



Sexual differences in life-history traits in the butterfly *Lycaena tityrus*: a comparison between direct and diapause development

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Abstract

During direct development the butterfly *Lycaena tityrus* was previously found to display sex-related reaction norms in response to temperature. Based on selection for protandry in males and fecundity selection for larger females, males favoured early emergence over large size, leading to a dramatic weight loss at higher temperatures, whereas females maintained similar weights throughout. Because males were able to avoid a weight reduction relative to females in spite of their shorter development at lower temperatures, sexual size dimorphism existed at higher temperatures only. In the present paper we compare sexual differences in life-history traits in *L. tityrus* between direct and diapause development at 25 °C. We demonstrate that, regardless of developmental pathway, protandry persisted and relative sexual size dimorphism, with females being larger, remained unchanged. Although diapausing individuals were less time-constrained, allowing them to grow to considerably higher final weights in both sexes, males were not able to reduce their weight loss relative to females. This is explained by the pressure to gain a developmental advantage solely during post-diapause development, whereas direct developing males may spread the burden over the whole larval period. Our results highlight the importance of considering sexual differences in selective pressures, which may influence central life-history traits in manifold ways.

Introduction

In a recent paper (Fischer & Fiedler, 2000a) we demonstrated that both sexes of the copper butterfly *Lycaena tityrus* (Poda, 1761) (Lepidoptera: Lycaenidae) respond in different ways to shorter development times forced by experimentally increased temperatures. Such differences had been predicted from sexual selection theory (Thornhill & Alcock, 1983; Andersson, 1994). For males a rapid development (due to selection for protandry; Fagerström & Wiklund, 1982; Zonneveld & Metz, 1991) as well as large size (enhancing competitive ability and thus promoting mating advantages; Enquist & Leimar, 1983; Thornhill, 1984; Wickman, 1985) should be of importance. If not simultaneously achievable (through plastic growth), the outcome of the resulting conflict

between development time and size will depend on the strength of selection for these two traits (Wiklund et al., 1991; Zonneveld, 1996). Since selection for protandry should be strongest in species exhibiting female monogamy (Wiklund & Fagerström, 1977; Fagerström & Wiklund, 1982), as is the case here (Fischer, 2000), we predicted a selective premium on early male emergence. For females, however, which are generally less time-constrained during larval development than protandrous males, a high weight, usually accompanied by a fecundity advantage, should be most important (Roff, 1992; Honek, 1993; García-Barros, 2000; Preziosi & Fairbairn, 2000).

In accordance with predictions, males favoured early emergence over body size, leading to a dramatic weight loss at high temperatures (Fischer & Fiedler, 2000a). On the contrary females favoured weight gain

(and hence fecundity), maintaining a similar weight over a broad range of temperatures. Concomitantly, sexual size dimorphism existed at higher temperatures only, because through increased growth rates males were able to achieve weights comparable to those of females at lower temperatures in spite of their shorter development (Fischer & Fiedler, 2000a). In the previous experiments, however, only directly developing individuals were considered. We therefore set out here to examine whether the patterns found also persist under diapause development. Within species, diapausing individuals were commonly found to be larger than directly developing ones (Mousseau & Roff, 1989; Nylin & Svård, 1990; Nylin & Gotthard, 1998). This has been attributed to more severe time constraints during direct development (Nylin, 1992, 1994). However, there is no universal relationship. In particular various studies on butterflies showed that size may be a highly canalised trait and development time may vary due to plastic growth (Nylin et al., 1996; Gotthard et al., 1994, 1999; Gotthard, 1998), which can lead to similar sizes regardless of developmental pathway (Nylin et al., 1989; Wiklund et al., 1991; Nylin, 1994).

In the present paper we compare sexual differences in life-history traits between direct and diapause development at 25 °C, a temperature at which a clear sexual size dimorphism was found during direct development. Since the advantages attached to protandry (maximisation of mating opportunities) do not rely on developmental pathway, we predict that (1) males should emerge before females throughout. If diapausing individuals are indeed less time-constrained (see above) and therefore exhibit longer development times, (2) this should result in higher final weights as, according to a fundamental trade-off frequently assumed in life-history theory, development time and size should be positively correlated (Roff, 1992; Stearns, 1992). However, this should not necessarily change the ratio between male and female weight. Due to the additional time available, diapausing males may cope better with the time stress imposed by selection for protandry and therefore reduce their weight loss relative to females even at higher temperatures. On the other hand, during indirect development protandry must be achieved solely in the period following diapause, as diapause leads to a complete synchronisation of the population thus eliminating any potential developmental differences (Nylin, 1994; Gotthard et al., 2000). Consequently, we predict (3) a similar relative size dimorphism between males and females in both developmental pathways.

