three to six weeks, depending on the temperature and host plant (C. Stefanescu, unpublished data).

Study site and sampling

The study was carried out in Can Liro (Sant Pere de Vilamajor, 41° 41' 16" N 2° 23' 07" E, 310 m a.s.l., NE Spain), an agricultural area surrounded by evergreen oak *Quercus ilex* forest. The climate is Mediterranean, with rainfall of ca. 650 mm per year and summer drought.

Immature stages of *I. podalirius* were monitored on their natural hostplants, and an attempt was made to follow any larva reaching the prepupal stage to the pupation site. Pupal colour, characteristics of the pupation site and the fate of pupae were subsequently recorded.

From 1996–1999, four blackthorns, two peach trees *Prunus persica* and one hawthorn were monitored in a hedgerow between two fields. All these plants were at least two years old in 1996. Their height ranged from 180–200 cm (trees) and 50–215 cm (shrubs). In 1999, 14 additional blackthorns in another hedgerow were also monitored. They

were young shoots resulting from the cutting, in February 1999, of a dense stand of blackthorn, and their mean height in April was 45.4 ± 8.1 cm (mean \pm SD, range: 35–64 cm). Their appearance differed strongly from that of shrubs, as they mainly consisted of a stem and a few branches.

Of over 700 larvae successfully hatched from 1033 naturally laid eggs, only 72 completed their development and were monitored throughout the pupal stage (see Stefanescu et al., 2003). To increase this sample, 65 fifth instar larvae were collected on nearby blackthorns and peach trees in 1998–2000 and released on the monitored host plants. These larvae were allowed to select a pupation site once their development was completed.

Correlates of pupal colour

To confirm that pupal colour and diapause are induced by the photoperiod and are correlated traits in *I. podalirius*, field data were complemented with data from the captive rearing of 112 larvae. Larvae were collected as eggs in 1996 and 1997 and were reared in clear plastic boxes in an outdoor yard (i.e. not exposed to direct sunshine but experiencing naturally fluctuating air temperatures and photoperiod). Larvae were allowed to pupate freely on the stems provided together with the food (blackthorn) or on the box sides. Preliminary rearings had shown that neither kind of substrate elicited differences in pupal colour (see Hazel & West, 1979, for a different situation), and therefore no attempt was made to control for this factor.

As noted by Hazel (1995), the colour of the green morph varies little in the pupae of swallowtail butterflies, while the brown morph can show considerable variation in accordance with the darkness of the pupation site (the so-called phenotypic modulation). In the present paper, however, pupae were classified as either green or brown, without taking into account the degree of darkness of brown pupae. Because the definitive colour appeared soon after pupation and then remained unchanged until adult emergence, colour assignment could be performed within the first 24 h after a larva had pupated.

Those factors most likely to influence (or to be associated with) pupal colour were measured: 1. Photoperiod, calculated at the pupation date (i.e. during the most sensitive period of the larval stage, according to Hidaka, 1961) from tables published by the Instituto Nacional de Meteorología (1991); 2. The generation to which the larva belonged, as deduced from the monitoring of immature specimens and from adult transect counts as part of the Catalan Butterfly Monitoring Scheme (Stefanescu, 2000a); 3. The developmental pathway, considering a pupa to have entered diapause if eclosion was delayed for more than 120 days (the shortest time necessary for passing the winter period, in the hypothetical case in which pupation and adult emergence occurred, respectively, by late October and early March); 4. The pupation site, its height off the ground and the diameter of the site measured at

the point of girdle attachment (following West & Hazel, 1979, 1996; West, 1995); and 5. Whether pupation occurred on or off the hostplant.

Pupal mortality

Once larvae selected pupation sites, positions were marked with plastic tags to allow subsequent relocation. Non-overwintering and overwintering pupae were inspected daily and at one-weekly intervals, respectively, and their fates were recorded as follows: 1. Alive and intact; 2. Attacked by a predator, remains found; 3. Gone and presumed preyed upon; 4. Dead but intact (physiological death); 5. Stepped on; or 6. Eclosed.

Although the identity of predators could not always be established with certainty, indirect evidence from characteristic forms of damage allowed the main sources of mortality to be estimated. Similar pupal remains to those described by Frank (1967) were considered to be the result of invertebrate predation. Bird predation was assessed by means of direct observation (a full account of observations is reported in Stefanescu, 2000b). Also, between 8 and 15 December 1997, 10 Sherman traps were placed in the experimental area from dusk to early morning to determine the most common small mammal predators.

