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Determination of female-biased sexual size dimorphism in moths with a variable instar number: The role of additional instars

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Abstract. While the ultimate causes and adaptive significance of sexual size dimorphism (SSD) have been extensively studied, the developmental mechanisms behind this phenomenon have received little attention. Going through an additional larval instar may form a specific way of achieving SSD in arthropods. In the present study, the mechanisms of SSD determination of two lymantriid moths, with marked SSD, were studied. In both species, females tended to go through an additional instar compared to males, and form pupae that were more than twice the weight of the males. To reveal the role of an extra instar, larval growth was monitored in the laboratory and the growth parameters were analysed as dependent on sex and developmental type (number of instars). Prolongation of growth by means of adding an additional larval instar in females turned out to be the key mechanism in the determination of the highly female-biased SSD in the species studied. There is thus a developmental mechanism available that permits achieving a larger size by means of extending the growth period. This provides evidence against constraint-based evolutionary explanations for body sizes in insects. There was no considerable accumulation of SSD during earlier larval life when females went through more instars than males. In contrast, in those cases in which males and females had the same number of instars, SSD accumulated gradually during the course of several larval instars. Longer growing period turned out to be a crucial mechanism leading to the female-biased SSD even when instar number did not differ between sexes, although higher instantaneous relative growth rates of females also played a complementary role in the latter case. Within sexes, an additional instar was characteristic of initially smaller larvae, as predicted by the "threshold size" hypothesis.

INTRODUCTION

Sexual size dimorphism (SSD), defined as the inequality in the body sizes of males and females, is a widespread phenomenon in animals (see Fairbairn, 1997; Badyaev, 2002 for reviews). Male-biased SSD predominates among birds and mammals (e.g., Cabana et al., 1982; Weatherhead & Teather, 1994; Lindenfors & Tullberg, 1998) while female-biased SSD is far more common among invertebrates (e.g., Hanken & Wake, 1993; Head, 1995; Fairbairn, 1997; Teder & Tammaru, 2005). SSD has been typically ascribed to sexual differences in the selection pressure on body size. Accordingly, sexual selection (e.g. male-male competition) tends to favour large males, while large size in females is often related to the strong fecundity selection (positive correlation between female size and fecundity) (e.g., Fairbairn, 1997; Blanckenhorn, 2000; but see Karubian & Swaddle, 2001; Isaac, 2005).

While there exists a vast body of literature dealing with the evolution and adaptive significance of SSD, most of these studies focus on the fitness effects that appear in the adult stage, and often ignore the developmental aspects leading to size differences between sexes (Fairbairn, 1997; Badyaev, 2002). However, without knowing the details of growth schedules, it may not be possible to fully understand the mechanisms underlying the evolution of SSD. This is because the different growth strategies leading to SSD, rather than adult SSD itself, may be the subjects of selection (Badyaev, 2002).

There are three basic mechanisms that can lead to sexual size dimorphism. Individuals of the ultimately larger sex could be larger at hatching/birth, grow faster or grow for a longer time. In insects, the first option is rarely investigated, and in the few studies available, no significant differences in egg/hatchling size between sexes were found in sexually dimorphic species (Mackey, 1978; Ernsting & Isaaks, 2002; Yasuda & Dixon, 2002). In various insect species with female-biased SSD, larval period is longer for females with no sex-related differences in the instantaneous growth rates (Mackey, 1978; Lederhouse et al., 1982; Nylin et al., 1993; Brakefield & Mazzotta, 1995; De Block & Stoks, 2003; Mikolajewski et al., 2005). In contrast, in some sexually dimorphic insects, growth rates, but not larval period differ between the sexes (Telang et al., 2001; Yasuda & Dixon, 2002), whereas in other species both the instantaneous growth rates and developmental periods differ between sexes (Bradshaw & Holzapfel, 1996; Ernsting & Isaaks, 2002). Thus, a prolonged larval period and higher growth rate of the eventually larger sex (typically females) are both available, though not universally used options in formation of SSD in insects.

In insects and other arthropods, going through an "additional" instar provides a specific way of prolonging the growth period. In order to grow, larvae of arthropods have to regularly replace their exoskeletons. This process

results in a growth curve, which is divided into discrete growing periods, i.e. larval instars. As the maximum mass increment per instar is likely to be limited, a fixed number of instars may form a constraint for achieving larger final size in insects (Tammaru, 1998). In contrast, adding an instar may provide a possibility to continue growing, prolong larval period, and achieve a larger final size. In fact, there are numerous unrelated insect species in which females invariably, or predominantly, have a higher number of larval instars than males and achieve larger final body sizes (e.g., Willis et al., 1958; Elliott, 1984; Larsson et al., 1986; Hassall & Grayson, 1987; Nagasawa, 1988; Gu et al., 1992). Moreover, in several species of spiders with extreme female-biased SSD, females often go through more instars than males (reviewed by Head, 1995; Coddington et al., 1997; Vollrath & Parker, 1997; Vollrath, 1998).

It is commonly believed that the additional instar in females enables them to attain a greater body size and forms thus the proximate causal mechanism behind a female-biased SSD (e.g. Head, 1995). However, we are unaware of studies that systematically explore the role of the additional instar. The question is not trivial, though. This is because, within sexes, an increased number of instars often serves a different purpose: it provides a "compensatory" mechanism for further growth in those cases where the necessary threshold weight for pupation is not attained by the "normal" number of instars (e.g., Wigglesworth, 1972; Nijhout, 1975, 1994). This typically results in the considerably longer developmental time of larvae with a higher number of instars when compared to those from the same sex having fewer instars (e.g., Frick & Wilson 1978; Shreeve, 1986; Strand, 1990). However, final sizes between the groups often do not differ notably (e.g., Liquido & Nishida, 1985; Shreeve, 1986; Strand, 1990).

At a more general level, the intuitive rule "achieve a larger size by growing for a longer time" appears not to be universally applicable. In particular, negative environmental correlations between developmental time and final size are ubiquitous (L-shaped reaction norms, Stearns, 1992; Day & Rowe, 2002). Moreover, there is increasing evidence, for insects, that genetic correlations between developmental time and final size also tend to be negative (Ueno, 1994; Kause et al., 1999, 2001; see also Roff, 2000). Thus, it may not always be possible to grow larger by extending the juvenile period. It is therefore an intriguing question if inserting an extra instar provides a mechanism unambiguously allowing to achieve larger sizes in means of growing for a longer time. In other words, does this mechanism lead to a positive correlation between size and time at maturity?

