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Life-history polyphenism in the Map butterfly (*Araschnia levana*): developmental constraints versus season-specific adaptations

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ABSTRACT

Hypothesis: Different generations of a seasonally polyphenic butterfly allocate their resources differently between dispersal ability and reproduction to fit the environmental circumstances specific to the seasonal environment of each generation.

Organism: Map butterfly (*Araschnia levana*).

Site of experiments: The Department of Zoology/Tovetorp Research Station, Stockholm University.

Methods: We estimated fecundity by assessing the amount of the limiting resource nitrogen that individuals of each generation allocated to their abdomens. We studied dispersal ability by assessing thorax nitrogen content, and by studying flight ability of both generations in a suite of temperatures.

Results: Individuals of the summer generation performed longer sustained flights than individuals of the spring generation in all temperatures except the warmest treatment. In males, abdomen nitrogen content was poorly correlated with thorax nitrogen content. Thus males do not need to trade off nitrogen between the tissues. However, female thorax and abdomen nitrogen contents were strongly positively correlated, and although summer females were better flyers than spring females, they still allocated disproportionately more nitrogen to the reproductive tissue in the abdomen. We conclude that the divergent allocation patterns between different Map butterfly generations are better understood in terms of developmental constraints acting on spring butterflies, rather than by season-specific adaptations.

Keywords: diapause, direct development, dispersal, flight performance, nitrogen analysis, phenotypic plasticity, reproductive output, seasonal polymorphism, trade-off.

INTRODUCTION

One interesting feature of temperate insects is the ubiquity of seasonal polyphenism (Shapiro, 1976). A polyphenic insect species appears in several temporally distributed generations or cohorts per year that differ in morphology (e.g. Moran, 1992; Windig and Lammar, 1999; Abouheif and Wray,

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2002; Fric and Konvicka, 2002; Van Dyck and Wiklund, 2002), life-history traits (e.g. Nylin *et al.*, 1989; Moran, 1992; Välimäki and Kaitala, 2007; Karlsson and Johansson, 2008; Karlsson *et al.*, 2008; Välimäki *et al.*, 2008), coloration (e.g. Windig and Lammar, 1999; Musolin and Numata, 2003) or reproductive behaviour (Friberg and Wiklund, 2007). Typically, seasonal polyphenisms are described as plastic responses to season-specific selection pressures, and each seasonal phenotype is thus predicted to be adapted to the ambient circumstances. Most of the detected polyphenisms are described in an adaptive framework (see references above), but it is important to also acknowledge the role of developmental constraints and challenges specific for the development of each seasonal morph (see, for example, Jacobs and Watt, 1994; Gotthard and Nylin, 1995). For example, preparation for winter diapause most likely makes more severe physiological demands to aid winter survival than continuing development into the reproductive stage under beneficial summer conditions.

One of the most striking examples of seasonal polyphenism is the Map butterfly (*Araschnia levana*). In this species, which overwinters in the pupal stage, a spring morph with the dorsal side of the wings coloured in a nuance closely resembling that of, for example, Tortoiseshells (*Aglais urticae*) or Comma butterflies (*Polygonia c-album*), ecloses in springtime (Fig. 1). In contrast, the dorsal side of the summer morph that develops directly in summer conditions is blackish with a contrasting horizontal white band present on both fore- and hindwings (Fig. 1). The decision to enter diapause or to continue development is induced in the penultimate instar (M. Friberg, I.M. Aalberg Haugen, J. Dahlerus, K. Gotthard and C. Wiklund, submitted), and is largely dependent on the ambient daylength; long days induce direct development, whereas shorter daylengths increase the propensity to enter diapause (Danilevskii, 1965). Furthermore, the ventral wing patterning differs between generations (Windig and Lammar, 1999) and, overall, the wing coloration of the two generations differs to the extent that Linnaeus described them as different species [a misconception that was corrected in the early nineteenth century by Hübner (1816)].

