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Hot summers, long life: egg laying strategies of *Maniola* butterflies are affected by geographic provenance rather than adult diet

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Abstract

Maniola butterflies undergo summer dormancy in dry and hot habitats and deposit their eggs only in early autumn when conditions become more favourable for their offspring. Female individuals of this genus are therefore relatively long-lived. For long-lived butterflies adult diet is of particular importance. We tested if added amino acids in nectar substitute fed to the butterflies affected timing of oviposition, fecundity and longevity. A hundred *Maniola* females were sampled from Mediterranean and Central European populations and made to oviposit under controlled laboratory conditions. Forty individuals were offered sucrose solution with additional amino acids while the remainder were fed with plain sucrose solution. We found that egg-laying strategies and longevity depended on geographic provenance rather than diet. Supplementary amino acids in adult diet did neither prolong lifetime nor increase total egg production. *Maniola* females from Sardinia started to lay eggs at least 20 days later relative to Central European *M. jurtina* and lived three times as long. Mediterranean individuals had on average twice the length of reproductive period and lifespan relative to Central European ones, and individuals of Pannonian origin lived longer than Alpine butterflies. Average total egg numbers were 200-350 eggs per female and did not differ significantly between populations. The fact that oviposition strategy could not be altered through diet may indicate that for univoltine butterflies, like *Maniola*, diet-quality at the adult stage is less important than endogenous factors, or factors the butterflies are exposed to in an earlier developmental stage than the imago. Oviposition strategy closely matched the climatic conditions that prevail in the geographic regions where these butterflies fly.

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Introduction

The Meadow Brown butterfly, *Maniola jurtina* L., is a widespread western Palaearctic butterfly that lays its eggs on various grass species (Brakefield, 1982a, b; Ebert und Rennwald, 1991, Schweizerischer Bund für Naturschutz, 1987). Adults are strictly univoltine and are on the wing from late May to early October, depending on altitude and climate. As typical for univoltine species (Danks, 2006; Košťál, 2006), this butterfly is adapted to the environmental conditions of its habitat to optimally use seasonally limited resources, in particular larval host plants. As a consequence, it exhibits large ecological plasticity with respect to oviposition strategies. In dry and hot conditions with no grass available for caterpillars to feed on during the summer months, females perform a summer dormancy (aestivation) accompanied by delayed ovarian maturation (Masetti and Scali, 1972). This means that eggs may be kept in the abdomen up to several months after mating and are deposited only in late summer or early autumn, when first rainfall allows for re-growth of grass. It also means that these females are relatively long-lived. For such long-lived butterflies adult diet is of particular importance.

Adult diet can extend lifespan and enhance fertility, as has been shown for example in tropical *Bicyclus* species (Brakefield and Kesbeke, 1995) and *Charaxes fulvescens* Aurivillius, 1891 (Molleman *et al*., 2008). These feed on rotting fruits and hereby enrich their diets with amino acids. Temperate-zone butterflies, like *Maniola jurtina*, usually rely on floral nectar containing mostly sugars.

The role of carbohydrates in butterfly nutrition is fairly well understood (Bauerfeind *et al.*, 2007 and references therein). Nectar-derived sugars obviously fuel the animals for flight and can also be incorporated into the eggs (Moore and Singer, 1987; Watanabe, 1992; Boggs, 1997; Fischer and Fiedler, 2001), but the role of other substances in adult butterfly nutrition is still a matter of controversy.