Experiments on bird predation pressure

In non-overwintering broods, pupation mostly occurred on the foodplants, except for one-year-old blackthorn shoots, in which case it predominantly occurred on the ground. On the other hand, in diapausing broods, larvae always pupated among the leaf litter (see Results). Because swallowtail pupae above ground level are commonly preyed upon by birds (e.g. West & Hazel, 1982; Stefanescu, 2000b), two field experiments were designed to test the possibility that larvae leaving the hostplant might have been selected to avoid bird predation.

Non-overwintering pupae

Four out of 10 larvae occurring on one-year old blackthorns in June–July 1999 pupated on the hostplants (one left its original foodplant but finally pupated on another sapling), at heights of 20–40 cm above the ground. To increase this sample, eight pupae were artificially glued on nearby shoots with a dab of colourless “Evo-stick” impact adhesive, using the same kind of pupation sites naturally selected (woody stems and the underside of leaves) within the same height range (20–40 cm). Their survival was compared with that of those pupae produced by the six larvae that left the same shoots and pupated among green grasses, plus another four pupae glued to grass stems to increase this data set. Survival was also compared with that of those pupae naturally occurring on shrubs in summers 1996–1999. All pupae monitored in this experiment (both naturally occurring and artificially placed) were green.

Overwintering pupae

The survival of two sets of overwintering pupae was compared in each of the winters 1997–98, 1998–99 and 1999–2000. One set was composed of pupae naturally occurring among the leaf-litter (i.e. those coming from larvae that could be followed to their pupation sites) and the other of pupae glued to hostplants at heights of 50–150 cm. To rule out the possibility that highly visible plastic tags on leafless shrubs and trees increased attraction for visual predators, relocation of artificially placed pupae was made with the help of a sketch showing their positions instead of the plastic tags. On 9 XI 97, 16 pupae were placed on three blackthorns and one peach tree to simulate the situation that would have arisen if the 17 wild larvae that survived to the prepupal stage had pupated on their hostplant instead of leaving to pupate among the leaf litter. This design prevented attaining an unnatural pupal density in the monitored hedgerow that might have led to an abnormally high level of predation. The same design was used in the following years. On 1 XI 98, 13 pupae were placed on two blackthorns, two peach trees and one hawthorn, and their survival was compared with that of 23 wild pupae. On 16 X 99, 25 pupae were placed on four blackthorns, one peach tree and one hawthorn and their survival was compared with that of 30 wild pupae. Wild pupae were inspected at weekly intervals, while artificially placed pupae were checked daily, and their fates recorded as described above. All pupae in this experiment (both naturally occurring and artificially placed) were brown.

The null hypothesis “pupal survival is unaffected by bird predation whether the pupa is on or off the hostplant” was tested by comparing proportions of successfully eclosed pupae by means of 2 x 2 contingency tables. In overwintering pupae, however, mortality was so high that the power of the test was much reduced; therefore, a second analysis was performed to compare another variable correlated with pupal survival: elapsed time (in days) before a pupa died. Non-parametric tests were used because this variable was not normally distributed even after data transformation.

Results

Seasonal polyphenism

First generation butterflies peaked in early May (fig. 1). Their offspring developed into green pupae (fig. 2A) that gave rise to a second generation of adults emerging from late June to early August (fig. 1).

The earliest offspring of the second generation produced green pupae that developed into a partial third generation of adults from early August to early September (fig. 1). The proportion of larvae entering the direct-developmental pathway decreased from 8:2 (non-diapausing green: diapausing brown) in 3–9 August to 4:12 in 10–16 August. The remaining pupae of the second generation and all those of the third generation (which appeared in early October) were diapausing brown

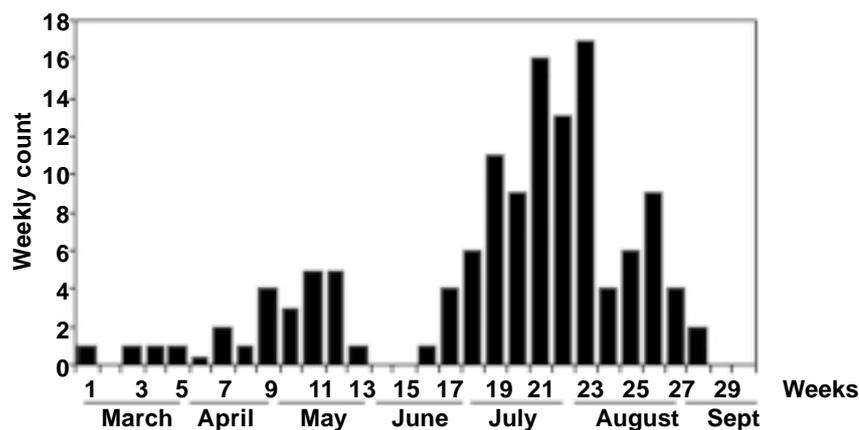


Fig. 1. Seasonal abundance of *Iphiclides podalirius* as recorded by weekly transect counts of adults at the study site in 1994–2000 ($n = 110$). Week 1 corresponds to 1–7 March and week 30 to 20–26 September.