Although the different wing pattern of the two Map butterfly morphs has been discussed and investigated for centuries (e.g. Hübner, 1816; Gerould, 1916; Danilevskii, 1965; Shapiro, 1976; Windig and Lammar, 1999; Fric and Konvicka, 2002; Fric *et al.*, 2004), the ultimate cause of the polyphenism has yet to be unravelled, and the number of untested hypotheses vastly outnumbers the available data supporting any explanation. For example, the underlying selection pressure favouring polyphenism in the Map butterfly has been suggested to be differences in predation pressure between generations (for a review, see Fric *et al.*, 2004). The spring morph appears to be the ancestral form, which might suggest that summer butterflies experience a different selection pressure, favouring induction of the dark and white phenotype (Fric *et al.*, 2004). It has been suggested that the two morphs have been selected to mimic different unpalatable nymphalid butterflies (Fric *et al.*, 2004) – the spring morph being more similar to, for example, the Tortoiseshell, and the summer morph resembling more, for example, the Poplar Admiral (*Limenitis populi*). So far, however, no studies have presented any evidence in favour of or against this or other hypotheses linked to predation pressure. We will not address this issue further in this paper. Instead, we focus on another set of hypotheses – those of temperature adaptations and season-specific demands on life-history traits.

In temperate areas, adults of the spring generation experience colder ambient temperatures than adults of the summer generation and it has been suggested that the polyphenism is the result of an asymmetry in spring and summer temperature conditions selecting for season-specific temperature adaptations (Fric *et al.*, 2004). In this case, it would be predicted that the spring morph would perform better in colder temperature, whereas the summer morph

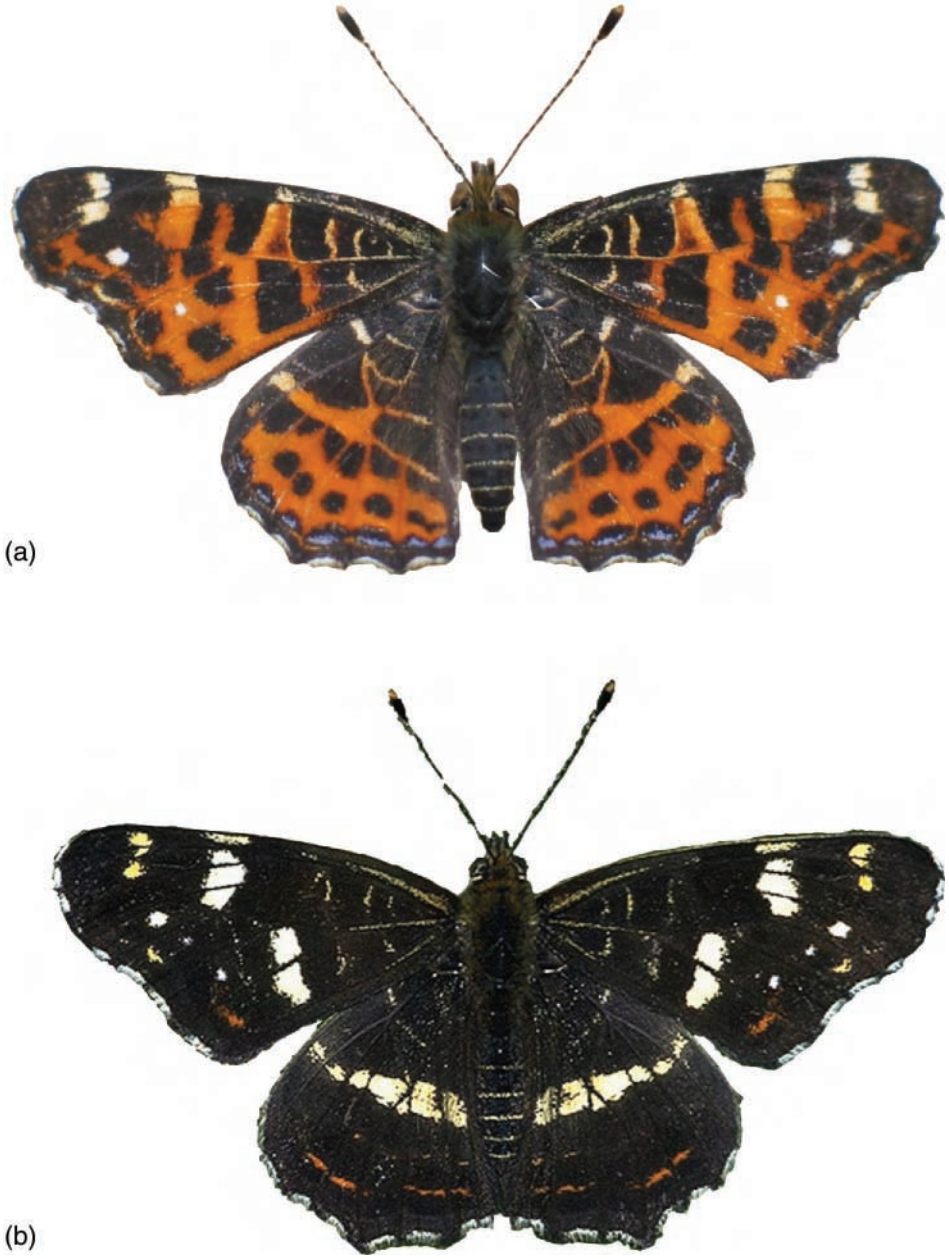

Fig. 1. The dorsal side of a female spring morph (a) and summer morph (b) Map butterfly.