In the present study, the role of additional instar(s) in females in the determination of female-biased SSD in two species of lymantriid moths, *Lymantria dispar* and *Orgyia antiqua*, was investigated. The females in both species tend to go through an additional instar compared to males while SSD in final size is among the greatest in insects (Teder & Tammaru, 2005). The primary goal was to understand how and when the sex related differences arise as dependent on instar number. If an additional instar is the main mechanism determining female-biased SSD, the following is expected: (1) SSD indices (female mass/male mass) should be notably lower earlier in larval life than at the pupal stage; (2) relative growth rates and instar-specific relative mass increments in earlier instars should not differ substantially between male and female larvae. In contrast, if an additional instar does not primarily serve the purpose leading to SSD, weight differences should accumulate earlier in larval life and relative growth rates as well as instar-specific mass increments of earlier instars should be higher in females than in males.

To analyse the ontogenetic determination of SSD, the body weights at different stages of larval development and pupae, mass increments within instars, developmental times and instantaneous growth rates of the two sexes were compared. As in both of the species studied, the number of larval instars varies also within sexes, it was possible to compare the determination of SSD in cases when the number of larval instars did not differ between sexes with these in which it did. Finally, the determination of the final size of larvae of the same sex that had different number of instars were compared. In addition, the significance of the results in the context of the more general question about the role of constraints on adaptive evolution of insect body size is discussed.

MATERIALS AND METHODS

Study species

The present study used larvae of the gypsy moth (*Lymantria dispar* L.) and vapourer moth (*Orgyia antiqua* L.), both belonging to the family Lymantriidae (Lepidoptera). Both species have a Holarctic distribution and are univoltine throughout the region (*L. dispar*), or at least in the northern areas, including the study area (*O. antiqua*). As is typical for capital breeders, adults of these species do not feed and have short lifespans. Moreover, females of both species are known for their behavioural simplicity, being wingless (*O. antiqua*) or at least functionally flightless (*L. dispar*). Both potential and realised fecundity are strongly positively correlated with female body mass in these species (Leonard, 1981; Tammaru et al., 2002). The availability of a reliable fitness estimate (pupal weight) makes these species promising model organisms for life-history studies.

Larvae of *L. dispar* and *O. antiqua* are highly polyphagous, feeding mainly on leaves of various deciduous trees and shrubs. Larvae of *L. dispar* are "spring-feeders", requiring young leaves for development, at least in early instars (e.g., Hunter & Elkinton, 2000). In contrast, larvae of *O. antiqua* start to develop later in spring and are not strongly affected by the phenological stage of their host (unpubl. data of the authors).

The number of larval instars varies within and between sexes in both of the species studied. Males of *L. dispar* may have 4 to 7 instars (most frequently 5), while females have 5 to 8 instars (most commonly 6) (Leonard, 1968; Nagasawa, 1988). In *O. antiqua*, males typically have 4 or 5 instars (rarely 6) while females have usually 5 or 6 instars (4 and 7 have also been recorded, Esperk & Tammaru, unpubl.). In both species the females have, on average, 0.5 to 1 additional instars compared to males. In insects, a higher number of instars within one sex has typically been associated with adverse developmental conditions (e.g., Wigglesworth, 1972). However, in both of the spe-

cies studied, variation in instar number occurs even in standardized rearing conditions.

Rearing procedure and experimental design

The data reported in this paper are based on 4 rearing trials, performed in the years 2001 and 2002 (Table 1). *L. dispar* larvae used in the trials were offspring of wild-collected females, while *O. antiqua* larvae originated from a non-inbred laboratory culture of Estonian origin. Larvae for both *L. dispar* trials originated from the same egg clutches, the difference in the timing of larval development was obtained by manipulating the length of egg diapause. Larvae were reared individually in 50 ml plastic vials at either 18°C (*O. antiqua* '2001' trial) or 22°C (*L. dispar* trials and *O. antiqua* '2002' trial), the difference was due to technical reasons. An artificial photoperiod of 18L : 6D was used in the *O. antiqua* 2001 trial and a natural light regime (close to 18L : 6D) in *L. dispar* trials and *O. antiqua* 2002 trial. During the pre-experimental rearing, larvae of *O. antiqua* 2001 trial and *L. dispar* were provided with leaves of goat willow (*Salix caprea* L.) and those of the *O. antiqua* 2002 trial with leaves of silver birch (*Betula pendula* Roth.).

In each of the trials, body mass of the larvae was recorded at 24 h intervals for one particular instar (the focal instar, Table 1), with the exception of the *O. antiqua* 2002 trial in which larvae were weighed daily from the beginning of the 3rd instar until they pupated. To be quantitatively comparable growth measurements had to be performed simultaneously. Therefore, the development of the larvae was synchronized so that all larvae moulted simultaneously to the focal instar. This was done by subjecting all larvae to low temperature (4–5°C) for 1–3 days just before moulting (identical manipulations for all broods and for both sexes). Such thermal manipulations are widely used to synchronize larval development in experiments and apparently do not have any detrimental effects (e.g., Ayres et al., 1987; Tammaru, 1998). During the focal instar, larvae were assigned to different host plant treatments (Table 1) and the leaves given to the larvae were renewed daily. Larvae that had passed through the focal instar were reared to pupation and sexed as adults. Number of instars was determined by inspecting larvae regularly and recording the moults.

Data sets and analysis

To investigate the proximate mechanisms of SSD determination, we compared body masses at different developmental stages (different instar larvae and pupae), relative within-instar mass increments, relative instantaneous growth rates and duration of instars of larvae belonging to different sexes and developmental types. In the present paper, the term developmental type is used to collectively designate individuals that had the same number of larval instars (e.g., larvae that had five larval instars are classified as belonging to the "5 instar type", abbreviated below as 5IT).

Four types of comparisons were performed. First, the determination of SSD regardless of the developmental type was investigated, i.e. all males were compared with all females within a particular trial (the total sample analyses, hereafter). Second, the growth curves of male and female larvae were compared in the cases when females had an additional instar, i.e. a subsample of males with n instars was compared with a subsample of females with $n + 1$ instars. The third comparison involved larvae in which the number of instars did not differ between sexes. Fourth, the growth curves of larvae of the same sex, but having a different number of instars were compared. To properly apply multiple comparison adjustment, three latter types of analyses were performed with data set including all developmental types, but the results are presented separately for all pair-wise comparisons between the types. Comparisons of growth parameters of larvae of different sexes, as well as comparisons of parameters of different developmental types, were performed using a two-way ANOVA (food treatment as an additional factor). Tukey-Kramer post hoc multiple comparison adjustment was applied to all pair-wise comparisons in analyses of particular developmental types.