Materials and methods

Study organism. *Lycaena tityrus* is a widespread temperate zone butterfly, ranging from western Europe to central Asia (Ebert & Rennwald, 1991). The species is bivoltine with two discrete generations per year in most parts of its range, although populations with one or three generations per year occur (Ebert & Rennwald, 1991; Tolman & Lewington, 1998). Larvae of the last brood enter diapause, overwintering half-grown in the third instar. Pupation occurs after completion of four larval instars. The principal larval host-plant is *Rumex acetosa* L., but some congeneric plant species such as *R. acetosella* L. and *R. scutatus* L. are utilised as well (SBN, 1987; Ebert & Rennwald, 1991; Tolman & Lewington, 1998). The animals for this study belonged to the alpine subspecies *L. tityrus subalpinus* (Speyer, 1851). Seven freshly emerged females were caught in the summer of 1998 in the central Alps (Senales valley, northern Italy) at an altitude of about 1800 m a.s.l. In this region, *L. tityrus* is monovoltine with adults being on the wing from mid-July through late August. As our own rearing experiments showed, even those alpine populations are potentially multivoltine (Fischer & Fiedler, 2000a).

Experimental arrangement. For oviposition, captured females were transferred to Bayreuth University and maintained in an environmental cabinet at a constant temperature (25 °C) and long-day conditions (L18:D6). They were placed individually in glass jars (1 l) lined with moistened filter paper and the jars covered with gauze. Each jar contained a bunch of the larval food-plant *R. acetosa* (in H₂O) as oviposition substrate as well as highly concentrated sucrose solution for adult feeding. Eggs were removed each day, pooled, and maintained in lots of about 100 in glass vials at a temperature of 20 °C (L18:D6). After hatching, young larvae were randomly divided among different groups, one of which was exposed to a constant day-length of L18:D6 and 25 °C (inducing direct development), the other to L12:D12 and 20 °C (inducing diapause). In the latter group, a combination of short day and lower temperature was used as both factors were found to influence diapause induction (Fischer & Fiedler, 2000a).

Hatchlings were reared singly in transparent plastic boxes (125 ml) containing moistened filter paper and fresh cuttings of *R. acetosa* in ample supply. The boxes were checked daily and supplied with new food when needed. Following the onset of diapause in the

short-day treatment, dormant larvae were transferred to another cabinet (T 4 °C, photoperiod L8:D16) for hibernation. After a diapause of about five months, larvae were reared in the way outlined above until adult eclosion under long-day conditions (L18:D6) and 25 °C. For the present comparison between direct and indirect development, a temperature of 25 °C was chosen because sexual size dimorphism depends on temperature and is apparent at higher temperatures only (Fischer & Fiedler, 2000a). The temperature change in the diapause group should not confound any of our results, as by far the most weight is gained during post-diapause development. Moreover, rearing temperatures of 20 vs. 25 °C did not affect diapause weight in the closely related species *Lycaena hippothoe* (Fischer, 2000).

We measured larval time from hatching to pupation (direct developers), pre- and post-diapause development time (indirect developers), pupal time (from pupation to adult eclosion), larval weight at the end of diapause (diapausing individuals), and pupal and adult weight. The onset of diapause was determined by the cessation of food intake and defecation. To compare pre-diapause development of indirect developers (at 20 °C) to the development in directly developing individuals, additional data of a group reared at 20 °C and L18:D6 were used (cf., Fischer & Fiedler, 2000a). Throughout, pupae were weighed on the day following pupation, adults on the day of eclosion after having excreted meconium. From the above data, we calculated relative growth rate of directly developing individuals according to Gotthard et al. (1994) as: growth rate = $\{[\ln(\text{pupal weight}) - \ln(\text{hatchling weight})]/\text{larval time}\} \times 100$ (for details see Fischer & Fiedler, 2000a), for diapausing individuals correspondingly as: growth rate = $\{[\ln(\text{pupal weight}) - \ln(\text{diapause weight})]/\text{post-diapause larval time}\} \times 100$. Proportional weight loss at metamorphosis was calculated using the formula given in Gotthard et al. (1994): Proportion weight loss = $[1 - (\text{adult weight} / \text{pupal weight})] \times 100$. As larval and total development times as well as growth rates in diapausing individuals are exclusively based on post-diapause development, these traits should not be compared across developmental pathways. Consequently, no such statistics are given.