would be better adapted to warmer, summer temperatures, although a phylogenetic study of the *Araschnia* clade suggests that the two phenotypes were already present before the dispersal into the Palearctic temperate zone, which might be held against this hypothesis (Fric *et al.*, 2004). Still, previous studies have shown that habitat-specific selection can result in

such flight performance patterns; woodland populations of the Speckled wood butterfly (*Pararge aegeria*) fly better in cold temperatures and worse in warm temperatures than their relatives from the open (and warmer) agricultural landscape (Merckx *et al.*, 2006), as does the forest/meadow generalist the Wood white (*Leptidea sinapis*) in comparison with its habitat specialist sister species Reál's wood white (*L. reali*), which thrives in warm meadow landscapes (Friberg *et al.*, 2008). The temperature conditions experienced by the spring and summer morphs of the Map butterfly can be described as a parallel to the conditions to the populations or species experiencing habitat-specific temperature regimes. Therefore, it is possible that season-specific selection has altered the temperature/flight reactions norms of the two generations to fit the climate conditions in which each morph appears.

A previous study on another seasonally polyphenic butterfly, the Green-veined white (*Pieris napi*), shows, however, a different pattern (Karlsson and Johansson, 2008). Here, summer generation individuals typically performed longer sustained flights than individuals of the spring morph at all test temperatures. This was contrasted to the generation-specific fecundity that was higher among spring females than among summer females, which implies that the two generations have been selected to balance the fecundity–dispersal trade-off differently. Such a trade-off has been suggested also in the Map butterfly. Fric and Konvicka (2002) showed that the two generations do not only differ in coloration, but also in wing loading and wing design. Their results further suggest that the two morphs might have allocated their resources differently between dispersal ability and reproduction. Field observations together with data on abdomen and thorax dry weight of field-collected specimens suggest that while the individuals of the summer generation were more active and dispersed longer distances, females of the spring generation had relatively heavier abdomens, which might be taken as evidence for different selection pressures having resulted in different allocation patterns in the different generations (Fric and Konvicka, 2002). The predictions from the field studies of the two generations following different selective allocation patterns remain, however, to be tested in controlled experiments in an attempt to clarify what between-generation differences can be ascribed to adaptive seasonal polyphenism versus effects of season-specific climate or random variation imposed in the field. Furthermore, abdominal and thoracic tissue is broken down throughout adult life (Stjernholm *et al.*, 2005; Saastamoinen *et al.*, 2009), and the breakdown process can often be asymmetric so that some tissue is broken down faster than others. This means that field studies risk imposing unnecessary variation, especially if the two seasonal morphs differ also in tissue breakdown pattern. Hence, controlled laboratory studies are needed to test the different predictions about seasonal polyphenism in temperature reaction norms and season-specific fecundity.

In this study, using an experimental approach, we test the flight reaction norms of individuals of the spring and summer generations of the Map butterfly. We test the prediction that the spring morph is better adapted to flight at lower temperatures at the expense of flight at higher (summer-like) temperatures. We contrast this prediction with the ideas outlined by Fric and Konvicka (2002) that the two generations show season-specific adaptations, so that the summer morph allocates more resources to dispersal ability at the expense of fecundity, whereas the allocation pattern is predicted to be the reverse in the spring generation. Finally, we highlight the role of developmental constraints for season-specific allocation patterns in this species.

