

Influence of diet and photoperiod on development and reproduction of European populations of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae)

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Abstract The current study examines the effect of photoperiod (16:08 or 12:12 h L:D) and diet (eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Phycitidae) or the pea aphid *Acyrtosiphon pisum* (Harris) (Hemiptera: Aphididae)) on the development and reproduction of the multicoloured Asian lady beetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). A long-term laboratory population of *H. axyridis* (since 1998) and a melanic and non-melanic population originating from field collected individuals of *H. axyridis* in Belgium were used in this study. Long day conditions (16 h photoperiod) shortened development of the field populations with 2–3 days when compared with short day conditions (12 h photoperiod). Oviposition in the field populations was delayed by 1–3 months when reared at a 12 h photoperiod. Dissections indicated that the females were in reproductive diapause. As compared with live pea aphids, a diet consisting of *E. kuehniella* eggs yielded heavier adult body weights (up to 12%) and increased the number of egg laying days (by 45–169%) for both field populations at a 16 h photoperiod and lengthened adult life span (by 45–92%) under both light regimens. The morph types differed in their response to the foods offered in terms of developmental rate, pre-oviposition period and number of oviposition days. The laboratory and field strains responded differentially to regimens of food and photoperiod. The study indicated a greater nutritional plasticity of the non-melanic morphs which may offer them a competitive

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advantage that may in part explain the predominance of non-melanic morphs in newly colonized areas.

Keywords *Harmonia axyridis* · Morph type · Establishment · Photoperiod · *Ephestia kuehniella* · *Acyrtosiphon pisum* · Development · Reproduction

Introduction

The multicoloured Asian lady beetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is an aphidophagous coccinellid indigenous to Central and Eastern Asia (Coderre et al. 1995; Koch 2003). Because of its high predatory effectiveness, the species was introduced as a biological control agent in both North America and Europe. However, the species succeeded in establishing wild populations on both continents (Koch 2003; Cuppen et al. 2004; Coutanceau 2006, Brown et al. 2007). Several authors recorded harmful side effects accompanying the invasion by *H. axyridis* in the USA: a dominance of the invader in coccinellid communities along with a decline in population numbers of some lady beetle species, aggregating adults causing nuisance to humans when overwintering en masse in occupied buildings and the inflicting of damage to agricultural crops (Koch 2003; Koch et al. 2004; Kenis et al. 2007; Pell et al. 2007). Due to these harmful side-effects the ladybird has lost its environment friendly image as a biological control agent and is increasingly being stigmatized as a major invasive alien species. The ecological mechanisms driving the successive colonisation of the invader in Europe are, however, still unclear.

Harmonia axyridis shows a high level of polymorphism, with more than 100 elytral patterns having been recorded (Soares et al. 2003). The morph types are commonly classified into a 'red' non-melanic group, comprising the *succinea* subgroup (0 to 21 dots, h), and a 'black' melanic group comprising the *conspicua* (two red dots, h^C), *spectabilis* (four red dots, h^S) and *axyridis* (more than four red dots, h^X) subgroups (Osawa and Nishida 1992; Serpa et al. 2003). A multi-allelic gene determines the colour polymorphism. Hosino (1940) and Tan (1946) together reported 15 alleles determining the large variation in colours and patterns. Black is generally the dominant colour, with a dominance order of $h < h^X < h^S < h^C$ in the four subgroups (Hosino 1936; Komai 1956; Sasji 1971). Colouration and maculation can to some extent also be influenced by larval diet (Grill 1999), and by temperature and humidity during pupation (Komai et al. 1950), with a higher quality diet causing a fuller red colour and a low temperature and high humidity increasing the extension of the melanic pigment. True (2003) indicates that melanic alleles, in addition to determining colour, can have pleiotropic effects on the developmental processes and life history of an individual. Soares et al. (2001, 2003, 2005) and Serpa et al. (2003) compared ecological and physiological characteristics of *H. axyridis* morphs and determined mutual dissimilarities that could support this theory. These dissimilarities could be the basis for the selection of certain genotypes in certain environments (Serpa et al. 2003; Soares et al. 2003, 2005), and consequently be the reason for both the spatial and temporal variations in the occurrence of the morphs and the maintenance of polymorphism in this species (Komai 1956; Osawa and Nishida 1992; Hodek and Honěk 1996; Krafur et al. 1997). In the Flanders region of Belgium, where established populations have been found since 2001, both melanic and non-melanic individuals occur, with a predominance of red *succinea* morph types (Adriaens et al. 2007). In Flanders, Hantson (2004) reported a proportion of 74.9% f. *succinea*, 23.1%