Statistical methods. Differences between groups were localised using Tukey's *post-hoc* comparison (Spjøtvoll-Stoline variant) after standard two-way ANOVAs with developmental pathway and sex as

factors (significance threshold $P < 0.05$ throughout). Since significant interactions between the two factors did not occur ($P = 0.09$ for adult weight, all other P -values > 0.53), these results are not shown. Larval times, pupal times, total development times, and growth rates were rank-transformed prior to analysis (see Conover & Iman, 1981). All statistical analyses were performed using StatSoft (1999). Throughout the text all means are given ± 1 s.d.

Results

Pre-diapause development (diapausing individuals). At 20 °C and short-day conditions, overwintering larvae needed 18.0 ± 0.4 days ($n = 283$) till onset of diapause. This is significantly longer (Mann–Whitney U test: $Z = 13.1$, $P < 0.0001$) than the time directly developing individuals took for completion of the first three larval instars (16.0 ± 1.0 days, $n = 90$) at the same temperature. Note that the difference between pathways given by these data is an underestimate, since *L. tityrus* hibernates in the third instar (continued after diapause), which is, therefore, not completely represented in the above given figure for the diapause pathway.

Protandry and development time. Total development time was in both developmental pathways significantly shorter in males than in females (sex effect: $F_{1,166} = 150.5$, $P < 0.0001$; Table 1). The developmental advantage of males was mainly achieved during larval development (sex effect: $F_{1,166} = 128.8$, $P < 0.0001$), whereas pupal time contributed to a lesser but still significant extent (sex effect: $F_{1,166} = 15.9$, $P = 0.0001$; Table 1). Pupal time did not differ between directly developing and diapausing individuals (effect of developmental pathway: $F_{1,166} = 1.9$, $P = 0.17$). Regardless of developmental pathway, protandry amounted to exactly 3.1 days. The shorter development of males was partly achieved by increased larval growth rates (sex effect: $F_{1,166} = 89.1$, $P < 0.0001$; Table 1).

Pupal and adult weight. Diapausing individuals reached significantly higher pupal (developmental pathway: $F_{1,166} = 253.0$, $P < 0.0001$; sex: $F_{1,166} = 50.9$, $P < 0.0001$) and adult weights (developmental pathway: $F_{1,166} = 170.6$, $P < 0.0001$; sex: $F_{1,166} = 179.3$, $P < 0.0001$) than direct developers (Table 1). Both sexes benefited from diapausing in the same way by achieving about 25% higher weights. Con-

Table 1. Life-history data (mean \pm 1 s.d.) for male and female *L. tityrus* compared between direct and diapause development at 25 °C. Figures within one row, followed by the same letter, do not differ significantly (*post-hoc* comparisons after two-way ANOVA; threshold for significance: $P < 0.05$). Note that data on larval time and concomitantly growth rate in diapausing individuals are exclusively based on post-diapause development. Therefore, these traits should not be compared across developmental pathways