Nitrogen is a limiting factor for many herbivorous insects, in particular butterflies (Morehouse and Rutowski, 2010 and references therein; Pellissier *et al.*, 2012). The reason for this are the discrepancies between the structural compounds used by plants to provide support for their tissues (carbon-based polysaccharides such as cellulose and lignin) and the protein complexes needed by insects to construct their bodies (Fagan *et al.*, 2002). As nectar contains little nitrogen (Baker and Baker, 1973, 1975), adults largely depend on larval resources for investment in reproduction, which leads to a depletion of nitrogen stocks during reproduction (Takeuchi, 2012). Additional amino acid content in adult food resources may compensate for this nitrogen loss and lead to greater longevity and larger quantity of eggs. That this can be the case, has been beautifully shown in an experiment with the Map Butterfly, *Araschnia levana* L. (Mevi-Schütz and Erhardt, 2005), where additional amino acids in the nectar increased the number of eggs laid. Similarly, Boggs (1986, 1997) pointed to the importance of adult nutrition for the reproductive potential, and Geister *et al.* (2008) have demonstrated that fecundity (measured as the number of eggs laid per lifetime) of tropical *Bicyclus anynana* (Butler, 1879) was higher when the individuals were fed banana fruits, containing amino acids, than when fed plain sucrose solution. In many cases, however, nitrogen income during the adult stage had no effect on fecundity (Fischer *et al.*, 2004; Moore and Singer, 1987; Hill and Pierce, 1989; Mevi-Schütz and Erhardt, 2003).

Here, we investigated if food resources available to adult *Maniola* butterflies affect fecundity (*i.e.* lifetime quantity of eggs), longevity and egg-laying behaviour. Particularly for females which undergo summer dormancy and consequently live about three times as long as their conspecifics from cooler climates, adult nutrition ought to be crucial for the reproductive success and additional amino acid intake should supposedly have beneficial effects on fecundity and lifespan.

To test this, we compared *M. jurtina* butterflies from populations with expected summer-dormancy with populations without summer-dormancy, including a population of the Sardinian endemic *M. nurag*, and tested experimentally if increased intake of amino acids could alter (a) timing of oviposition (*i.e.* days from capture to oviposition), (b) fecundity (*i.e.* total number of eggs laid), (c) lifespan of adult females, (d) the length of the reproductive period (*i.e.* number of days while eggs were deposited), or (e) post reproductive lifespan.

Material and methods

A hundred adult female butterflies were collected 'fresh' (*i.e.* shortly after onset of population emergence in the field, but already mated as shown by the deposition of fertile eggs later on) from the following localities (Table 1): the island of Sardinia, Italy (10 individuals: 7 *M. nurag*, 3 *M. jurtina*); vicinity of Illmitz (Burgenland), eastern lowland Austria (12 individuals in

Table 1. Number of individuals sampled per geographic region/species.

Locality	Climatic provenance	Altitude (m a.s.l.)	Diet	Species	Individuals	Year (n)	Latitude	Longitude
Sardinia	Mediterranean	700	sugarwater	*M. jurtina*	3	2011	39.239	9.386
Sardinia	Mediterranean	703	sugarwater	*M. nurag*	7	2011	39.248	9.393
Burgenland	Pannonian	114	added amino acids	*M. jurtina*	16	2005	47.765	16.866
Burgenland	Pannonian	114	sugarwater	*M. jurtina*	5	2005	47.765	16.866
Burgenland	Pannonian	110	sugarwater	*M. jurtina*	12	2011	47.794	16.771
Krk	Mediterranean	50	added amino acids	*M. jurtina*	12	2005	45.032	14.498
Krk	Mediterranean	50	sugarwater	*M. jurtina*	16	2005	45.032	14.498
Mariahilferberg	Alpine	700	added amino acids	*M. jurtina*	12	2005	47.870	15.877
Mariahilferberg	Alpine	700	sugarwater	*M. jurtina*	17	2005	47.870	15.877
Total					100			

2011 and 21 individuals in 2005: *M. jurtina*); the island of Krk, Croatia (28 individuals: *M. jurtina*); and Mariahilfer Berg/Unterberg (Lower Austria), eastern montane Austria (29 individuals: *M. jurtina*). Individuals from lowland Austria hereafter are referred to as 'Pannonian', individuals from Krk and Sardinia are termed 'Mediterranean', and individuals from Mariahilfer/Unterberg are referred to as 'Alpine' populations.