f. *spectabilis* and 2% f. *conspicua*. This indicates a potential role for polymorphism in the adaptation of the species to a new environment.

In the current study the effects of photoperiod and diet on the development and reproduction of European populations of *H. axyridis* were investigated. A first objective was to compare the responses of a melanic and non-melanic field population in order to judge possible differences in adaptiveness of the morph types. Second, performance of these field populations was compared to that of a long-term laboratory population that had originally been established using insects from a commercial culture that was one of the sources of biological control releases in the area since 1997 (Brown et al. 2007).

Materials and methods

Populations of *H. axyridis*

All experiments were conducted in the laboratory using three populations: a laboratory population and two field populations.

The laboratory population was started in September 1998 at the Laboratory of Agrozoology of Ghent University using larvae acquired from Biobest NV (Westerlo, Belgium). The origin of the commercial culture and the rearing methods used at its facilities were not disclosed by the company. The laboratory culture at Ghent University was infused with individuals from Biobest NV on several occasions thereafter; the last infusion was done in August 2000. Individuals of the 50th generation of laboratory rearing were used in the experiments. The population originally consisted of an even mixture of morphs, but at the time of this study, it consisted of more than 99% melanic *spectabilis* and *conspicua* morphs.

The field populations were started in April 2005 with individuals collected from an established wild population in an orchard in Wippelgem, near Ghent, Flanders. A first melanic population was established using *conspicua* and *spectabilis* individuals (referred to as the “black field population”), whereas the second non-melanic population only consisted of *succinea* individuals (referred to as the “red field population”). The very few (<5%) non-melanic offspring that occurred in the melanic field population during the process of laboratory rearing were discarded from the population upon adult emergence. Experiments were done using individuals of the 4th generation of both field populations.

All populations were reared on frozen eggs of the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Phycitidae), as described by Ongagna et al. (1993) and De Clercq et al. (2003).

Experimental design

To study the effects of diet and photoperiod on developmental time and reproductive capacity of *H. axyridis*, experiments were conducted in Petri dishes (diameter 9 cm, height 2 cm), kept in incubators held at $23 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH. The insects were exposed to two diet regimens (deep-frozen *E. kuehniella* eggs or a mixture of live late instars and adults of the pea aphid, *Acyrtosiphon pisum* (Harris) (Hemiptera: Aphididae)) and two photoperiod regimens (16 and 12 h light). Hodek and Honěk (1996) mention *A. pisum* as a natural and essential prey of *H. axyridis*, whereas *E. kuehniella* eggs are considered a suitable substitute food source. Pea aphids, fed on faba bean *Vicia faba* L., and

E. kuehniella eggs were obtained from Biobest NV (Westerlo, Belgium) and Koppert BV (Berkel en Rodenrijs, The Netherlands), respectively. Foods were always offered ad libitum and were replaced every other day. Water was provided by way of a moist paper plug fitted into a 1.5-cm plastic dish. The 16 and 12 h photoperiods were chosen to simulate late spring/early summer and early spring/early autumn light conditions, respectively. A photoperiodicity of 16 h occurs in Belgium on 25 May and 18 July, while 12 h occurs on 18 March and 25 September (Anonymous 2007).