Initial body mass of an instar was measured immediately after moulting, i.e. before the larvae resumed feeding. Final body mass of an instar was recorded one day before moulting into the next instar if the focal instar was not the final instar (i.e., 4th instar *L. dispar* in the spring trial and 3rd instar *O. antiqua* in the 2002 trial). In the trials in which at least some of the larvae pupated at the end of the focal instar, body mass on the 4th day (*O. antiqua* 4th and 5th instar in 2002 trial) or on the 6th day (*L. dispar* summer trial and *O. antiqua* 2001 trial) was used as the final mass of the instar. These days were selected as representing time points one day before the first larvae started to moult to the next instar. Relative mass increment within an instar was calculated as the final mass divided by the initial mass of the instar. Pre-experimental thermal manipulations prevented the use of the actual time from hatching to pupation as an indicator of larval developmental time. Therefore, time from the start of the focal instar to pupation was used in the analyses. However, an estimation of approximate larval periods was still possible by adding the average durations of earlier instars at the same temperature regime, to the recorded developmental time.

TABLE 1. Experimental design of the trials. Percentage of different developmental types (number of larval instars) of larvae of *L. dispar* and *O. antiqua* are presented for each trial. Number of individuals in each group is given in parenthesis.

Trial (Species, time)	Broods[a]	Focal instar[c]	Food plants (treatments)[d]	Developmental type					
				4[e]		5[e]		6[e]	
				Males	Females	Males	Females	Males	Females
L. dispar 2001 spring (May)	4[b]	4	*Betula pendula*, *Cotoneaster lucidus*	—	—	100.0 (49)	10.9 (5)	—	89.1 (41)
L. dispar 2001 summer (June)	4[b]	5	*Betula pendula*, *Acer platanoides*	—	—	100.0 (30)	100.0 (4)	—	—
O. antiqua 2001 (June)	5	4	*Betula pendula*, *Cotoneaster lucidus*	63.8 (30)	—	36.2 (17)	84.0 (21)	—	16.0 (4)
O. antiqua 2002 (July)	4	3, 4, 5	*Betula pendula*, *Quercus robur*, *Salix caprea*, *Salix myrsinifolia*, *Salix viminalis*	25.9 (14)	—	70.4 (38)	35.9 (14)	3.7 (2)	64.1 (25)

[a] Number of females whose progeny were used in the trial; [b] Larvae originated from the same broods (see Methods for details); [c] instar in which body masses of the larvae were regularly recorded (see Methods); [d] Provided in the focal instar(s); [e] Number of larval instars.

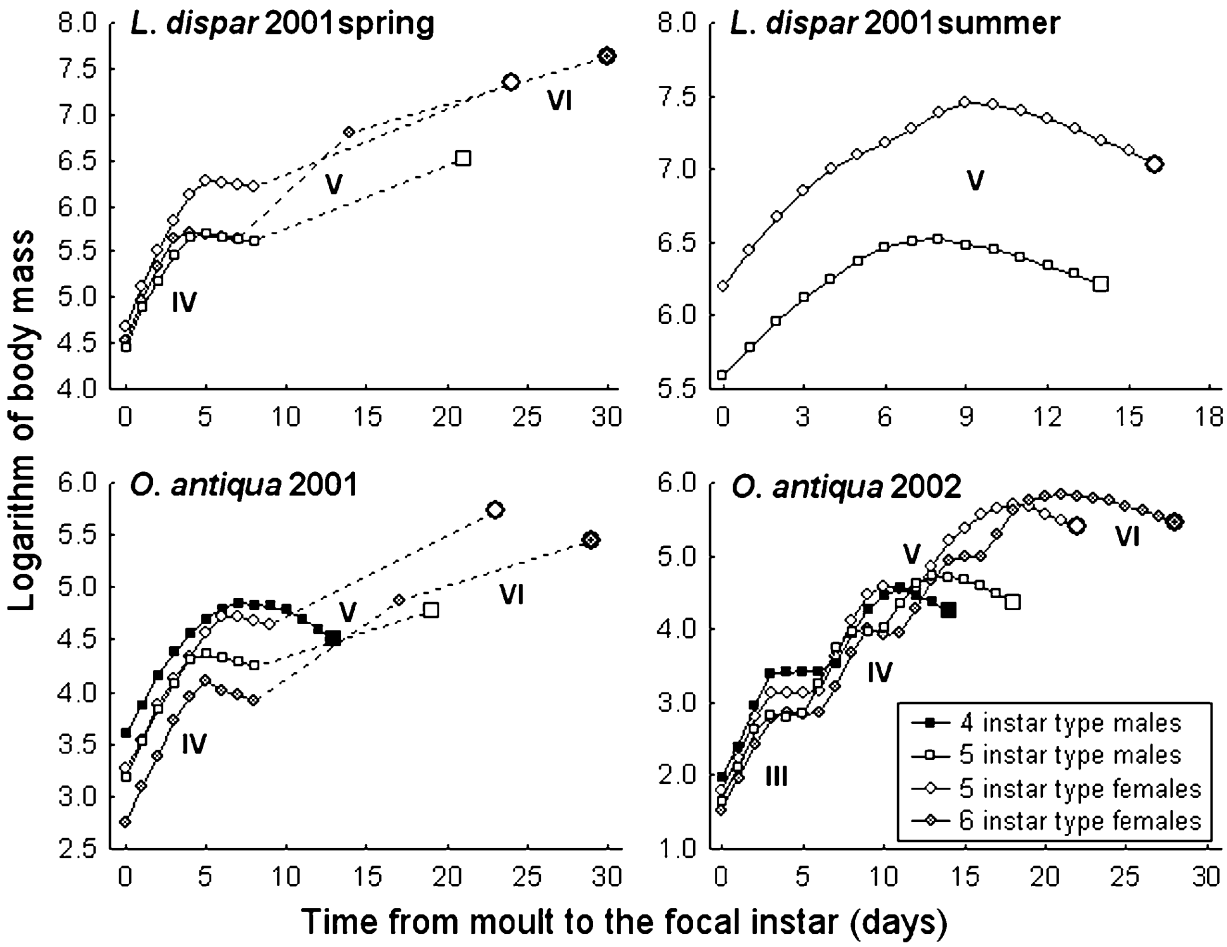

Fig. 1. Pooled average growth curves of *L. dispar* and *O. antiqua* larvae of different sexes and developmental types (total number of instars). Each point represents mean body mass of larvae in a particular rearing experiment. Instars are indicated by roman numerals. Day '0' is the time of the moult to the focal instar, i.e. the instar in which regular measurements were performed (see Methods and Table 1 for details). Instars other than the focal ones, i.e. the instars for which only initial and final mass were recorded, are represented by dashed lines. Last (and largest) point on each growth curve indicates the average time and size at pupation. See Table 1 for sample sizes and Figs 2–5 for statistical details.

Instantaneous relative growth rate – RGR, log (mass at the end of the day/mass at the beginning of the day) – was calculated for each day of the focal instar. RGR values of the second and third day of the instar were used in the analyses, as these represented the "free", exponential growth phase of the instar (Esperk & Tammaru, 2004). When comparing the RGR values of the developmental types, a repeated measures ANCOVA design was used in order to avoid overestimation of degrees of freedom. To eliminate any trivial allometric influence of body weight on growth rates, initial masses on the second and third days of the focal instar were included as covariates in the ANCOVA models. The compound symmetry option was used (temporal autocorrelations not modelled, SAS PROC MIXED, Littell et al., 1996).