	Direct development		Diapause development	
	Males (<i>n</i> = 59)	Females (<i>n</i> = 33)	Males (<i>n</i> = 46)	Females (<i>n</i> = 32)
Larval time (days)	17.4 \pm 2.2 ^a	20.1 \pm 2.4 ^b	9.5 \pm 0.6 ^c	12.2 \pm 1.9 ^d
Pupal time (days)	7.9 \pm 0.7 ^a	8.3 \pm 0.5 ^{b,c}	8.0 \pm 0.6 ^{a,b}	8.4 \pm 0.6 ^c
Larval + pupal time (days)	25.2 \pm 2.1 ^a	28.3 \pm 2.4 ^b	17.5 \pm 0.6 ^c	20.6 \pm 1.7 ^d
Pupal weight (mg)	108.9 \pm 12.1 ^a	120.5 \pm 12.7 ^b	136.2 \pm 8.6 ^c	150.0 \pm 11.5 ^d
Adult weight (mg)	40.8 \pm 7.3 ^a	52.7 \pm 6.0 ^b	52.3 \pm 5.1 ^b	67.6 \pm 6.8 ^c
Weight loss (%)	62.7 \pm 4.0 ^a	56.2 \pm 3.2 ^b	61.6 \pm 3.1 ^a	54.8 \pm 4.5 ^b
Growth rate (%/d)	42.2 \pm 4.4 ^a	37.0 \pm 3.6 ^b	28.8 \pm 1.6 ^c	23.6 \pm 3.4 ^d

sequently, the ratio between male and female weight remained unchanged, being 90.4% (direct) and 90.8% (indirect development), respectively, in the pupal phase, and 74.4% (both pathways) of the females' weight in the adult stage. Since weight loss during metamorphosis was higher in males than in females (developmental pathway: $F_{1,166} = 4.5$, $P = 0.04$; sex: $F_{1,166} = 123.4$, $P < 0.0001$; Table 1), sexual dimorphism is more pronounced in the adult as compared to the pupal stage. Correlation analyses (Pearson) between individual growth rates and weight loss at metamorphosis within developmental pathways showed significantly positive relationships between the two traits (direct development: $r = 0.55$, $P < 0.0001$, $n = 92$; diapause development: $r = 0.44$, $P < 0.0001$, $n = 78$).

Discussion

Overall, our results on sexual differences in life-history traits revealed a surprisingly strong correspondence between the two developmental pathways. As predicted, protandry persisted throughout and was at least partially achieved by increased male growth rates. The same developmental advantage of three days in either direct or diapause development emphasises the strength of selection on the trait leading to highly co-ordinated development within and between both sexes (cf., Zonneveld & Metz, 1991; Gotthard et al., 2000). Data on pre-diapause development suggest that direct developers are indeed more time-constrained than diapausers, as is indicated by

shorter development times already during early larval growth. Consequently, both sexes achieved about 25% higher final weights under diapause development, which should be related to the longer development time. However, in some other butterfly species weight remained similar regardless of developmental pathway, in spite of shorter development times during direct development (Nylin et al., 1989; Wiklund et al., 1991; Nylin, 1992). In these studies the shorter development was exclusively attained through increased growth rates. In contrast, *L. tityrus* obviously used the additional time to increase weight. Adult size is therefore a phenotypically variable rather than a strongly canalised trait.

Nevertheless, relative size dimorphism between males and females, with females showing higher weights, remained completely unchanged in both treatment groups. Because males lost more weight during metamorphosis, this dimorphism is more pronounced in the adult than in the pupal stage. Similar results were previously found in *Pararge aegeria* and *L. tityrus* (Gotthard et al., 1994; Fischer & Fiedler, 2000a,b), and have been interpreted as representing a potential cost of the males' accelerated development. In line with this interpretation of weight loss during metamorphosis as a physiological cost of rapid growth, weight loss increased significantly with growth rate in both developmental pathways taken by *L. tityrus*.

The independence of sexual size dimorphism from the developmental pathway seems to be related to the pressure for diapausing males to gain a developmental advantage during post-diapause development

only, whereas direct developers are able to spread this burden over the whole larval period. Although the overall longer development time offers potentially a higher scope for a plastic response and concomitantly a reduced weight loss relative to females (see Nylin, 1992, 1994), the above constraints cause an equal size dimorphism regardless of the respective pathway. Trade-offs, such as the one found here between short development time (protandry) and final size in males, should be expected only in stressful situations (Nylin & Gotthard, 1998). In recent years it has become increasingly clear that there are a number of reasons why negative correlations are not always seen between traits expected to be involved in a trade-off (Stearns, 1989; Nylin, 1992; Nylin & Gotthard, 1998). In particular adaptive plasticity in growth and developmental rates may account for inconsistencies with predictions (Nylin, 1994; Nylin & Gotthard, 1998). Moreover, our results reported here demonstrate that selective pressures may differ with respect to sexes, i.e., that selection acts differently on males and females, which may strongly influence central life-history traits.

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