Butterflies were kept individually in 1-litre plastic containers lined at the bottom with humid paper towels, under 24h light-dark cycles with a photoperiod of L12:D12 at 24°C (light) and 16°C (dark). Sugar water (5% sucrose solution dissolved in tap-water) was available *ad libitum* to the animals as a source of carbohydrates, and fresh cut grass was provided as egg-laying substrate. For 40 of the individuals collected in 2005, a commercial mixture of twenty amino acids (Alanine, Arginine, Asparagine, Aspartic acid, Cysteine, Glutamic acid, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, Valine) as used as human diet supplement was added to the sugar water as extra nitrogen source for these unusually long-lived butterflies. Containers were checked for eggs daily, and all eggs were removed after counting so that each count indeed reflected the number of eggs deposited at a given date.

Data were first analyzed separately for each population from the two different years. When we found no significant differences between years in populations from the Pannonian region, we pooled the data from 2005 and 2011 for subsequent analysis.

Only individuals that, once in captivity, had a reproductive period longer than five days and produced 10 or more eggs were included in the analyses.

For each individual we recorded the following five variables:

(a) timing of oviposition (= the number of days from capture to first egg laid)
(b) fecundity (= total number of eggs laid during entire lifetime)
(c) lifespan (= number of days from capture to death)
(d) reproductive period (= number of days from when first egg was laid till the day when the last egg was laid)
(e) post reproductive lifespan (= number of days lived after deposition of last egg)

To obtain normal distribution of the variables they were square-root transformed or log(*e*) transformed (for total number of eggs). Square-root or log transformed data were tested for significant effects of geographic provenance or diet in one-way ANOVAs followed by Tukey's post hoc test for multiple comparisons. To test for the effects of diet and population membership on lifespan, fecundity and timing of oviposition in *M. jurtina* (*M. nurag* did not receive extra amino acids, as there were too few individuals to create a control group) we used two-way ANOVA. All statistical analyses were performed in the program Statistica 8.0 (StatSoft, 2005).

Results

No diet effects were found (Fig. 1, Table 2). A diet spiked with additional amino acids did not influence fecundity, lifespan or any of the other variables recorded from our butterflies. The only significant effects very strongly supported by GLM analysis, ANOVAs and Tukey's post hoc tests ($p < 0.001$) regarded lifespan and oviposition strategy relative to population provenance (Tables 2-3).

Oviposition strategies

Timing of oviposition clearly depended on population membership, which in this study equals geographical provenance. Tukey's post hoc tests (Table 3) showed significant pair-wise differences ($p < 0.001$) between *M. nurag* and *M. jurtina* females from Sardinia and all the other populations; Pannonian populations did not differ from Alpine ones with regard to the start of egg-laying (Fig. 1c, 2 and 3a). The Pannonian sugar-fed group started egg-laying later than the amino acid-fed group (Fig. 1c), but this effect was only significant at the 0.02-level (Table 2).

Maniola nurag and *M. jurtina* from Sardinia started ovipositing not before 22 days after capture, whereas *M. jurtina* from all other regions started immediately or a few days after capture (Fig. 2). Mean number of days until the first egg was deposited was significantly different between individuals from Sardinia (*M. jurtina* and *M. nurag*) and the rest (Fig. 3a). Length of the reproductive period was on average ten days longer in *M. nurag* and Mediterranean *M. jurtina* compared to Pannonian individuals, and in those again about five days longer than in Alpine individuals. It varied significantly between Central European (= Alpine and Pannonian) and Mediterranean (= populations from Krk and Sardinia) populations (Fig. 3c).