Distributions of the dependent variables were approximately normal in all cases. There was a limited heteroscedasticity in some data sets, which could be removed by logarithmic transformation. However, as this transformation had only a weak quantitative effect on the results and did not affect any of the qualitative conclusions, all statistical analyses were performed with untransformed data. The data on larvae belonging to different food treatments were pooled in the analyses. This was done because no qualitative differences were detected between food treatments. However, food treatment was included as an additional variable in respective models unless its influence was clearly non-significant ($P > 0.5$). All the analyses were performed both with and without the effect of brood (offspring of individual females) included in the models. As the former design did not lead to any different conclusion and in order to simplify the presentation, the results presented below are those of the models with the effect of brood not included.

RESULTS

Number of instars

The number of larval instars varied both between and within sexes in both species (Table 1). The *L. dispar* summer trial was the only exception, as all males and all females had 5 instars, however this may be related to the extremely small sample size of females (Table 1). All male larvae of *L. dispar* had 5 instars in both trials, whereas females had either 5 or 6 instars in the spring trial. *O. antiqua* males had either 4 or 5 instars while females developed through 5 or 6 instars. In the 2002 trial, two *O. antiqua* males had as many as 6 instars, but due to the very limited sample size, they were excluded from further analyses. The average instar number for

TABLE 2. Ontogenetic determination of sexual size dimorphism in *Lymantria dispar* and *Orgyia antiqua*. SSD indices (female weight/male weight) are cross-tabulated for the total samples for each trial, as well as separately by developmental types. SSD index value higher than 1 indicates that females are larger than males. For example, the figure 1.08 in the upper left corner of the table implies that, in the *L. dispar* spring trial, females were 1.08 times heavier than males when developmental type was ignored. The 1.24 just below indicates that 5 instar type females were 1.24 times heavier than 5 instar type males. Significance levels[a] of weight differences between the males and females belonging to particular developmental types are given based on Tukey-Kramer post hoc multiple comparison tests (corrected for different food treatments) while the results of two-way ANOVA are presented for comparisons between sexes for the total sample (all males vs all females).

Trial	Focal instar	Developmental type[b]	Sexual size dimorphism (female weight/male weight)								
			Beginning of instar[c]			End of instar[d]			Pupae		
		Females / Males	all[e]	5 instars	6 instars	all[e]	5 instars	6 instars	all[e]	5 instars	6 instars
L. dispar 2001 spring	4	all[e]	1.08**			1.12**			2.83***		
		5 instars		1.24***	1.06*		1.83***	1.03^{NS}		2.27***	2.89***
L. dispar 2001 summer	5	all[e]	1.83***			2.23***			2.60***		
		5 instars		1.83***	—		2.23***	—		2.60***	—
O. antiqua 2001	4	all[e]	0.75***			0.93^{NS}			3.15***		
		4 instars		0.70***	0.42***		0.88^{NS}	0.55***		3.68***	2.39***
		5 instars		1.09^{NS}	0.66^{NS}		1.26*	0.78^{NS}		2.87***	1.86***
O. antiqua 2002	3	all[e]	0.90*			0.92^{NS}			3.29***		
		4 instars		0.88^{NS}	0.71***		0.70***	0.50***		3.30***	3.25***
		5 instars		1.11^{NS}	0.89^{+}		1.45***	1.04^{NS}		3.33***	3.27***
	4	all[e]	0.87^{NS}			1.03^{NS}					
		4 instars		0.65***	0.45***		0.98^{NS}	0.52***			
		5 instars		1.43**	0.99^{NS}		1.82***	0.96^{NS}			
	5	all[e]	1.28*			1.55***					
		5 instars		1.84***	0.97^{NS}		2.19***	1.22^{NS}			

[a] Levels of statistical significance: ^{NS} – p > 0.1; + – p < 0.1; * – p < 0.05; ** – p < 0.01; *** – p < 0.001; [b] Male (column) and female (row) larvae developing through different number of larval instars; [c] First day of the focal instar; [d] Last day (in the case of 4th instar *L. dispar* and 3rd instar *O. antiqua*), 4th day (4th and 5th instar of *O. antiqua* in 2002 trial) or 6th day (in the case of 5th instar *L. dispar* and 4th instar *O. antiqua* in 2001 trial) of the particular (manipulated instar), see Methods for details; [e] Total sample (ignoring developmental type).

male *L. dispar* larvae was thus 5.0 whereas females, on average, had 5.0 (summer trial) or 5.9 instars (spring trial). *O. antiqua* male larvae developed, on average, through 4.4 (2001 trial) or 4.8 (2002 trial) instars, while females had 5.2 (2001 trial) or 5.6 (2002 trial) as the average instar number.

Determination of SSD in the total sample

To investigate SSD in the total sample, all males were compared to all females within a particular trial, disregarding their developmental type (number of instars). There was a notable female-biased sexual size dimorphism in pupal weights in both species (Fig. 1, Table 2). In contrast, earlier in larval life (i.e. until the beginning of the last instar in males) *O. antiqua* males were about equal in size, or even heavier than females, whereas female larvae of *L. dispar* were only slightly (though significantly) heavier than males in the 4th instar (Table 2).

Although female larvae of both species tended to increase their body weight relative to males during all inspected instars (Table 2), mass increments (final weight/initial weight of instar) in earlier instars did not differ significantly between sexes (Fig. 3). In particular, there were no significant differences between sexes in relative mass increments in *O. antiqua* 3rd instar (2002 trial, $F_{5,85} = 1.60$, $P = 0.21$) or *L. dispar* 4th instar (spring trial, $F_{2,92} = 0.87$, $P = 0.35$). In the 4th instar, female *L. dispar* larvae had significantly higher relative growth rates than males (spring trial, $F_{1,89} = 96.65$, $P < 0.0001$; see also Fig. 4). In *O. antiqua*, comparisons of RGR values between sexes gave inconsistent results. In particular, there were no significant differences in RGR values between sexes in *O. antiqua* 3rd instar larvae (2002 trial, $F_{1,85} = 0.31$, $P = 0.58$) or 4th instar larvae in the 2001 trial ($F_{1,69} = 0.02$, $P = 0.90$). However, 4th instar female *O. antiqua* larvae had significantly higher RGR values than males in the 2002 trial ($F_{1,85} = 0.92$, $P = 0.029$).