Twenty pairs of adults (3–5 days old) were randomly taken from stock cultures of each population. The pairs were placed in individual Petri dishes, held at $23 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH and a 16 h photoperiod and left to mate and reproduce for 1 week on a mixed diet of *A. pisum* and *E. kuehniella*, to prevent food source conditioning. After this week, an accordion-pleated carton board strip (15 by 2 cm, folded 6–8 times along its length) was placed in each Petri dish to serve as an oviposition substrate and egg batches deposited during the following day were collected. The egg batches (F1) of each pair were divided into four equal sub-batches which were assigned to one of four experimental regimens: 16 h + *A. pisum*; 16 h + *E. kuehniella*; 12 h + *A. pisum*; 12 h + *E. kuehniella*. On the first day of hatching, three larvae were randomly collected per sub-batch. Each first-instar larva was then transferred to an individual Petri dish and allowed to develop to adulthood under the same regimen as experienced by the egg stage. By way of this design, 60 individuals of each population were subjected to each regimen and allowed to complete immature development. Sex of the resulting F1 adults was determined (McCornack et al. 2007) and per population 20–25 pairs were formed from the cohort of individuals that had been exposed to the same regimen. The pairing of brothers and sisters was carefully avoided when composing adult pairs. Each pair was then placed in an individual Petri dish and subjected to the same regimen as during immature development in order to monitor reproductive performance.

Fitness parameters

Several fitness parameters were assessed to evaluate effects of the tested regimens on the developmental and reproductive performance of the F1-individuals of the three *H. axyridis* populations.

Developmental duration of the immature stages was monitored on a daily basis. The total developmental period was defined as the period from oviposition to adult emergence. Newly emerged adults were starved for one day and weighed on a semi-microbalance Sartorius Genius ME215P (Sartorius AG, Goettingen, Germany) (± 0.01 mg).

Body length of adults was measured at the time of death. The distance between the anterior margin of the pronotum and the posterior margin of the elytra was measured with an ocular micrometer.

Reproduction of females was characterized using four parameters: pre-oviposition period, number of oviposition days, size of egg batches and egg hatch. To determine the pre-oviposition period and the number of oviposition days, each Petri dish was checked daily for oviposited eggs. The values for egg batch size and egg hatch were determined on a weekly basis. Once a week a fresh accordion-pleated carton board was introduced into each Petri dish; the following day the carton board was removed and the eggs were collected and counted to calculate mean batch size. The collected eggs were transferred to a new Petri dish, placed under the same regimen as that experienced by the ovipositing female adult and were checked daily for hatching. In addition, longevity of female adults

was determined. The experiment was terminated 210 days after F1 egg hatching. At that moment all individuals of the laboratory population had died, whereas about 15% of the individuals of both field populations fed *A. pisum* at 16 h light and between 0 and 5% of the individuals in the remaining treatment groups were still alive.

Dormancy

A significant delay in oviposition in the main experiment described above (see “Results”) suggested the occurrence of dormancy in the field populations reared at a 12 h photoperiod. A second experiment was set up to confirm this finding. In this experiment a cohort of 60 larvae from either field population was allowed to develop to adulthood and reproduce, while fed *E. kuehniella* at a 12 h photoperiod. The state of dormancy of the resulting adult females was judged by performing dissections 4 and 7 weeks after emergence. Ten randomly chosen females of each population were dissected each time. The state of dormancy was evaluated in a qualitative manner, by visually inspecting gut content and the developmental status of the fat body and ovaria in comparison with reproducing females of the same populations fed *E. kuehniella* eggs at 23°C and a 16 h photoperiod. At the start of diapause, female adults of *H. axyridis* have a well developed fat body and a full gut. Fat body degenerates and the gut becomes emptied of food during dormancy. Ovarian development is also suppressed during dormancy (Sakurai et al. 1992; Iperti and Bertand 2001).