MATERIALS AND METHODS

Study species and rearing protocol

The Map butterfly (*Araschnia levana*) is distributed throughout most of Eurasia. In Europe, it is expanding its previously central European distribution both to the south (into Spain) and north. This species arrived in Sweden from Denmark in the late 1990s and is now an established species in the southern counties, each year continuing its spread to the north. Eggs are laid in clutches exclusively on stinging nettle (*Urtica dioica*) and larvae are gregarious until the penultimate or ultimate instar. The winter is spent in the pupal stage and in Sweden spring generation butterflies eclose in late April to mid-May, whereas the summer generation is on the wing from mid-July to August. The Map butterfly functions well in the laboratory, with one important exception: it is difficult to induce mating behaviour (Fric and Konvicka, 2002; P. Brakefield and H. Van Dyck, personal communication; M. Friberg, personal observation). Thus to assess generation-specific variation in allocation pattern between dispersal and fecundity, we needed to use a measure of fecundity other than lifetime number of eggs. Therefore, we used the nitrogen content in the abdomen of newly eclosed laboratory-reared adult butterflies as a measure of the investment in egg production, and the nitrogen content in the thorax as an assessment of resources allocated to wing muscles. This approach has been successful in previous studies of butterfly allocation patterns (e.g. Karlsson, 1998; Stjernholm *et al.*, 2005), and the amount of nitrogen allocated to the abdomen has been shown to be the limiting factor for female fecundity (Mattson, 1980; Boggs, 1981; Karlsson and Wickman, 1990), and the amount of nitrogen allocated to the thorax the limiting factor for wing muscle size (e.g. Karlsson, 1998; Stjernholm *et al.*, 2005).

On 27 July 2008, 19 females were collected from Ålstorp in Skåne (55°49′0″N, 12°58′0″E), the southernmost county in Sweden. Females were allowed to lay eggs in one-litre jars with access to stinging nettle host plants. Egg strains were removed from the leaves and incubated on moist filter paper in Petri dishes in climate cabinets maintained at 20°C. At hatching, larvae were transferred in groups of five into one-litre jars with unrestricted access to stinging nettle. Larvae were reared to pupation in light conditions set to induce diapause (12 : 12 h light/dark). Diapausing pupae were incubated outdoors on the balcony of the Zoological Department of Stockholm University over winter. On 25 April 2009, pupae were incubated at 20°C until adult eclosion. The eclosing adults belonged to the spring generation (Fig. 1a). Ten males and ten females were euthanized by freezing directly after the release of the meconium and stored at −20°C until nitrogen analyses (see below). Fifteen individuals of each sex were assigned to the flight experiment (see below).

On 29 July 2009, 16 females were collected in the Ålstorp area (see above). We followed the previous year's collection, egg-laying, and rearing procedure (see above), with the exception that larvae were reared in conditions inducing direct development (20°C, 20 : 4 h light/dark). Eclosing adults thus belonged to the summer generation morph (Fig. 1b). Again, 10 of the eclosing males and females were immediately frozen and later used for nitrogen analysis, and 15 individuals of each sex were randomly selected for the flight experiment.

Nitrogen analysis

The 40 individuals (10 females and 10 males of each generation) were transferred from the freezer into an incubator maintained at 60°C. After 7 days, their dry weight was measured, as well as abdomen and thorax weight. Thereafter, each abdomen and thorax was transferred into separate tin capsules in preparation for nitrogen analysis. The nitrogen content of the different tissues was then assessed through flash combustion with a LECO CHNS-932 elemental analyser (Leco Corp, 3000 Lakeview Av., St. Joseph, MI, USA).

Flight experiment

Flight performance was tested on two occasions with spring generation individuals (5 May 2009) and in autumn on summer generation individuals (1 September). Experiments were conducted at the Tovetorp field station 100 km south of Stockholm. In the study, we used six completely empty constant rooms ($2 \times 4 \times 2.4$ m) set to 15°C, 17°C, 19°C, 21°C, 23°C, and 25°C respectively that were illuminated by six 28-W fluorescent lamps. All participating butterflies (15 individuals of each generation and sex; $n = 60$ individuals in total) were 1 day old and were not fed before the experiment to avoid food intake effects on flight performance. The flight performance of all butterflies was tested in all constant rooms and each individual was randomly assigned one of the constant rooms in which to start. Each trial began with the release of the butterfly in the centre of the room at approximately 2 m height, and the time from the release until the butterfly landed to rest was recorded. After the trial, the butterfly was randomly assigned a new room, transferred there, and allowed to rest for 30 min in darkness, and at the same time given the chance to acclimate to the new temperature, before being tested in that room (cf. Merckx *et al.*, 2006).