No significant differences were found between sexes in the durations of 3rd instar in *O. antiqua* (2002 trial, $F_{5,85} = 1.60$, $P = 0.21$). However, the 4th instar in *L. dispar* females was significantly shorter than in males (spring trial, $F_{1,92} = 27.02$, $P < 0.0001$; see also Fig. 5). Developmental time (measured as the time from 3rd or from 4th instar to pupation, see Methods) of female larvae was more than 7 days longer than in males in both species (statistically significant in all cases: $F > 110.0$, $P < 0.0001$). Approximate larval period (developmental time plus average durations of earlier instars, see Methods) of female larvae of both species was 25–35% longer than that of males.

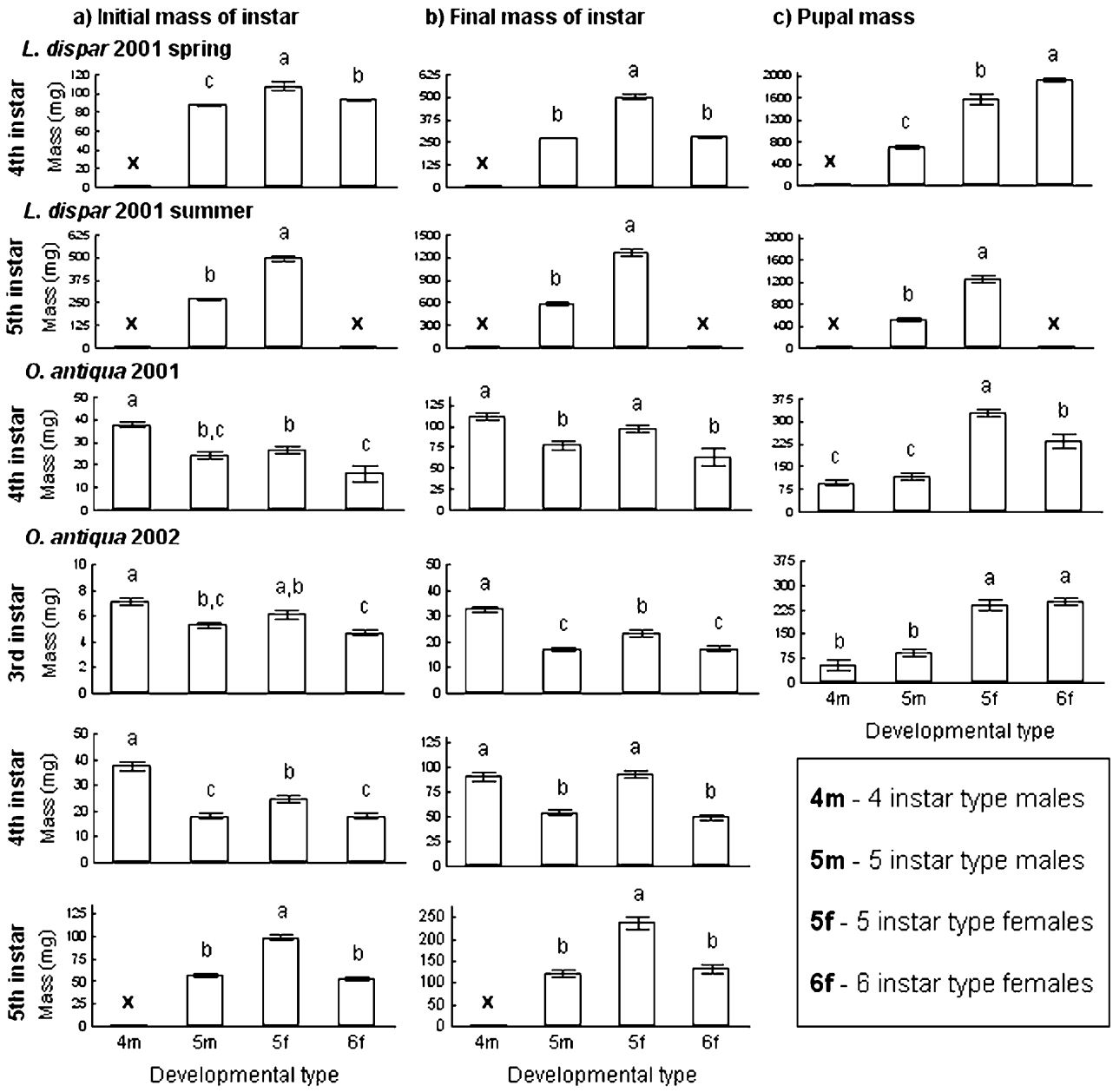

Fig. 2. Comparison of body masses (± SE; corrected for food treatments by SAS, Proc GLM, LS Means) in different larval instars of *L. dispar* and *O. antiqua* larvae belonging to different sexes and developmental types. Letters above the columns indicate significant differences based on Tukey-Kramer post hoc multiple comparison tests. Groups marked with the same letter are not significantly different at the 0.05 level. Missing values are marked with an 'x'. Initial mass of instar is the mass on the first day of a particular (focal) instar. Final mass of instar is the mass on either the last day (4th instar *L. dispar* and 3rd instar *O. antiqua*), 4th day (4th and 5th instar of *O. antiqua* in the 2002 trial) or 6th day (5th instar *L. dispar* and 4th instar *O. antiqua* in 2001 trial) of the instar (see Methods). See Table 1 for sample sizes.

SSD determination in the case of an additional instar in females

In this section, for *L. dispar*, the results of comparisons between 5IT (= 5 instar type) male (i.e. all males) and 6IT female developmental types are presented. For *O. antiqua*, 4IT males were compared with 5IT females and 5IT males with 6IT females. To keep the presentation concise, the cases in which females had two additional instars compared to males (i.e. 4IT males and 6IT females) are not discussed.

No significant differences in larval weights at the end of 4th instar were observed in either of the species studied, in none of the trials (Figs 1, 2, Table 2). Consistently, there were no significant differences in 5th instar final weights of 6IT female and 5IT male larvae in *O. antiqua* 2002 trial (Fig. 2, Table 2). Interestingly, there was a tendency of females with an additional instar to be lighter than males earlier in larval life (up to the beginning of the 4th instar) in the case of *O. antiqua*, while the opposite seemed to be the case in *L. dispar* (Fig. 1, Table 2).