Data analysis

Data analysis was carried out in Stata/SE 9.2 (StataCorp 2005). A generalised linear model was used with the link function and error distribution depending on the nature of the data (Poisson distribution for count data and negative binomial distribution in case of over dispersion). This generalized linear model is an extension of the general linear model and allows the use of error distributions other than the normal distribution. The model uses the principle of the regression analysis and thus tests each time the difference between the average of the reference group and the average of the group in question. Thus, each test consists of a regression coefficient being calculated and tested for being significantly different from zero, for which *P*-values are presented (McCullagh and Nelder 1989). If none of the parametric models could be used, a bootstrapped median regression was applied. Analysis started with a saturated model and interactions and non-significant main factors were dropped at a significance level of 0.05. Each saturated model contained four factors: population (laboratory, red field or black field), photoperiod (16 or 12 h), diet (*E. kuehniella* or *A. pisum*) and sex (male or female). The most parsimonious model is reported, using likelihood ratios to assure model fit.

Results

Fitness parameters

The results of the fitness assessment for the three populations exposed to the four tested regimens are presented in Figs. 1 and 2. A significant correlation between body weight and

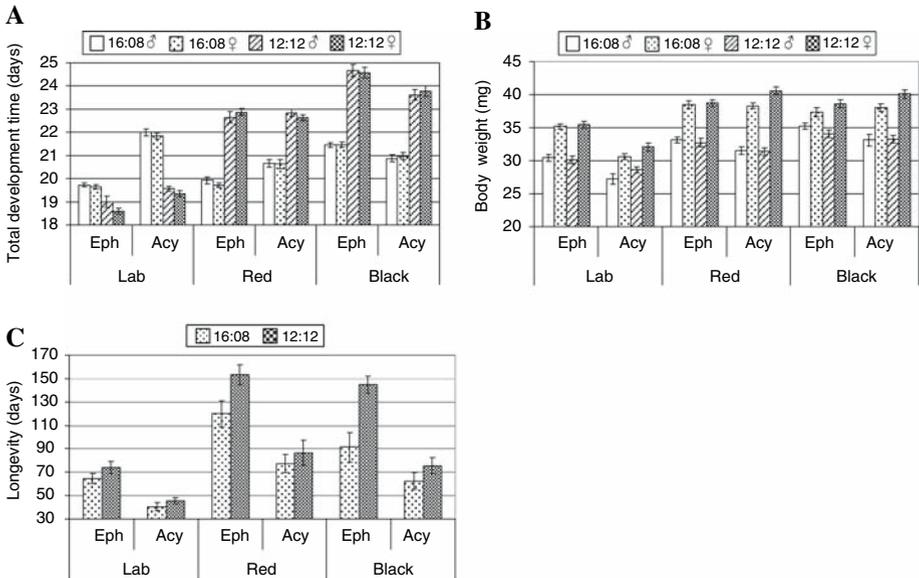


Fig. 1 Total development time (means ± SE, days) (a) and body weight (means ± SE, mg) (b) of males and females and longevity of females (means ± SE, days) (c) of a laboratory population (Lab) and a red (Red) and black (Black) field population of *Harmonia axyridis* at different photoperiods (16:08 and 12:12 h L:D) and diets (*Ephesthia kuehniella* (Eph) and *Acyrtosiphon pisum* (Acy))