Statistical analysis

Data on dry weight and nitrogen content were log-transformed to meet the test assumptions of linear models (ANOVA II). As females were significantly heavier than males (see Results), the two sexes were tested in separate models. The fecundity dispersal allocation pattern was assessed by using either abdomen dry weight or amount of nitrogen invested in the abdomen as the response variable in separate models, generation affiliation as the categorical factor, and thorax weight or the amount of nitrogen invested in the thorax as a continuously distributed covariate in each model, respectively. A significant main effect of generation would mean that the two generations differ in abdomen weight or amount of nitrogen, a significant effect of thorax weight or amount of nitrogen in the thorax would mean that allocation into the two types of tissues is interdependent, while a significant interaction between generation and nitrogen invested in the thorax would imply that the two generations invest their reserves differently into reproductive (abdomen) and wing muscle (thorax) tissue (see Results). Thus, a lack of relationship between thorax and abdomen weight or nitrogen content would imply that nitrogen is not a limiting factor and that each individual can allocate sufficient amounts both to the thorax and abdomen. If nitrogen is limited, a positive relationship between the absolute amounts of nitrogen present in the abdomen and thorax would be expected, as larger individuals with more resources would have more nitrogen to divide between the different tissues. The proportional investment in each tissue between the thorax and abdomen would then be decided by the slope of the

correlation between the investment in the two tissues (see Results). A correlation coefficient deviating from 1 implies that females of different size (or resource levels) allocate their resources differently between the reproductive tissue in the abdomen and the thorax wing muscles.

The flight experiment data were analysed using a repeated-measure approach (ANOVA III), with butterfly generation affiliation and sex as categorical factors, and flight times in each temperature as the repeatedly measured response variable. Data were transformed by subtracting 1 divided by the square root of the flight time from 1 [$1 - (1/\sqrt{\text{flight time}})$] to meet the test assumptions of equal variances.

RESULTS

Nitrogen analysis

Females were consistently larger than males, and individuals of the summer generation were significantly larger than those of the spring generation (Linear model: generation: $F_{1,36} = 11.35$, $P = 0.0018$; sex: $F_{1,36} = 109.5$, $P < 0.001$; generation × sex: $F_{1,36} = 2.25$, $P = 0.14$) (Fig. 2). This was especially true for females ($F_{1,18} = 14.77$, $P = 0.0012$), whereas male size did not differ significantly between the two generations when tested separately ($F_{1,18} = 1.46$, $P = 0.24$) (Fig. 2).

Abdomen weight and nitrogen content were at most poorly correlated with thorax weight and nitrogen content in males (Table 1; Fig. 3a,c). Nitrogen content was, however, larger in the abdomen of summer generation males ($P = 0.024$) (Table 1; Fig 3c), although the nitrogen allocation pattern between the thorax and abdomen was similar in males of both generations (as indicated by a non-significant interaction term in the statistical model; $P = 0.92$) (Table 1; Fig. 3c). Whereas nitrogen allocation patterns were highly similar between generations, there was a near significant interaction term between generation and the abdomen/thorax weight curves ($P = 0.062$) (Table 1; Fig. 3c). This would indicate generation-specific differences in allocation pattern, and this result highlights the need not only to rely on dry weights, but also to perform nitrogen analyses when estimating fecundity–dispersal allocation patterns in seasonally polyphenic insects.

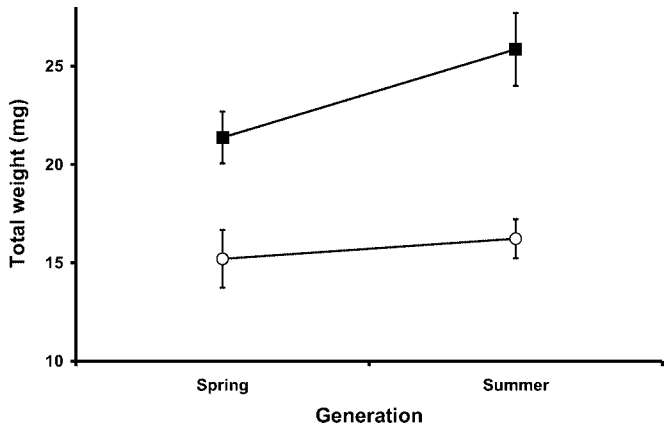

Fig. 2. Average dry weight (mg) of females (■) and males (○) of the spring and summer generation (±95% confidence intervals).

Table 1. The resulting ANOVA (II) table from the analysis of (a) abdomen dry weight in relation to generation affiliation and thorax dry weight, and (b) abdomen nitrogen content in relation to generation affiliation and nitrogen content in the thorax in males and females

	Males				Females			
	SS	d.f.	*F*	*P*	SS	d.f.	*F*	*P*
(a)								
Generation	0.0084	1	1	0.33	0.0039	1	2.81	0.11
Thorax weight	0.00047	1	0.056	0.82	0.054	1	39.02	<0.001
Generation × thorax weight	0.0034	1	4.02	0.062	0.00022	1	0.16	0.7
Residuals	0.13	16			0.022	16		
(b)								
Generation	0.054	1	6.24	0.024	0.03	1	29.76	<0.001
Thorax nitrogen	0.036	1	4.12	0.059	0.033	1	32.69	<0.001
Generation × thorax nitrogen	0.000097	1	0.011	0.92	0.0053	1	5.27	0.036
Residuals	0.14	16			0.016	16		