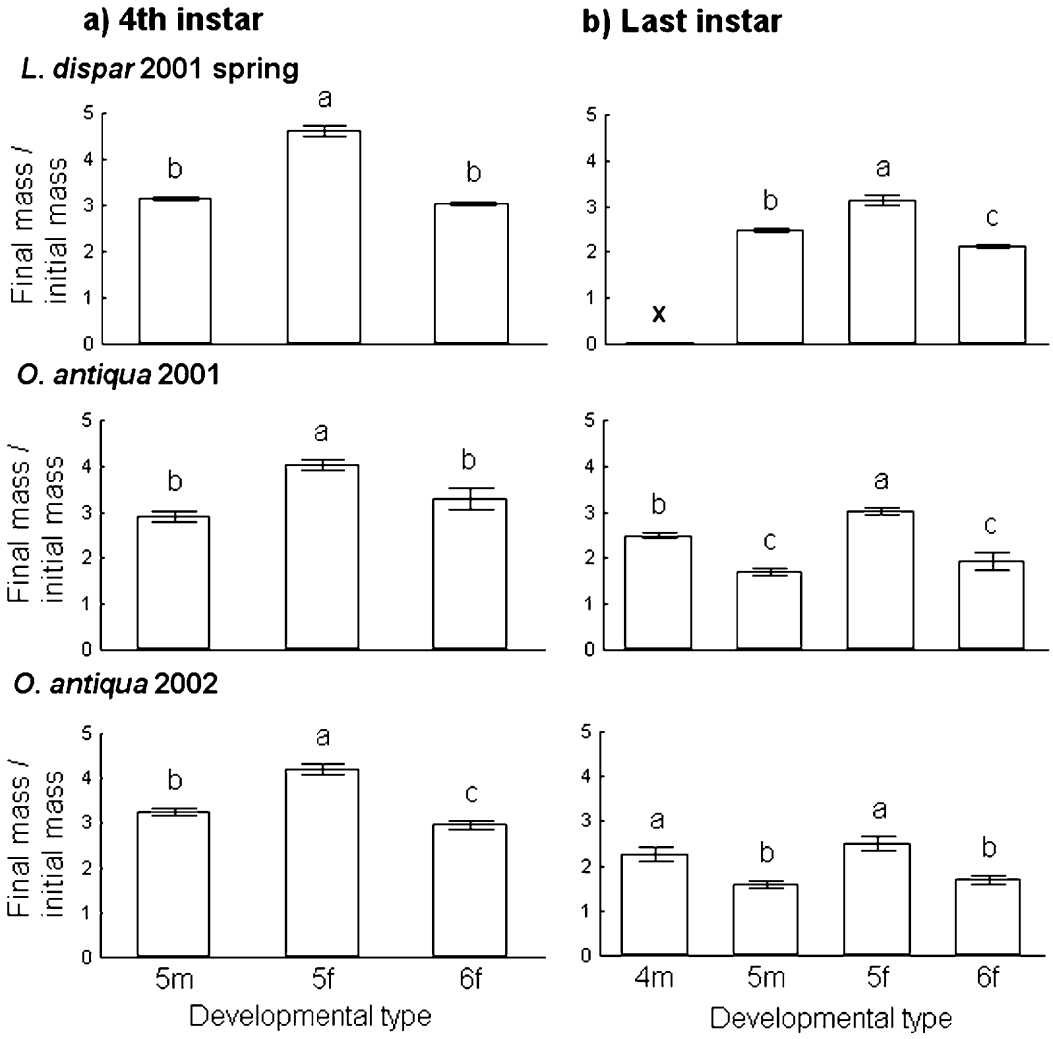

Fig. 3. Final mass / initial mass ratio (± SE; corrected for different food treatments) of the 4th and last instar of *L. dispar* and *O. antiqua* larvae. Final mass is the mass on the last day of the instar (4th instar final mass) or as the pupal mass (last instar's final mass). See Fig. 2 for more details.

Comparisons of instar-specific mass increments between sexes in the case of additional instar of females gave inconsistent results, while the differences between sexes remained mostly non-significant (Fig. 3). RGR values of the 4th instar of 6IT female *L. dispar* larvae were significantly higher than those of 5IT males (Fig. 4). However, results were inconsistent in the case of *O. antiqua* when the instantaneous growth rates of males were compared to those of females with an additional instar. In some cases, males grew faster, whereas the opposite was true in others and in some cases no significant differences were recorded (Fig. 4).

Duration of the 4th instar was significantly shorter in 6IT *L. dispar* females compared with 5IT males, whereas there were no significant differences between these developmental types in *O. antiqua* (Fig. 5). Developmental time was significantly longer in females with an additional instar compared to that of males in both species (Fig. 5). The estimated total larval period (from hatching to pupation) was 25% (*L. dispar*) or 25–35% (*O. antiqua*) longer in females with an additional instar than in males.

SSD determination in larvae having equal instar number

In both species, a considerable proportion of larvae of both sexes pupated after 5 instars (Table 1). 5IT female larvae were significantly heavier than 5IT males in all inspected instars (except at the beginning of the 3rd instar in the *O. antiqua* 2002 trial and beginning of the 4th instar in the *O. antiqua* 2001 trial; Figs 1, 2, Table 2). Moreover, differences in the body weights between 5IT males and 5IT females increased notably during development (Figs 1, 2, Table 2). In both species and in all trials, relative mass increments in the 4th and 5th instar were significantly greater in 5IT females than 5IT males (Fig. 3). Furthermore, as early as in the 3rd instar, the relative mass increment of *O. antiqua* 5IT females was already significantly higher than that of 5IT males ($T = 3.6$, $P = 0.0033$).

RGR values of 5IT *L. dispar* female larvae were significantly higher than those of 5IT males (Fig. 4). Consistently, *O. antiqua* 5IT females had higher instantaneous growth rates than 5IT males in 3rd and 5th instar (Fig. 4). However, in the latter species, RGR did not differ signifi-