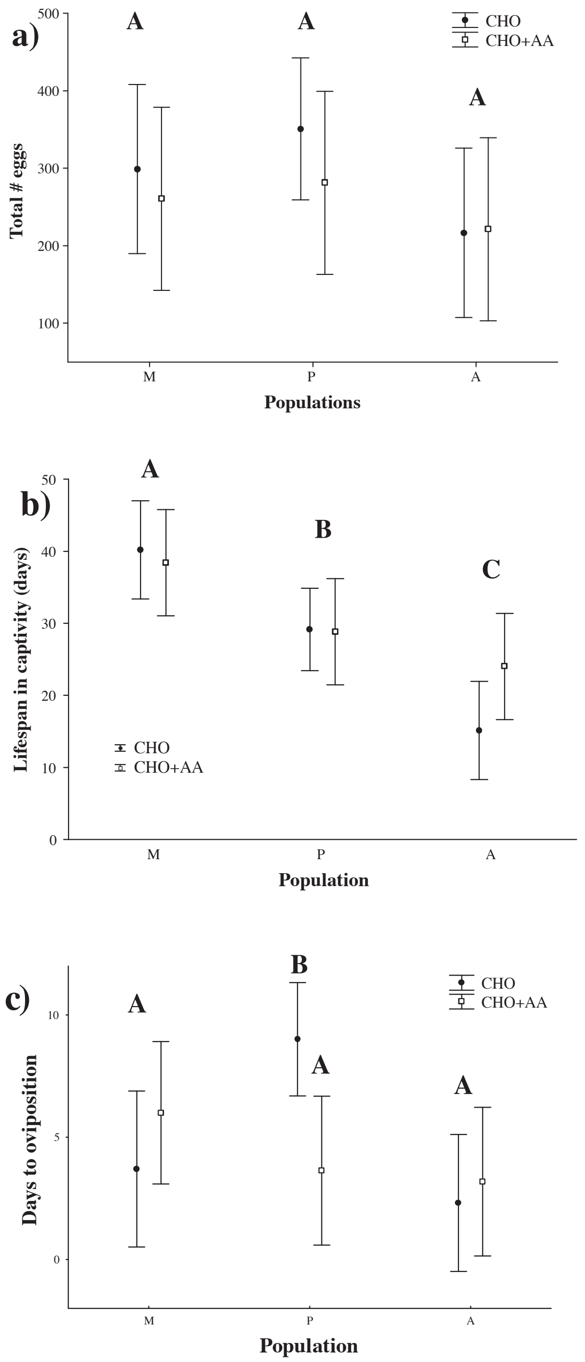


Fig. 1. Mean (± SD) total number of eggs (a), lifespan (b) and days to oviposition (c) of *Maniola jurtina* from different geographic provenance. M = Mediterranean, P = Pannonian, A = Alpine. Supplementary amino acids in the carbohydrate solution fed to (open squares) the adult butterflies' did not enhance egg production and longevity, or speed up oviposition, compared to individuals fed only with carbohydrate solution only (black circles). Significant differences ($p < 0.02$) between populations are indicated with different letters (compare Table 2).

Total numbers of eggs deposited did not differ between populations, nor were they augmented by a diet of supplementary amino acids (Fig. 1a, Table 3).

Lifespan

For lifespan, Tukey's post hoc tests showed a clear differentiation ($p < 0.001$) between Alpine and all other *M. jurtina* populations, and between *M. nurag* and Pannonian and Alpine *M. jurtina* populations (Fig. 3b, Table 3).

Females of *M. nurag* and Sardinian *M. jurtina* lived on average longer (max. 76 and 74 days, respectively) than Central European *Maniola*. *M. nurag* females lived around 60 days (Fig. 3b), that is twice as long as Pannonian *M. jurtina* females and three times as long as females from the Alpine population. *M. jurtina* individuals from Mediterranean populations (max. 52 days) lived on average ten days longer than Pannonian populations (max. 62 days) and twice as long as individuals from Alpine populations (max. 44 days), which typically lasted for around 20 days.

Only in *M. nurag* longer lifespan was paralleled by delayed oviposition and a longer reproductive period. Mediterranean *M. jurtina* lived longer and had a longer reproductive period than Alpine individuals, but no delayed oviposition (Figs 3-4). The latter, however, might be an artefact resulting from a late collection date of the individuals from Krk plus the time required transporting them from the field to the lab.

Post-reproductive lifespan (*i.e.* after cessation of oviposition) lasted between 0 and 32 days and did not differ with regard to provenance.

Discussion

Does adult nutrition affect adult longevity and fecundity?