Fig. 1. Abundancia estacional de *Iphiclides podalirius* en la zona de estudio entre 1994–2000 ($n = 110$), según los contajes semanales a lo largo de un transecto. La semana 1 corresponde a 1–7 de marzo y la semana 30 a 20–26 de septiembre.

pupae (fig. 2A). Very similar results were obtained in the rearing experiment, with the single exception of a non-diapausing brown pupa occurring in early July (fig. 2B). Photoperiods are shown in fig. 2C.

The data confirm the existence of a seasonal polyphenism, with the colour and developmental pathway of the pupae being correlated traits. Mean duration (in days) of non-diapausing green pupae was 16.82 ± 0.46 (mean \pm SD, $n = 17$; range: 14–21) and 18.18 ± 0.59 ($n = 37$; range: 13–25) for wild and captive pupae, respectively. For brown diapausing pupae, these values were 214.13 ± 13.8 ($n = 8$; range: 167–269) and 258.73 ± 2.3 ($n = 74$; range: 209–302), respectively. There was no difference in the duration of this stage between captive and wild non-diapausing pupae (t test = 1.82, $df = 53$, $p = 0.074$), but captive diapausing pupae took longer to develop than wild diapausing pupae (t test = 3.19, $df = 80$, $p = 0.014$).

Pupation site

Pupal colour and whether pupation site was on or off the hostplant were strongly associated (table 1). Although the plant species did not affect the behaviour shown by non-diapausing broods, the size of the hostplant did. Prepupal larvae tended to leave their hostplants when these consisted of small one-year old blackthorns (78.9% of green pupae stayed on shrubs vs. 27.2% on shoots; Fisher's exact test, $p = 0.009$).

The main pupation sites are summarized in table 2. Green pupae occurred much higher above ground level and on broader substrates than brown pupae. Twigs and thorns hidden by dense foliage were chosen on blackthorns while the underside of leaves were preferred on peach trees. In eight out of 13 cases in which larvae left the hostplant, they pupated on woody stems of nearby blackthorn shrubs. The background colour of pupation sites was always green (including those cases in which pupation occurred off the hostplant) and hence pupae were cryptically coloured.

Brown pupae strongly preferred slender grass stems (mainly dry stems of *Cynodon dactylon*) and weed stalks (mainly brown stems of *Medicago sativa* from a nearby field) close to the ground. They were cryptically coloured during the winter because pupation sites consisted of predominantly dead vegetation.

Pupal mortality

Non-overwintering pupae

Of 46 non-diapausing pupae, 29 successfully eclosed (63%) and 17 died (fig. 3). Survival ranged from 42.9% to 85.7% in 1996–1999, but differences among years were not significant ($P^2 = 6.174$, $df = 3$, $p = 0.1$). It should be noted, however, that there might well have occurred differences in survival that were undetected due to the small sample sizes and the reduced statistical power of the test.

Bird predation was the main mortality factor, accounting for at least 47% of losses. Included in

this estimation are confirmed records of predation by the Great Tit *Parus major*, as well as pupae that disappeared in the early morning leaving no trace other than the broken girdle (see Stefanescu, 2000b, for details). Impact by avian predators could indeed be much higher (nearly 80% of total losses), if pupae disappearing without trace had also been preyed upon by birds. Invertebrate predation was apparently unimportant and was only recorded once when the wasp *Polistes gallica* killed a second day prepupa.

Comparison of pupal survival on blackthorn shrubs as opposed to shoots (including experimental data of glued pupae) revealed strong differences: 13 out of 19 green pupae on shrubs successfully eclosed, but none of 12 green pupae on shoots survived (Fisher's exact test, $p = 0.0001$). Difference in survival rates between green pupae occurring on shoots (0 out of 12) and those that naturally left from the same shoots and pupated among the leaf litter (7 out of 10, the latter including four green pupae glued to equivalent sites on grass stems) was also significant (Fisher's exact test, $p = 0.02$). On the other hand, neither the survival of pupae occurring on blackthorn shrubs as opposed to the leaf litter (Fisher's exact test, $p = 1$), nor between blackthorn shrubs and peach trees and hawthorn (Fisher's exact test, $p = 1$) were significantly different.