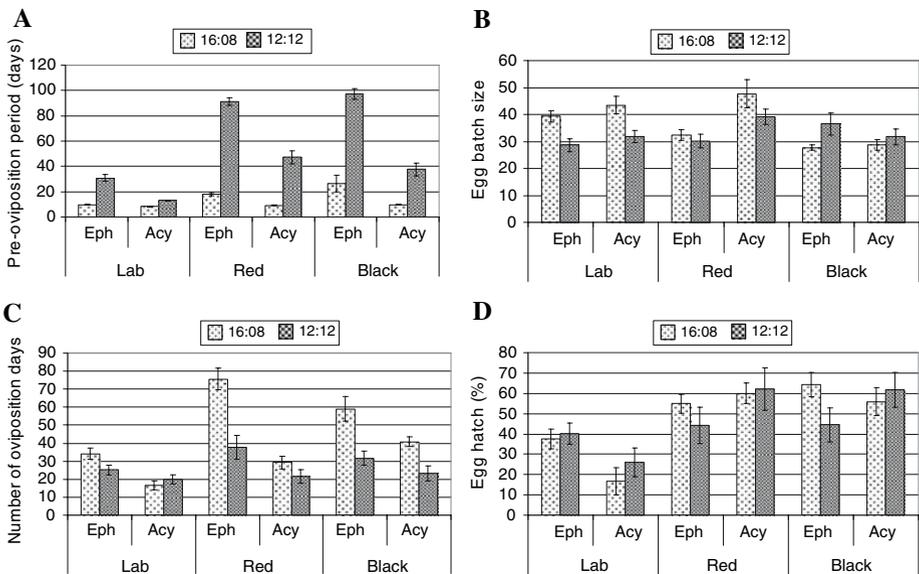


Fig. 2 Pre-oviposition period (means ± SE, days) (a) egg batch size (means ± SE) (b) number of oviposition days (means ± SE) (c) and egg hatch (means ± SE, %) (d) of a laboratory population (Lab) and a red (Red) and black (Black) field population of *Harmonia axyridis* at different photoperiods (16:08 and 12:12 h L:D) and diets (*Ephesthia kuehniella* (Eph) and *Acyrtosiphon pisum* (Acy))

body length was found for adults of either sex ($r = 0.68$, $P < 0.001$ and $r = 0.475$, $P < 0.001$ for males and females, respectively). For this reason only data on body weight are presented.

A late spring/early summer photoperiod of 16 h shortened larval and pupal development ($P < 0.001$ for both sexes) compared to an early spring/early autumn photoperiod of 12 h (Fig. 1a). Equally, the pre-oviposition periods of all three populations were shortened at 16 h light ($P = 0.002$ for the laboratory population fed *A. pisum*, $P < 0.001$ for all other cases) (Fig. 2a). Females of all populations lived longer at 12 h than at 16 h light ($P = 0.002$) (Fig. 1c). The 16 h light regimen increased the number of oviposition days of both field populations fed *E. kuehniella* ($P < 0.001$ for the red population and $P = 0.009$ for the black one) and of the black field population fed *A. pisum* ($P < 0.001$) (Fig. 2c).

Although *E. kuehniella* eggs caused longer pre-oviposition periods than aphids ($P < 0.001$ in all cases except for the laboratory population reared at a 16 h photoperiod) (Fig. 2a), they yielded an increase in the number of egg laying days for both field populations at a 16 h photoperiod ($P < 0.001$ for the red field population, $P = 0.001$ for the black field population) (Fig. 2c). A diet of *E. kuehniella* eggs furthermore increased life span of the females under both light regimens ($P < 0.001$) (Fig. 1c) and yielded heavier adult body weights at long day conditions ($P < 0.001$ for both sexes) (Fig. 1b).

Two marked differences between the red and black morphs were found in this study. First, there was a differential effect of the diet on development and reproduction of the morphs. When fed *E. kuehniella* eggs, the red individuals developed faster than the black ones, a difference that was marginally significant at a 16 h photoperiod ($P = 0.055$ for both sexes), but significant at a 12 h photoperiod ($P = 0.016$ for both sexes) (Fig. 1a); there was, however, no difference in developmental time between the populations when fed *A. pisum* (Fig. 1a). The black field population had a shorter pre-oviposition period than the red field population when reared at a 12 h photoperiod on *A. pisum* ($P = 0.044$), but the inverse was the case at 16 h light on *E. kuehniella* ($P = 0.003$) (Fig. 2a). When fed *A. pisum* under long day conditions the black field population had a higher number of oviposition days than the red population ($P = 0.035$) (Fig. 2c). Furthermore, the difference in the number of oviposition days between diets was greater for the red field population than for the black field population (Fig. 2c), whereas the opposite was found for the pre-oviposition period (Fig. 2a). Second, in all treatment groups the red morphs were longer lived than the black morphs ($P = 0.043$) (Fig. 1c).