The abdomens of spring females constituted on average 70% (±1.6, S.D.) of their total weight (measured as abdomen + thorax), and the proportion of nitrogen allocated to the abdomen was 63% (±4.2) in these females. The abdomens of summer generation females constituted 69% (±2.1) of the total weight and contained 67% (±1.3) of the nitrogen. Both abdomen dry weight and nitrogen content were strongly positively correlated with thorax weight and nitrogen content (Table 1; Fig. 3b,d), which indicates the presence of a trade-off between investing nitrogen into reproductive or flight muscle tissue (see pp. 608–609). Again, individuals of the summer generation invested more nitrogen into their abdomens in absolute amounts ($P < 0.001$; Table 1; Fig. 3d). Perhaps more interestingly, a significant interaction term between generation and nitrogen content in the thorax ($P = 0.036$) (Table 1; Fig. 3d) implies that females of the two generations allocate resources differently between the thorax and abdomen. The flatter curve of summer generation females indicates that this generation allocates disproportionately more resources to the wing muscle tissue in the thorax than females of the summer generation (Fig. 3d), and this was especially true for the five largest females of the spring generation (Fig. 3b,d).

Flight experiment

Flight duration varied from a few seconds in the coldest constant rooms to more than 10 min in the warmest treatment, and was thus strongly dependent on temperature in butterflies of both generations and sexes (Table 2; Fig. 4a,b). Furthermore, males flew for significantly longer than females at all temperatures ($F_{1,55} = 46.54$; $P < 0.001$), and both males and females of the summer generation performed longer sustained flights than individuals of the spring generation at all temperatures but the warmest test temperature of 25°C (Table 2; Fig. 4).