The data indicate that the experimental manipulation did not produce a bias in the results. Survival rates among the leaf litter did not differ between naturally occurring and glued pupae (Fisher's exact test, $p = 0.67$). Similarly, no differences in elapsed time before a pupa died were found between those pupae naturally occurring and those artificially placed on shoots (Mann-Whitney U test = 9, $df = 4,8$; $p = 0.22$).

Overwintering pupae

Three ground sites could not be relocated; of the remaining 88 overwintering pupae, eight eclosed (9.1%) and 80 were killed as the result of several mortality factors (fig. 3). Predation by the Great Tit was observed in one occasion. In another 15 cases, bird predation by several species was the most plausible explanation (see Stefanescu, 2000b). 50 pupae disappeared leaving no remains, birds and small mammals (mainly the shrew *Crocidura russula*, the only species trapped in the area in December 1997, and the hedgehog *Erinaceus europaeus*, a common species at the studied site) being the most probable predators. Minor mortality factors included physiological death during the abnormally rainy winter of 1997–98 (in which some pupae were submerged for long periods following winter flooding), trampling by humans and invertebrate predation. Pupal survival was 0% in 1996 (eclosed/total monitored: 0/11), 1998 (0/23) and 2000 (0/7), 13.3% in 1999 (4/30) and 23.5% in 1997 (4/17). Even though the sample sizes were small, differences in these proportions were marginally significant among years