Several differences were found between the performances of the field populations and the laboratory population. In contrast to the field populations, longer day length resulted in a significantly longer developmental time for the laboratory population ($P = 0.008$ for both sexes) and *E. kuehniella* eggs shortened the developmental time of the laboratory population compared with *A. pisum* ($P = 0.017$ for both sexes) (Fig. 1a). Both field populations had a longer developmental time ($P < 0.001$ for both sexes) (Fig. 1a) and pre-oviposition period ($P < 0.001$) (Fig. 2a) than the laboratory population when reared at a 12 h light regimen, irrespective of diet, and a longer pre-oviposition period at 16 h light on *E. kuehniella* ($P < 0.001$). When fed *E. kuehniella* eggs at a 16 h photoperiod both field populations oviposited on more days than did the laboratory population ($P < 0.001$ for the red field population and $P = 0.006$ for the black field population) (Fig. 2c). On *A. pisum*, egg hatch in the laboratory population was lower than that in both field populations ($P < 0.001$) (Fig. 2d). Females of both field populations lived longer than those of the laboratory population in all cases ($P < 0.001$) (Fig. 1c). Males and females of the field populations weighed more than those of the laboratory population under all diet and light regimens ($P \leq 0.001$ for both sexes) (Fig. 1b).

Dormancy

As females of the field populations showed prolonged pre-oviposition periods at a 12 h photoperiod in the main experiment, a second experiment was done to confirm if rearing at 12 h light on *E. kuehniella* eggs induced a state of reproductive diapause in the red and black field populations. Females of either morph again went through a prolonged pre-oviposition period like those in the first experiment. Dissections done 4 and 7 weeks after adult emergence revealed that females reared at 12 h light had a lower amount of fat body compared to those kept at a 16 h photoperiod. All females maintained under short day conditions had empty ovaries and empty or quasi empty guts. Suppressed activity, aggregation behaviour and a minimal food uptake of male and female adults reared at a 12 h photoperiod were also observed during the prolonged pre-oviposition period.

Discussion

Photoperiod had a significant influence on developmental and reproductive performance of *H. axyridis*. Ongagna and Ipert (1994) also observed a significant decrease in the developmental duration of *H. axyridis* at a 16 h photoperiod compared to shorter photoperiods varying between 9 and 12 h of light. Omkar and Pathak (2006) reported similar findings for the lady beetle *Coelophora saucia* (Mulsant) (Coleoptera: Coccinellidae) and ascribed the greater fitness of the insect at a 16 h photoperiod to the feeding behaviour of lady beetles. Coccinellidae are diurnal organisms and thus most likely only feed during light conditions (Hodek and Honěk 1996). The individuals can therefore consume more prey under a 16 h light regimen and ad libitum food conditions, which in turn benefits development and reproduction.

In the field populations, oviposition was delayed by 1–3 months when held at short day conditions. A shortening of day length in the autumn, prey scarcity and low temperatures are cues for adult *H. axyridis* and other lady beetles to prepare for their winter dormancy (Ongagna and Ipert 1994; Hodek and Honěk 1996). The prolongation of the pre-oviposition period found in this study indicates a state of reproductive diapause induced by the short day length during larval development. This is supported by the physiological state (gut content, fat body and ovarian development) of the dissected adult individuals and the behaviour observed during the prolonged pre-oviposition period (low activity, aggregation and minimal food uptake). The reproductive diapause in the field populations lasted longer when *E. kuehniella* was provided as food. These observations may indicate that *E. kuehniella* eggs are not nutritionally optimal for maturing eggs directly out of dormancy. The delay of oviposition at short day conditions was much less pronounced for the laboratory population. This can be due to the loss of photoperiodic response after prolonged rearing at a 16 h light regimen. As also reported by Michaud and Qureshi (2006) for *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), the adults of *H. axyridis* undergoing a reproductive diapause lived longer in our study, suggesting a trade-off between the reproductive capacities and survival.