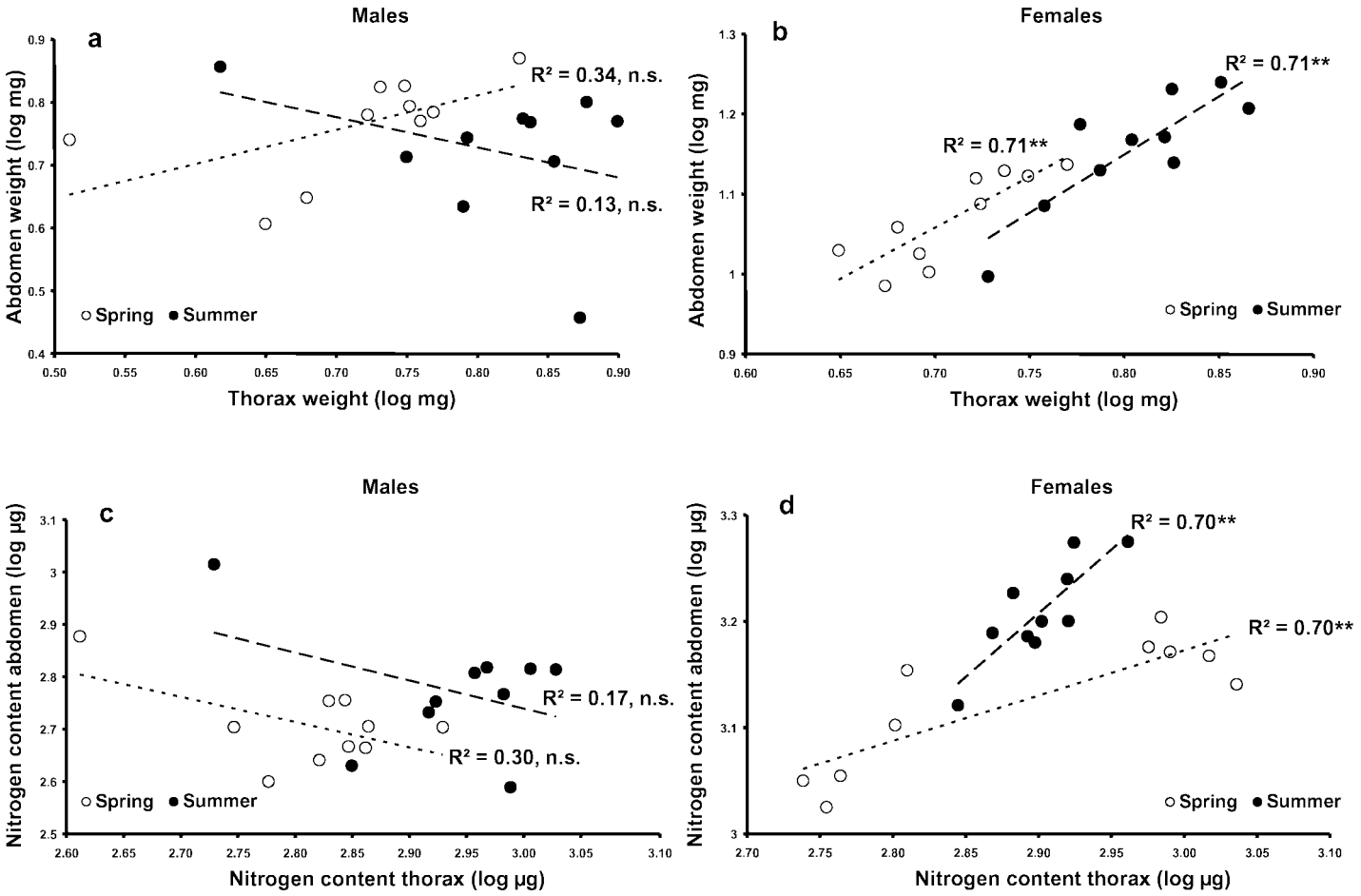

Fig. 3. Allocation patterns between reproduction and dispersal measured as the interaction of generation affiliation and the correlation between abdomen and thorax dry mass (log mg) in males (a) and females (c), and as the interaction of generation affiliation and the correlation between nitrogen content (log μg) in the abdomen and thorax in males (b) and females (d). Trend lines and R^2-values indicate the strength and directions of the allocation pattern. Significant correlations are indicated by: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Table 2. The resulting repeated-measures ANOVA (III) table with males and females analysed separately, showing the flight performance dependent on butterfly generation affiliation and interactions with the different temperatures (15°C, 17°C, 19°C, 21°C, 23°C and 25°C) experienced

	Males				Females			
	SS	d.f.	*F*	*P*	SS	d.f.	*F*	*P*
Generation	0.44	1	37.4	<0.001	0.31	1	25.6	<0.001
Residuals	0.33	28			0.33	27		
Temperature	3.523	5	139.0	<0.001	3.03	5	127.9	<0.001
Temperature × generation	0.173	5	6.81	<0.001	0.063	5	2.56	0.03
Residuals	0.71	140			0.64	135		

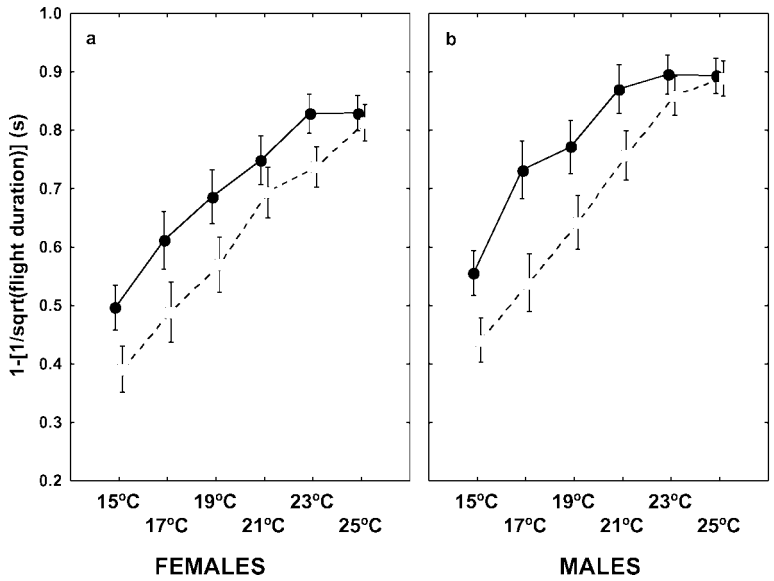

Fig. 4. The average transformed flight time [1 – (1/√flight time)] (±95% CI) in six different rooms maintained at 15°C, 17°C, 19°C, 21°C, 23°C or 25°C, for (a) males and (b) females of the spring (□) and summer (●) generation.