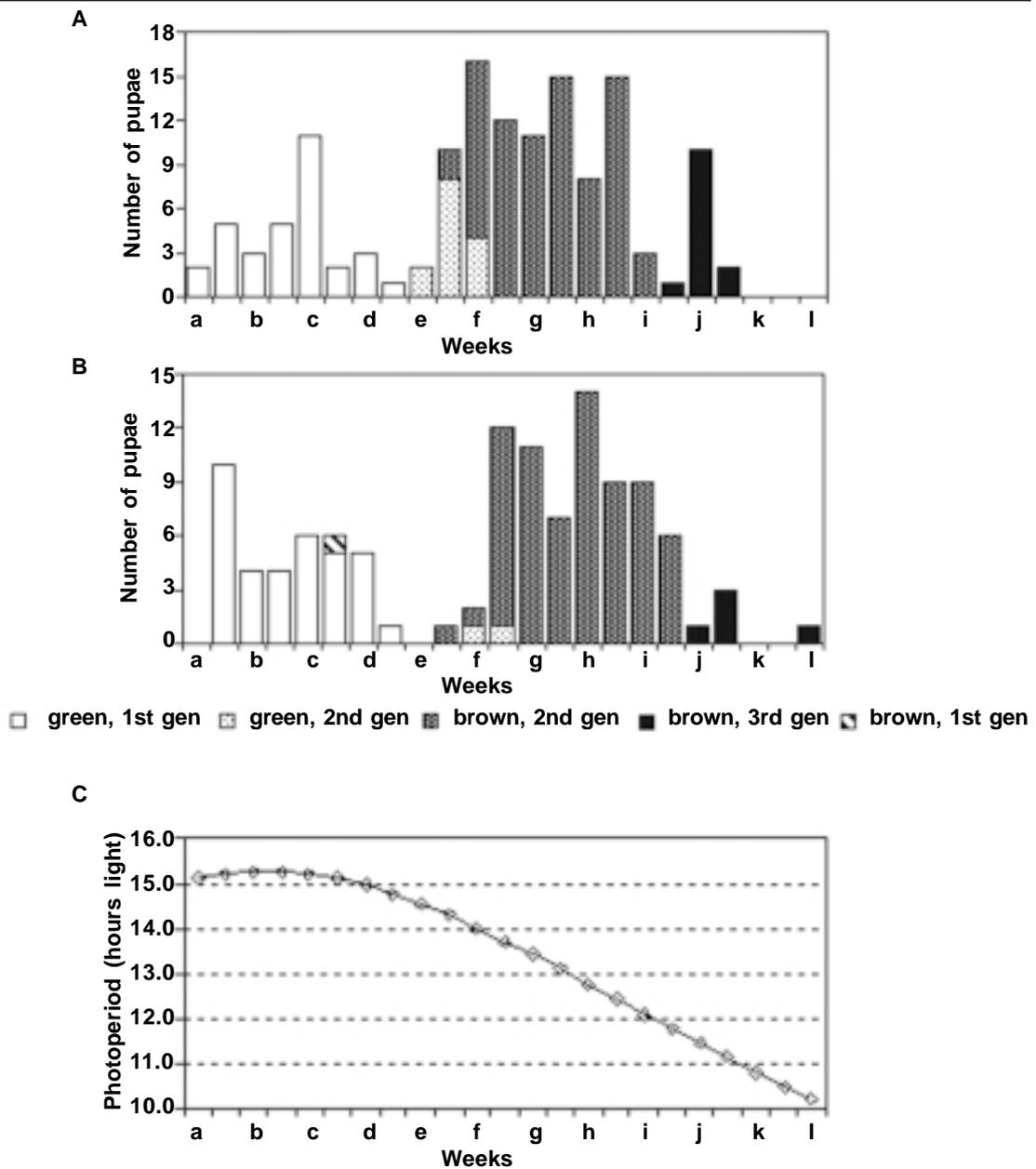


Fig. 2. Seasonal distribution of pupal colour in *Iphiclides podalirius* as recorded from the monitoring of: A. One hundred thirty-seven wild larvae; B. The rearing outdoors of 112 larvae; C. Changes in day length during the pupation period. All surviving green pupae from (A) and (B) developed into adults in ≤ 21 and ≤ 25 days, respectively (i.e. were non-diapausing). All brown pupae with the exception of the single captive pupa from the first generation entered in diapause: a. 1–7 June; b. 15–21 June; c. 29–5 July; d. 13–19 July; e. 27–2 August; f. 10–16 August; g. 24–30 August; h. 7–13 September; i. 21–27 September; j. 5–11 October; k. 19–25 October; l. 2–8 November.

Fig. 2. Distribución estacional del color de la pupa de *Iphiclides podalirius* obtenida a partir de: A. Ciento treinta y siete larvas seguidas en el campo; B. La cría en el exterior de 112 larvas; C. Cambios en la duración del día durante el período de pupación. Todas las pupas verdes supervivientes de (A) y (B) dieron lugar a adultos en ≤ 21 y ≤ 25 días, respectivamente (es decir, no entraron en diapausa). Todas las pupas marrones, con la excepción de la única pupa de este color criada en cautividad y perteneciente a la primera generación, entraron en diapausa: a. 1–7 junio; b. 15–21 junio; c. 29–5 julio; d. 13–19 julio; e. 27–2 agosto; f. 10–16 agosto; g. 24–30 agosto; h. 7–13 septiembre; i. 21–27 septiembre; j. 5–11 octubre; k. 19–25 octubre; l. 2–8 noviembre.

Table 1. Green (non-overwintering) and brown (overwintering) pupae of *Iphiclides podalirius* recorded on or off the hostplant. Significance for G test of independence between pupal colour and pupation site: * $p < 0.05$; *** $p < 0.001$; # Including one pupa monitored in 1998. (Abbreviations: G. Green pupae; B. Brown pupae; S. Significance; Bs. Blackthorn (shrubs); B1. Blackthorn (one-year old shoots); Pt. Peach tree; Hs. Hawthorn (shrub); T. Total.)

Tabla 1. Número de pupas verdes (no hibernantes) y marrones (hibernantes) de *Iphiclides podalirius* halladas sobre la planta nutricia o entre la vegetación circundante. Se indica la significación de un test G para la independencia entre el color de la pupa y el lugar de pupación: * $p < 0,05$; *** $p < 0.001$; # incluye una pupa estudiada en 1998. (Abreviaturas: G. Pupas verdes; B. Pupas marrones; S. Significación estadística; Bs. Endrinos (arbustivos); B1. Endrinos (rebrotos de un año); Pt. Melocotonero; Hs. Majuelo (arbusto); T. Total.)

	G		B		S
	on	off	on	off	
Bs	15	4	0	52	***
B1	3	8 [#]	0	18	*
Pt	14	1	0	17	***
Hs	1	0	0	4	*
T	33	5	0	91	***

($P^2 = 9.042$, $df = 4$, $p = 0.06$), indicating, most probably, a real variation in overwintering survival depending on the season.