Ephestia kuehniella eggs appeared to be a better food for *H. axyridis* than live pea aphids based on the results of this study. Eggs of *E. kuehniella* have been shown to be an effective unnatural food for the production of different insects, including coccinellids (Specty et al. 2003; De Clercq et al. 2005). Specty et al. (2003) found that *E. kuehniella* eggs were nutritionally superior to *A. pisum* in terms of amino acid and fatty acid content and composition.

Better performance of the *H. axyridis* populations tested in this study on *E. kuehniella* eggs under long day conditions may be the result of artificial selection. The laboratory population was reared on *E. kuehniella* and at long day photoperiods for ca. 50 generations at our facilities and originated from a commercial source that most probably used the same conditions during mass culturing (Kabiri 2006). Also the field populations were reared on *E. kuehniella* eggs and at a 16 h photoperiod in the laboratory for three generations prior to the experiments and may originally have sprung from the same commercial source.

Like in the current study and in Berkvens et al. (2007), Soares et al. (2001, 2005) also found dissimilar reproductive and food assimilating responses among morph types of *H. axyridis* to various food sources. The findings imply that red morphs may be nutritionally more adaptive and may be more able to exploit new and unknown food sources, such as the factitious food *E. kuehniella* in our study. Their greater nutritional plasticity may offer non-melanic morphs a competitive advantage for the exploitation of food sources during establishment and may in part explain the predominance of non-melanic morphs in newly colonized areas (Koch 2003; Hantson 2004). Surprisingly, however, a laboratory population of *H. axyridis* was predominated by black morphs after some years of laboratory rearing on a factitious host, suggesting that other factors are involved in morph determination. Selective factors that could have played a role in this increase in melanics during laboratory culturing include the genetic dominance of melanism in *H. axyridis* (Komai 1956), a potentially higher mating success of *H. axyridis* melanics in the summer conditions used in the laboratory (Osawa and Nishida 1992), and the reported increase in melanism at high population densities in several insects (True 2003).

Serpa et al. (2003) also observed a difference in adult longevity between certain morph types of *H. axyridis*. They found that the redder morph, *aulica*, had a longer life span compared to a completely black morph, *nigra*. One of the explanations might be thermal melanism, which suggests that when insolated, dark ectothermic insects heat up faster and reach a higher equilibrium temperature. This results in a higher level of activity and a reproductive advantage, but equally a lower longevity (Serpa et al. 2003). However, since our experiments were carried out in climate chambers using fluorescent lamps with low radiation, thermal melanism is not a valid explanation for the shorter life span observed in the black morphs.

A laboratory population of *H. axyridis* was included in the experiments in order to compare its performance with that of the field populations. Individuals of the laboratory population showed different developmental and reproductive responses to the tested regimens of diet and photoperiod from those of the field populations. The observed differences between the field and laboratory populations may be due to inter-population variation within *H. axyridis*. However, the broad consistency in the morph frequency data from Europe provides some evidence of the genetic similarity of the populations found in different European countries and suggests that the species has spread from limited points of origin in Europe (Brown et al. 2007). If the field and laboratory populations used in this study would thus have the same origin, the differences observed here may also indicate that prolonged rearing may have altered the biological characteristics of the insect as a result of genetic and non-genetic adaptation (Mackauer 1972, 1976; Chambers 1977; Grenier and De Clercq 2003). As noted above, the long term use of an unnatural food like *E. kuehniella* eggs and long day photoperiods in the laboratory may have led to unintentional selection in *H. axyridis*.

In conclusion, this laboratory study has demonstrated differential effects of photoperiod and diet on the developmental and reproductive fitness of the morph types of a European population of *H. axyridis*. A different capability to exploit available food sources may lead

to a competitive advantage that may in part explain the predominance of non-melanistic morphs in newly colonized areas. Further research is needed to fully understand the bearing of the ecophysiological differences among the morph types of *H. axyridis* on the establishment potential of the species.

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