DISCUSSION

Map butterflies of the summer generation of both sexes performed longer sustained flights in almost all temperatures. Hence, this study does not provide any support for the idea that spring butterflies are adapted to perform better in colder temperatures than summer generation butterflies. Instead, the experimental assessment of generation-specific flight performance supports the observations of Fric and Konvicka (2002) that summer generation butterflies are more dispersive and willing to fly also in nature. A similar pattern, with the summer generation morph showing better flight performance, was recently shown in the Green-veined white butterfly [*Pieris napi* (Karlsson and Johansson, 2008)]. In this species, however,

the higher dispersal ability of the summer morph appears to have been traded off against fecundity, as spring females performed shorter sustained flights but laid a significantly higher number of eggs than females of the summer generation (Karlsson and Johansson, 2008).

In the Map butterfly, the allocation pattern is less obvious. It even appears that butterflies of the summer generation not only are better flyers and dispersers, which is further corroborated by their larger nitrogen investment (in absolute amounts) into wing muscles in the thorax, the summer females also invest disproportionately more nitrogen into their abdomens. Abdomen nitrogen content is positively correlated with female fecundity (Mattson, 1980; Boggs, 1981), which means that the two generations most likely differ in overall resource availability, so that summer generation individuals can invest more energy both into wing muscle construction and fecundity. In fact, the between-generation difference in allocation pattern is especially distinct for the five largest spring generation females studied (Fig. 3b,d), which appear to have allocated disproportionately more resources to their thoraces and thus were not able to allocate as many resources into the reproductive parts in the abdomen. Potentially, this is due to an allometric cost of growing larger as a spring female, if the demands on flight ability in the cold spring are larger than the fecundity benefits of being large. This might thus be one selective agent for smaller size of the spring generation, although this idea warrants more studies, and cannot be further tested with the current material. Either way, the results from the experiments presented here do not support the predictions from the descriptive study of Fric and Konvicka (2002), where it was suggested that spring butterflies invested more resources into their abdomens, whereas summer generation butterflies invested more resources into building flight muscles in their thoraces.

Instead, our results imply that the allocation pattern between fecundity and dispersal might be guided less by season-specific adaptations and more by developmental constraints acting with varying intensity on the two generations during larval and pupal development. In this case, the largest constraints on development would then act on the spring generation butterflies, which appears both to be less fecund and less prepared for long bouts of flights. The most obvious difference in the development of the two generations is that the spring generation spends the winter in the pupal stage, having to survive harsh environmental conditions, whereas the summer generation continues pupal development into adults without interruption. Hence, diapausing individuals have to meet the demands of the long winter by incorporating additional anti-freeze compounds (see, for example, Danilevskii, 1965; Hodkova and Hodek, 2004), building thicker pupal cuticles (Yata *et al.*, 1984) and denser body tissues (Kaneko and Katagiri, 2006). The adaptations for surviving the long winter are likely costly; for example, larvae set for diapause spend almost a day longer in the fifth instar before pupating than larvae set for direct development (M. Friberg *et al.*, submitted). Furthermore, Map butterfly larvae that are transferred from long-day conditions that induce direct development into short-day diapause-inducing conditions typically cannot switch pathway later than the middle of the penultimate instar, whereas larvae transferred in the opposite direction can switch pathway from diapause to direct development as late as the middle of the ultimate instar (M. Friberg *et al.*, submitted). Our results show that summer generation individuals simply outperformed spring generation butterflies in all fitness-related traits measured. Hence, the diapause pathway appears to make more substantial demands, which means that diapausing individuals might have to trade overall adult fitness parameters (fecundity, dispersal, etc.) against being able to survive the long and cold winter. A previous study on Comma butterflies (*P. c-album*) implied similar costs of hibernation for other butterfly species. In

this species, which overwinters in the adult stage, the diapausing individuals trade off reproduction against longevity, compared with individuals entering direct development (Karlsson *et al.*, 2008). Further studies of more species will show to what extent this is a general pattern in bivoltine butterflies and other insects.

Although our results suggest that developmental constraints guided by costs of surviving the long winter months might be responsible for seasonal polyphenisms in dispersal behaviour and fecundity, there is still no explanation for the striking polyphenism in wing coloration in this species. This study, as well as that of Fric and Konvicka (2002), shows how spring generation butterflies are less active than butterflies of the summer generation. Furthermore, as spring weather conditions typically are colder, even less time will be available for egg-laying and dispersal among spring generation butterflies. Future studies would benefit from investigating to what extent this generation-specific difference in lifestyle affects patterns of predation. By comparing season-specific attack rates and escape abilities of the two generations, it might be possible to further unravel the underlying cause of the seasonal polyphenism experienced by Map butterflies and how adaptations and constraints interplay to shape the seasonal morph diversity in this species.

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