Additional amino acids in the adults' nutrition had no measurable effects on longevity and fecundity of *Maniola* butterflies. Given their relatively long adult lifespan, we would have expected the Mediterranean females to benefit from additional nitrogen intake and live longer or produce larger quantities of eggs. An explanation for the lack of such a benefit could be that the butterflies had sufficient nitrogen intake at the larval stage and therefore supplementary amino acids did not affect adult butterflies, similarly as shown for *Araschnia levana* by Mevi-Schütz and Erhardt, 2005).

Table 2. Results of two-way ANOVA for effects of diet and population membership on lifespan, fecundity and timing of oviposition in *M. jurtina*. Diet = sucrose solution with ($N = 40$) or without ($N = 53$) additional amino acids.

		df	F	p
Lifespan	constant	1	424.685	< 0.001
	population	2	15.348	< 0.001
	diet	1	0.625	0.433
	population x diet	2	1.330	0.271
	error	78		
Fecundity	constant	1	165.913	< 0.001
	population	2	2.318	0.106
	diet	1	0.396	0.532
	population x diet	2	0.161	0.852
	error	70		
Days to oviposition	constant	1	61.170	< 0.001
	population	2	3.215	0.046
	diet	1	0.379	0.540
	population x diet	2	4.160	< 0.019
	error	70		

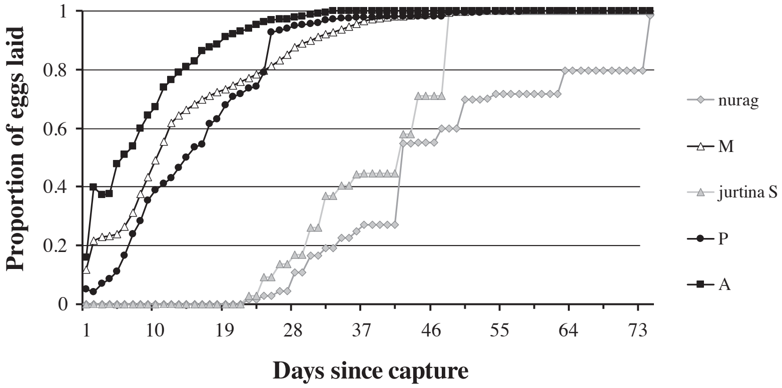
Table 3. Results of one-way ANOVAs (presented are values of the F statistic accompanied by their p-values) followed by Tukey's HSD post hoc tests for multiple comparisons (given are p-values for pairwise comparisons) to analyze the effects of population membership on days to oviposition, fecundity, lifespan in captivity, reproductive lifespan, and post-reproductive lifespan for populations of *M. jurtina* and *M. nurag* of different geographic origin (Mediterranean: $N = 28$; Pannonian: $N = 33$, Alpine: $N = 29$, *M. nurag*: $N = 7$, *M. jurtina* Sardinia: $N = 3$).

Trait	df	F	p
one-way ANOVAs			
days to oviposition (days)	4	17.048	< 0.001
fecundity (n total number of eggs)	4	1.636	0.175
lifespan (days)	4	14.276	< 0.001
reproductive period (days)	4	5.691	< 0.001
postreproductive lifespan (days)	4	2.586	0.044

Tukey's post hoc test

Days to oviposition	Mediterranian	Pannonian	Alpine	*M. nurag*
Pannonian	0.785			
Alpine	0.559	0.053		
M. nurag	< 0.001	< 0.001	< 0.001	
M. jurtina Sardinia	< 0.002	< 0.006	< 0.001	0.975
Fecundity				
Pannonian	0.867			
Alpine	0.822	0.219		
M. nurag	0.683	0.351	0.947	
M. jurtina Sardinia	0.999	0.963	1.000	0.977
Lifespan				
Pannonian	0.125			
Alpine	< 0.001	0.004		
M. nurag	0.053	< 0.001	< 0.001	
M. jurtina Sardinia	0.988	0.607	0.047	0.680
Reproductive lifespan				
Pannonian	< 0.020			
Alpine	< 0.001	0.493		
M. nurag	0.997	0.676	0.213	
M. jurtina Sardinia	0.651	1.000	0.991	0.875
Postreproductive lifespan				
Pannonian	0.991			
Alpine	0.271	0.080		
M. nurag	0.979	0.997	0.458	
M. jurtina Sardinia	0.512	0.394	0.934	0.435

Fig. 2. Relative cumulative number of eggs (means) in relation to day since capture for populations from different geographic provenance: 'nurag' = *M. nurag*, 'jurtina S' = *jurtina* from Sardinia, 'A' = *jurtina* from mountain populations, 'P' = *jurtina* from Pannonian populations, 'M' = *jurtina* from the island of Krk.