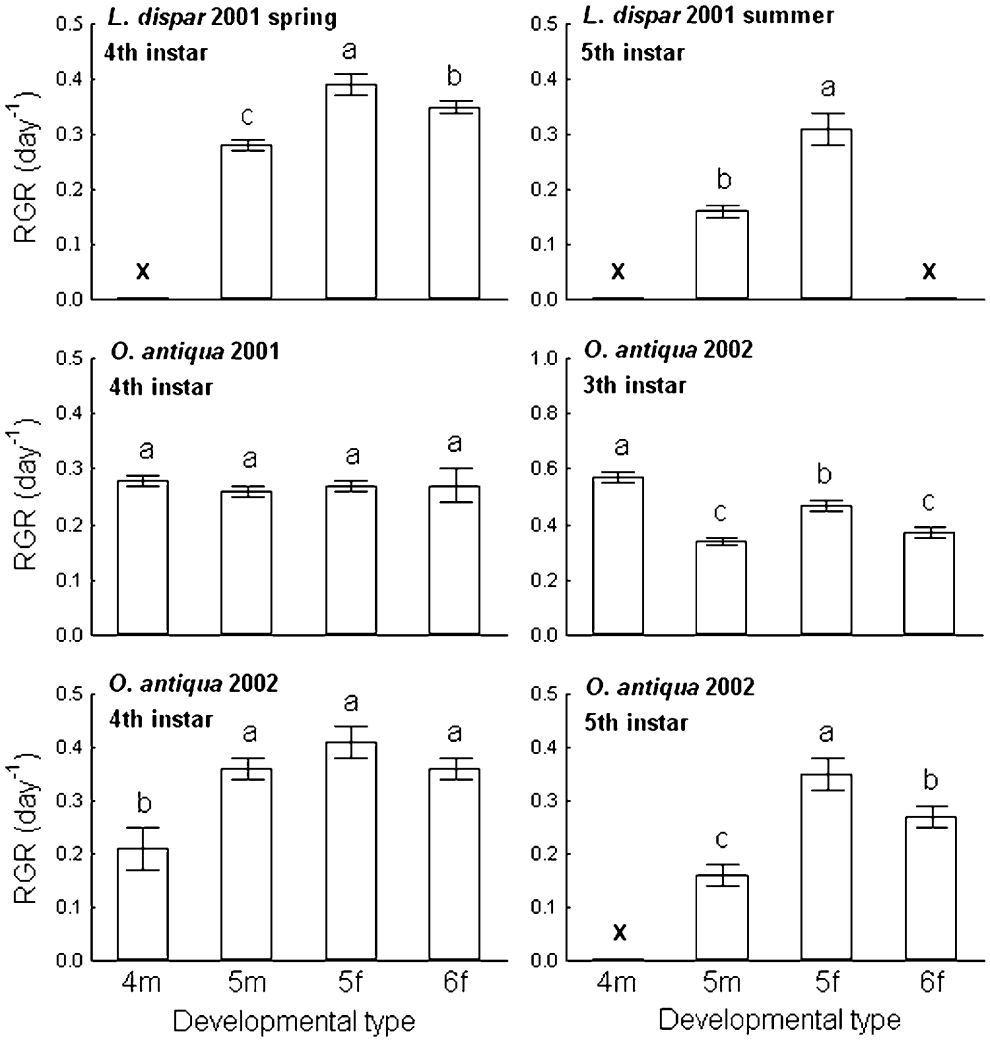

Fig. 4. Comparison of instantaneous RGR (± SE, corrected for different food treatments) of different larval instars of *L. dispar* and *O. antiqua*. RGR values for the second and third day of particular instars are considered (see Methods). See Fig. 2 for more details.

cantly between these groups, in 4th instar, in either of the trials (Fig. 4).

In *L. dispar*, there were no significant differences in durations of the 4th instar between 5IT males and 5IT females. However, 5th instar of 5IT female larvae was significantly longer than that of 5IT males (Figs 1, 5). In *O. antiqua*, durations of all instars, from 3rd to 5th, were significantly longer in the 5IT females than in 5IT males (Figs 1, 5; $T = 2.77$, $P = 0.035$ for 3rd instar of 2002 trial). In both species the developmental time of females was significantly longer than that of males even when instar number did not differ (Figs 1, 5). Approximate larval period of 5IT females was 10–15% longer than that of 5IT males in both species.

Within-sex comparison of different developmental types

In both species and in all instars examined, larvae that went through an additional instar were significantly lighter at the same developmental stage than larvae of the same sex that did not have additional instar (Figs 1, 2). With respect to pupal weights, the results were, however, qualitatively different for the two species studied. In *L. dispar*, 6IT females achieved notably heavier pupal weights than 5IT females (Figs 1, 2, Table 2). In *O. antiqua* pupal weights either did not differ significantly between developmental types, or larvae with an additional instar even formed significantly lighter pupae (females in 2001 trial) (Fig. 2, Table 2).

In both species, the relative mass increment in the 4th instar was significantly higher in 5IT than 6IT females (Fig. 3). RGR of 5IT females was significantly higher than that of 6IT females in the 4th instar of *L. dispar*, as well as in the 3rd and 5th instar of *O. antiqua* (Fig. 4). However, there were no significant differences in RGR values of the 4th instar between different developmental type *O. antiqua* female larvae in either trial (Fig. 4). Results of RGR comparisons between different developmental types of the *O. antiqua* males were qualitatively inconsistent (Fig. 4).

In both species, the duration of the 4th instar was significantly longer in 5IT than 6IT females (Figs 1, 5). Larval period was approximately 15–20% longer (signifi-