Pupae artificially placed on hostplants disappeared within a few days in all three years, as a result of bird predation (fig. 4). The Great Tit was repeatedly observed inspecting the hedgerow in search of food and, occasionally taking pupae for consumption. Another very common forager was the Sardinian Warbler *Sylvia melanocephala*, although no direct observations confirmed that it was actually a predator.

Survival of pupae naturally occurring among the leaf litter and those artificially placed on the hostplants was significantly different when data were pooled for all years (Fisher's exact test, $p = 0.009$), but not for individual years. Nevertheless, elapsed time before a pupa died was always much longer for pupae in the leaf litter (fig. 4). When comparisons were restricted to pupae presumably preyed upon by birds or small mammals (i.e. after discarding the few cases of invertebrate

Table 2. Pupation sites, height from the ground and diameter of the site at the point of girdle attachment for green (non-overwintering) and brown (overwintering) pupae of *Iphiclides podalirius*. Significance for Mann-Whitney U test: *** $p < 0.001$. (Abbreviations: G. Green pupae; B. Brown pupae. Pupation sites: Ul. Underside of leaf; Gs. Grass stem; We. Weed stalk; Wo. Woody stem; Ull. Underside of living leaf; Udl. Underside of dead leaf; Ur. Underside of rock.)

Tabla 2. Lugares de pupación, altura desde el suelo y diámetro de lugar de pupación en el punto de fijación con el cíngulo para pupas verdes (no hibernantes) y marrones (hibernantes) de *Iphiclides podalirius*. Significación de un test de U Mann-Whitney: *** $p < 0.001$. (Abreviaturas: G. Pupas verdes; B. Pupas marrones; Lugar de pupación: Ul. Reverso de una hoja; Gs. Gramínea; We. Tallo de planta herbácea; Wo. Tallo de planta leñosa; Ull. Reverso de una hoja viva; Udl. Reverso de una hoja muerta; Ur. Bajo una roca.)

	G	B	S
Pupation site			
On the hostplant			
Twig	18	–	
Ul	15	–	
Total	33	0	
Off the hostplant			
Gs	2	56	
We	1	23	
Wo	8	3	
Ull	2	4	
Udl	–	4	
Ur	–	1	
Total	13	91	
Height above the ground (cm)			
mean±SD	63.66±9.32	4.09±0.51	***
median	33.5	3	
range	1–180	1–35	
n	44	87	
Diameter of substrate (mm)			
mean±SD	10.09±1.26	3.49±0.62	***
median	5	2	
range	1–30	2–50	
n	46	91	

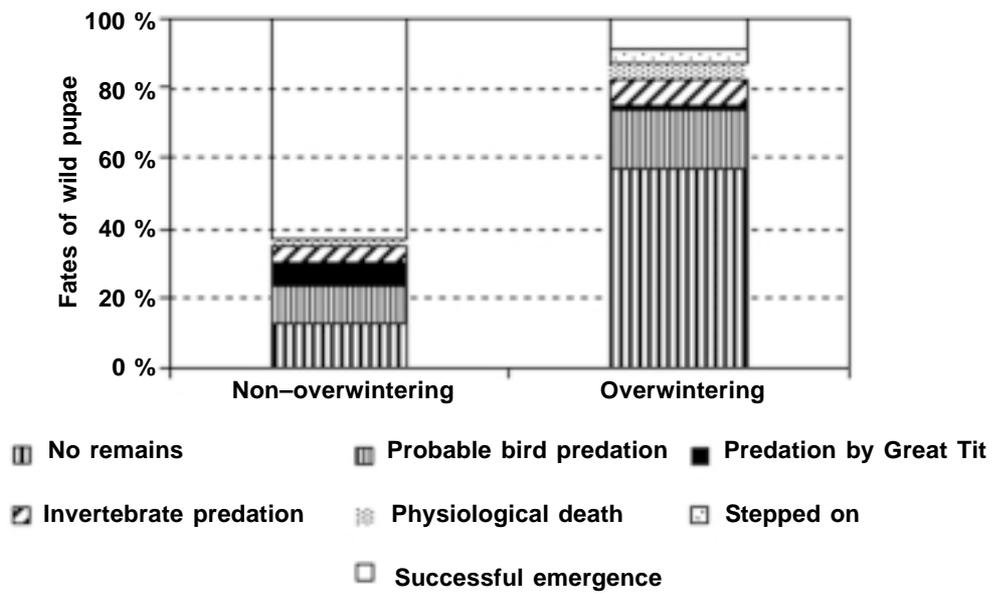


Fig. 3. Fates of 46 non-overwintering and 88 overwintering wild pupae of *Iphiclides podalirius* monitored in 1996–2000 (see text for more details).