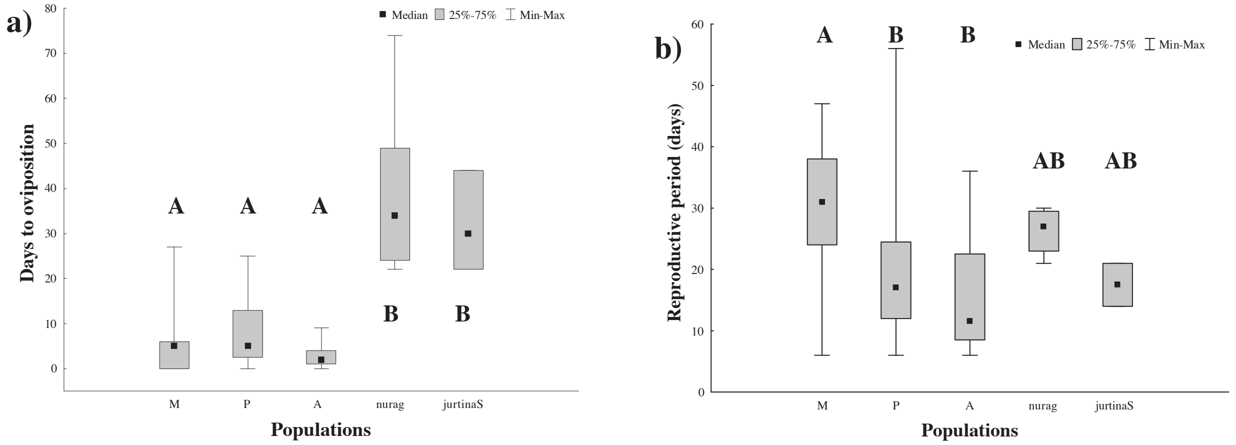
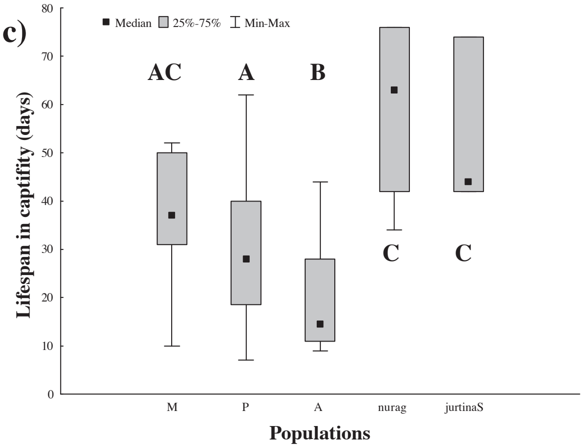


Fig. 3. Mean number of days (a) till the onset oviposition, (b) total lifespan in captivity, and (c) duration of reproductive period in relation to geographic provenance of Meadow Brown populations. M = Mediterranean, P = Pannonian, A = Alpine, nurag = *M. nurag*, jurtinaS = *M. jurtina* from Sardinia. Significant differences ($p < 0.001$) between populations are indicated with different letters (compare Table 3).



In Mevi-Schütz and Erhardt's study those butterflies which were raised on nitrogen-poor larval food resources reacted positively to nectar enhanced with amino acids, while butterflies from caterpillars raised on nitrogen-rich food did not react to increased amino acid intake. In *Araschnia levana*, a lack of amino acid intake at one life history stage can thus be compensated at a later stage.

The *Maniola* adults we used in our experiment did most probably not suffer from nitrogen limitation at the larval stages. Meadows from where they were collected were all situated in the proximity of fertilized farmland, or they were grazed by sheep and goats, which also produces additional nitrogen input. Moreover, although widespread across Europe, *M. jurtina* has been reported to suffer from too much nitrogen input at highly fertilized meadows, from where it usually disappears, moving to more natural and nitrogen-poor grasslands (Ebert and Rennwald, 1991; Schweizerischer Bund für Naturschutz, 1987). *Araschnia levana* typically occurs in nitrogen-rich humid habitats such as floodplain forests, where its larval host plant, *Urtica dioica*, thrives. It is thus also not nitrogen limited in its natural habitats. For *Pieris rapae* (Morehouse and Rutowski, 2010), nitrogen availability is the key factor for growth and development at the larval stage, whereas carbohydrates are less constraining (for the larvae!) and can be compensated by increased adult feeding with sugar. Larvae of the Meadow Brown, *M. jurtina*, do not appreciate too much nitrogen in their diet, and the same has been shown for a number of other grassland butterfly species (*e.g.* Fischer and Fiedler, 2000).

Additional amino acids in the nectar can thus be favourable for butterflies, but only under certain conditions (Boggs, 1986); in many cases nitrogen income during the adult stage has no effect on fecundity (Fischer *et al*., 2004; Moore and Singer, 1987; Hill and Pierce, 1989; Mevi-Schütz and Erhardt, 2003).

A very recent study on *Coenonympha pamphilus* (Cahenzli and Erhardt, 2012) found that females of this species increased the quality of their offspring (*i.e.* heavier larvae) when receiving additional amino acids. Only if larvae had been raised on scarce resources also the quantity of the eggs laid by *C. pamphilus* was increased through amino acid intake as adults. An amino acid enhanced diet might also have effects on offspring weight in *Maniola jurtina*, but this was not investigated in the frame of our study.

Coenonympha pamphilus, *P. rapae* and *A. levana* are all multivoltine species. Additional amino acids in the nectar may be more likely to have a positive fecundity effect in multivoltine, but not in strictly univoltine species. A number of studies observing no fitness effects of nectar amino acids on butterfly fecundity would support this idea (Murphy, 1983; Moore and Singer, 1987). It is evolutionarily plausible that species with only one generation per year need to be less dependent on fluctuations in resources than species with more generations per year. So it seems logical that multivoltine species react more strongly and instantaneously to improvement of adult food resources than univoltines, like *M. jurtina*.

However, there are also studies which detected no nectar amino acid effects for bivoltine (Mevi-Schütz and Erhardt, 2003) and multivoltine species (Hill and Pierce, 1989). In *Lasiommata megera*, total number of eggs laid depended on the emergence weight of the female and the amount of carbohydrates (*i.e.* nectar mimic without amino acids) ingested (Mevi-Schütz and Erhardt, 2003). Interestingly, O'Brien *et al*. (2004) showed for the univoltine butterfly *Euphydryas chalcedona* that egg provisioning (*i.e.* carbon intake) takes place before adult emergence and that the extent to which larval diet contributed to egg carbon can depend on the timing of the oviposition.

How does geographic provenance relate to oviposition strategy and longevity?

Only in one population the timing of oviposition was weakly affected by the addition of amino acids. Pannonian females oviposited earlier when they received amino acids in their nectar substitute (Fig. 1c). However, these females did not oviposit earlier than those of other populations, rather the sucrose-fed females from Pannonia oviposited later than the amino acid-fed individuals of the same population. In the other populations there was no detectable effect of amino acids on the timing of oviposition. Similarly, the duration of the reproductive period and total lifespan were not significantly affected by the addition of amino acids.