Fig. 3. Destino de 46 pupas no hibernantes y de 88 pupas hibernantes de *Iphiclides podalirius*, seguidas en el campo entre 1996–2000 (ver el texto para más detalles).

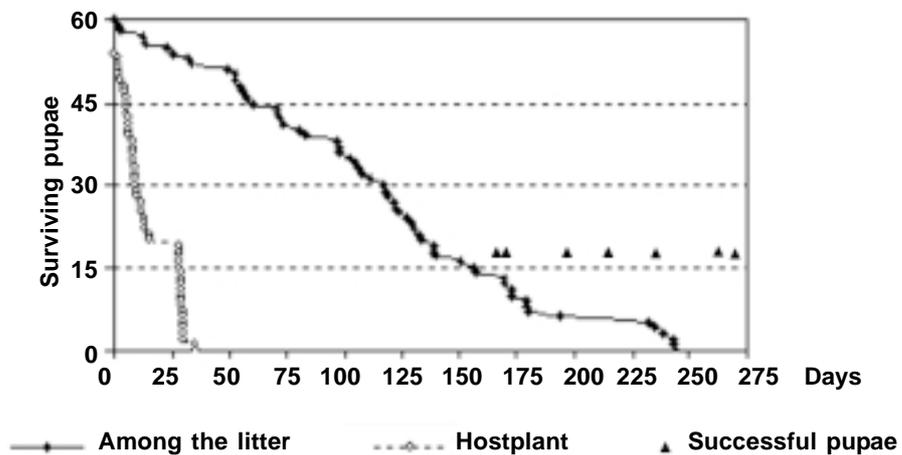


Fig. 4. Elapsed time before death for 62 pupae of *Iphiclides podalirius* naturally overwintering among the leaf litter and 54 pupae artificially placed on the hostplant in 1997–1999. The emergence time of eight successful pupae naturally overwintering among the leaf litter during the same period, is also indicated for comparison.

Fig. 4. Tiempo transcurrido antes de la muerte de 62 pupas de *Iphiclides podalirius* hibernantes entre la hojarasca, y de 54 pupas colocadas artificialmente sobre las plantas nutricias, entre 1997–1999. Se indica también, con fines comparativos, el tiempo de emergencia de los imagos procedentes de ocho pupas hibernantes entre la hojarasca, durante el mismo período.

predation, trampling and physiological deaths), the median of the elapsed time for pupal death was 109 days ($n = 52$; range: 2–238) and 11 days ($n = 54$; range: 2–36) for litter (wild) and host (artificially placed) pupae, respectively (Mann–Whitney U test = 4017.5, $df = 52, 54$; $p < 0.0001$; $p < 0.001$ in each year).

Discussion

This study supports Wohlfahrt's (1957) general conclusion that green non–diapausing and brown diapausing pupae of *I. podalirius* are produced when larvae are reared under long and short day length conditions, respectively. However, the occurrence of a single brown non–diapausing pupa in the rearing experiment (fig. 2b) suggests that pupal colour is not entirely controlled by photoperiod under long day conditions. This finding may be interpreted in the context of what is known from *Papilio polyxenes* and *P. troilus* (Hazel & West, 1979, 1983), in which short photophases cause the obligate production of brown pupae, while long photophases allow for pupal colour to vary depending on the pupation site. Therefore, though a seasonal polyphenism with respect to pupal colour seems to be the rule in *I. podalirius*, further research is required to determine which environmental cues may elicit the production of brown pupae under long day length conditions.

In *I. podalirius*, fifth–instar larvae seem to be the most sensitive to photoperiod, with the critical day length for diapause induction being positively correlated with latitude (Friedrich, 1986). In northern Spain, at a latitude of 41° 41' N, field data showed that the critical photoperiod lies between 13.7 and 14.5 h (corresponding to the first three weeks of August).

In this area, the population is bivoltine with a partial third generation. Offsprings of the first generation pupate before August and produce monomorphic green pupae (but see above), while offsprings of the third generation pupate by the end of September at the earliest and produce monomorphic brown pupae. On the other hand, offsprings of the second generation pupate in August and September and produce a mixture of green and brown pupae. This situation provided an excellent opportunity to test and confirm the prediction raised by Clarke & Sheppard (1972), that is, the existence of differences in pupation sites in successive generations related to differences in pupal colour (first and third generation) and, by extension, between green and brown pupae of the second generation. Green and brown pupae strongly differed in their pupation sites: the first were usually found on the hostplant, well above ground level, while the second were always found on the ground, among the leaf litter (tables 1 and 2).

It is broadly accepted that pupal colour in swallowtails has evolved in relation to pupation site preference (Hazel & West, 1979) and pupae are thus cryptically camouflaged in their habitats, thereby

increasing their chances of survival (Wiklund, 1975; West & Hazel, 1982; Hazel et al., 1998). In the present study, pupal mortality was very high and ranged from 14.3–57.1% for green pupae and 76.5–100% for brown pupae. However, mortalities would have been even higher if pupation site selection had not occurred. For instance, larvae that left the hostplant to produce green pupae did so mainly when they fed on one–year–old blackthorns, on which no pupal survival was recorded.

Data from the second field experiment also indicate that natural sites for overwintering pupae are adaptive: all brown pupae artificially placed on the hostplants in three consecutive years disappeared within the first month, while most of those naturally occurring among the leaf litter survived for much longer periods. Although mortality was very high in wild overwintering pupae, it is important to note that each year a considerable proportion (ca. 25%) survived long enough to have eclosed, at least in theory (fig. 4). Habitat selection also meant that pupation usually occurred in the warmer south–facing side of the hedgerow (C. Stefanescu & J. Navarro, unpublished data), allowing a faster post–diapause development of pupae, reducing their time of exposure to predation.

Although many wild pupae disappeared without trace (especially during the winter, fig. 3), direct observations and indirect evidence seem to indicate that bird predation was the major mortality factor for non–diapausing pupae and may also have a strong impact on diapausing pupae (Stefanescu, 2000b). In particular, the Great Tit regularly includes Lepidopteran pupae in its diet (e.g. Baker, 1970; Cowie & Hinsley, 1988; Cramp & Perrins, 1993); in the present study, it was the main predator of green pupae but it was also observed capturing a brown pupa among the leaf litter.

Visual (avian) predators have long been recognised as the main source of mortality for swallowtail pupae above ground–level, while non–visual predators (small mammals) are thought to be the primary predators of pupae on the ground (West & Hazel, 1982). Hazel (1995) and Hazel & West (1996) further developed this idea and reconstructed the possible events leading to the evolution of plasticity in pupal colour in swallowtail butterflies, starting with the ancestral condition of brown pupae occurring among the leaf litter. Results in the present study somewhat contradict this view: though non–visual predators (especially the shrew and hedgehog) may be responsible for a substantial part of the losses experienced by overwintering pupae, field observations indicate that birds are also probably a very important cause of mortality (see also Stefanescu, 2000b). In my study, other insectivorous birds in addition to the Great Tit (e.g. *Anthus pratensis*, *Turdus merula*, *Turdus philomelos* and *Sylvia melanocephala*) also spent many hours searching in the vegetation around overwintering pupae and should be consid-

ered as serious potential predators. Furthermore, it should be noted that the role of invertebrate predators (e.g. carabid beetles and ants) as potential predators of pupae among the leaf litter cannot, of course, be disregarded.

In relation to the hypothesis of Hazel (1995) and Hazel & West (1996), this study suggests that the selection pressure for pupation sites above ground-level in summer generations was the combination of non-visual (by invertebrates and small mammals) and visual (avian) predation that affected pupae among the leaf litter. Moreover, selective pressure would also have favoured prepupal larvae staying on the hostplant, thereby reducing mortality during the critical period of wandering in search of another pupation site (e.g. Baker, 1970; C. Stefanescu, unpublished data). The opposite behaviour, however, would have been selected in diapausing broods, taking into account the extremely high mortality to which pupae artificially placed on the hostplant are subject during the winter.

Further insight into the mechanisms leading to the selection of distinct pupation sites may be gained from detailed studies on the behaviour exhibited by larvae from either developmental pathway during the wandering phase. For instance, in another related species, *Papilio machaon* L., the duration of the pre-pupation larval wandering phase seems to be much longer for larvae of the diapausing pathway as compared to larvae of the non-diapausing pathway (C. Wiklund, pers. comm.). A similar behaviour in *I. podalirius* may well result in larvae from the non-diapausing pathway pupating on and off the hostplants when the plants are large and small, respectively, as indeed occurs. On the other hand, long wandering phases in larvae from the diapausing pathway would always result in pupae away from the hostplant. This and other aspects of the wandering phase (e.g. the timing at which it takes place and the larval orientation) will be considered in a future paper.

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