Development hold-up before oviposition was very pronounced in Sardinian *Maniola* (*M. nurag* and *M. jurtina*) which showed a clear delay in egg-laying with respect to all other *M. jurtina*. These observations are completely in line with earlier investigations on *Maniola jurtina* from Mediterranean origin (*e.g.* Scali, 1971; Grill *et al*., 2006). As individuals from Krk were not collected immediately after eclosion, but towards the end of August, many of them oviposited immediately after capture, which is rather an artefact than a result of the experiment. Very interestingly, Pannonian individuals significantly differed from their relatives from nearby Austrian mountains in lifespan (see Figs 3b, 4b), and their egg-laying curve (Fig. 2) resembled that of Mediterranean individuals. This may follow from the climatic circumstances in Pannonian landscapes, where summers are considerably hotter, drier and last longer than in the adjacent alpine upland and winters are only moderately cold (Neuwirth, 1976; Köllner, 1983; Harlfinger and Knees, 1999). We do not exclude that also Pannonian individuals experience an (at least short) dormancy period before egg-laying, similar to Mediterranean ones.

Compared to the aforementioned species of *Pieris* which are known migrants, *Maniola* butterflies are quite sedentary. Very few individuals move more than a hundred meters within their lifetime (Grill *et al*., 2006). Moreover, they are restricted to one adult generation per year. This obliges these butterflies to match their survival and reproductive strategy with the climatic conditions at the location where they have grown up.

Delayed oviposition is an ecological trait of various Lepidoptera species (see for example García-Barros, 1988, 1992; Grüner and Sauer, 1988; Sauer and Grüner, 1988) and has been known from *M. jurtina* butterflies since the 1970s (Scali, 1971). It has also been observed in other species of the genus *Maniola* occurring in Mediterranean habitats, like the island endemic *M. nurag* (Grill *et al*., 2006). Even though Meadow Browns have been studied by various authors (Brakefield, 1982a, b; Goulson, 1993), it remains unclear what proximate cues trigger the butterflies to deposit their eggs immediately after mating, or keep them in store for ecologically better times.

Developmental hold-ups are usually under endogenous control in univoltine insect species (Košťál, 2006). Multivoltine species, on the other hand, like the Large White butterfly *Pieris brassicae*, are known to react more plastically to exogenous factors (Spieth *et al*., 2011 and references therein), giving them the advantage to finely adjust the number of generations per year depending on environmental conditions. For univoltine *Maniola* we would thus expect endogenous factors rather than actual habitat conditions to regulate egg-laying strategies. These could be genetic or influenced by the conditions the butterflies had been exposed to in an earlier developmental stage than the imago. If only the photoperiod during the flight period of the adults were to influence oviposition and induce aestivation, as has been shown for a number of other butterfly species like *Pieris brassicae* (Held and Spieth, 1999), *P. melete* (Xiao *et al*., 2009), or *Polygonia c-aureum* (Fujita *et al*., 2009), all butterflies in our experiment should have oviposited more or less synchronously, as they were kept under identical light conditions. But they did not. Photoperiod will surely have a certain effect but is definitely not the only regulatory mechanism for the great variation in oviposition behaviour observed in *Maniola*.

Maniola butterflies spend the winter in the larval stage. Day-lengths during winter time do not vary substantially between, say, Sardinia and Central Europe, but average temperatures do. These larvae hibernate in strict dormancy under cold climatic conditions, while they remain active also during wintertime in warmer regions. In our experiment we saw that even individuals from geographically very close populations can have different oviposition strategies if these populations live in areas with differing local climate.

We consequently suggest that temperature regimes experienced during the larval stage could be a crucial factor determining the oviposition strategy of a *Maniola* female for the following summer. If this is true, it would also allow a certain developmental flexibility as a response to variation in weather conditions between years and may be good news for aestivating butterflies in a climate warming scenario.

It has been shown that individuals of *Danaus plexipus* enter reproductive diapause in the adult stage when as caterpillars they are exposed to short day photoperiods and low temperature conditions (Barker and Herman, 1976). Generally, short-day photoperiods and low-temperature regimes encountered during embryonic or post-embryonic stages often determine whether an insect species undergoes a diapause during one of its development phases, be that egg, larvae, pupae or imago (*e.g.* Danks, 1987).

We conclude that timing of oviposition and lifespan of the adult female depend on geographic provenance rather than on the nutrition the adult butterfly receives or the conditions it is kept in. Meadow Brown butterflies from Mediterranean regions live much longer and have considerably longer reproductive periods than butterflies from Central European mountain areas, but deposit similar numbers of eggs through their lifetimes.

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