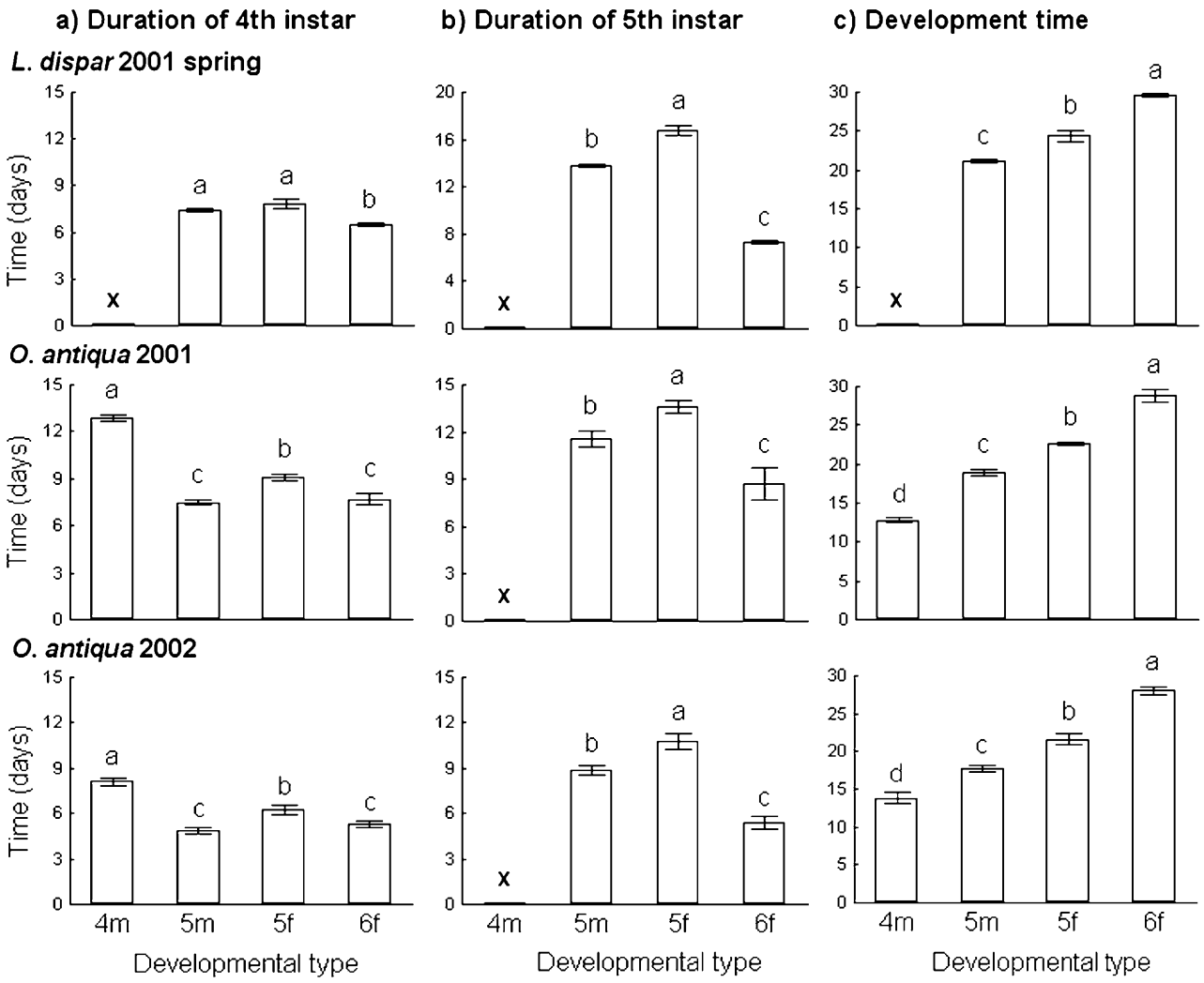

Fig. 5. Comparison of the durations (± SE) of the 4th and 5th instars as well and developmental times (corrected for different food treatments) of *L. dispar* and *O. antiqua* larvae. Developmental time is the time from the beginning of the 4th instar to pupation for *L. dispar* and *O. antiqua* 2001 trial larvae and from the beginning of the 3rd instar to pupation for *O. antiqua* 2002 trial larvae. See Fig. 2 for more details.

cant in all cases; Fig. 5) in those individuals of the same sex that developed through a higher number of instars.

DISCUSSION

Results of the present study clearly demonstrate that, in the two lymantriid moths studied, the high female-biased SSD can be largely ascribed to a prolonged growth period of female larvae that is achieved by adding an extra instar to the growth schedule (Fig. 1). Females of *Lymantria dispar* and *Orgyia antiqua* had, on average, one additional larval instar, and a 25–35% longer larval period than males. Pupal weights of females, in turn, were more than 2.5 times higher than those of males in all trials (Figs 1, 2, Table 2). In contrast, earlier in life, female *L. dispar* larvae were only slightly heavier than males, whereas even the opposite tended to be true in *O. antiqua* (Figs 1, 2, Table 2). There are consistent results from other insects in which females tend to have an additional instar compared to males: body sizes of the sexes do not differ earlier in larval life despite the marked SSD of the adults (Tanaka & Hasegawa, 1979; Roe et al., 1982; Dunbar & Wagner, 1992). In the present study, one of the *L. dispar* trials constituted the only exception, with the female larvae being considerably heavier than males already in the beginning of 5th instar (Table 2). This result is, however apparently related to the fact that all the females in that trial developed through the same number of instars as the males (Table 1).

Relative mass increments in the 3rd instar of *O. antiqua* and 4th instar of *L. dispar* did not differ between sexes. These results further emphasize the importance of an additional instar in the formation of female-biased SSD as they indicate that female-biased SSD did not substantially accumulate during earlier larval instars. However, despite of the tendency of SSD being formed predominantly by means of an additional instar in females, there were some differences between the sexes present already in earlier larval development. In particular, *L. dispar* females had higher instantaneous relative growth rates and spent less time in the 4th instar than males (Figs 1, 4, 5).

Analysing the determination of SSD by subsets of the data allowed us to reveal the two different strategies leading to a high female-biased SSD that coexisted in both of the species studied. When females had a higher number of larval instars than males, SSD was formed mainly during the additional instar. In contrast, when larvae of both sexes developed through the same number of instars, SSD existed already early in larval life and progressed during the larval period. In the latter case, prolongation of the single instars (and the whole larval period) as well as the higher instars-specific mass increments and instantaneous growth rates of the females were important determinants of SSD. These results strongly suggest that one should not ignore variation in instar number when analysing insect growth schedules.

There is, however, a potential methodological problem in comparing larvae of different developmental types. The results of the present study indicate that within the sexes, at least in *O. antiqua*, the number of instars may be associated with the different "quality" of the larvae, as discussed in more detail below. In particular, within the sexes, an additional larval instar was characteristic of larvae that were smaller and had a lower growth rates. Therefore, in comparing 5 instar type male with 5 instar type female larvae, one may actually compare "low quality" males with "high quality" females. Thus, for instance, the result that the RGR of males was lower than that of females might be because of the different quality of the larvae, rather than their sex. However, comparisons of the RGR values and instar specific mass increments between 5IT *O. antiqua* male and 5IT female larvae led to qualitatively different results in the different instars, and in the different trials. Therefore, these traits seem to be robust to the methodological pitfall in focus. Moreover, for example, the fact that the 4th instar of *O. antiqua* 5IT males was significantly shorter than that of 5IT females cannot be due to the lower quality of males, as exactly the opposite would be expected in this case. We thus conclude that even if the quantitative results might be affected by the quality of individual larvae, there is no reason to expect the qualitative conclusions to be biased.

It is suggested that a fixed number of instars may be a constraint on achieving a larger final size in some insect species (Tammaru, 1998). It should then follow that insects with a variable instar number have overcome this constraint. Furthermore, because of the typically strong positive relationship between body weight and fecundity in insects (Honěk, 1993), females of such species should go through a higher number of larval instars than males and so a female-biased SSD is free to evolve. The results of the current study partially support this hypothesis: females of both species studied had, on average, more larval instars than males and formed notably heavier pupae. However, when SSD determination was analysed separately by developmental types, it appeared that some females achieved considerably higher pupal weights than males even without adding an extra instar, i.e. having the same number of larval instars as males. This finding seems to contradict the hypothesis of Tammaru (1998), as it indicates that, at least in the species studied, a fixed number of instars does not form an absolute constraint on achieving a higher body size in females. The formation of high female-biased SSD may thus occasionally be possible even when instar number between the sexes does not differ.

Nevertheless, it has to be noted that in the cases when a SSD was achieved without a sex-related difference in instar number, the differences in weight accumulated gradually over the course of several larval instars (Fig. 1). This may imply that the formation of a high SSD may still be impossible during one (final) instar and the constraint hypothesis should not be dismissed. Moreover, it is notable that in the cases in which females had an additional instar, instar durations tended to be even shorter in females. This may be cautiously interpreted as evidence of costs associated with prolonging development within one instar: this option is not used if not "needed". The nature of such costs is not clear (e.g. Esperk & Tammaru, 2004) and may deserve further study.

Within the sexes, larvae with an additional instar were significantly lighter throughout the larval period and had lower mass increments within instars than larvae that developed through the lower number of instars (Figs 2, 3). These results are consistent with the so-called "threshold-size" hypothesis, according to which there exists a certain size that larvae have to achieve to be able to pupate. Failing to achieve that threshold with a "normal" number of instars, larvae have to go through additional instars until they attain the critical size (Nijhout, 1975, 1994). This threshold however, may be different for males and females (Nijhout, 1994).

The present results for *O. antiqua* support the idea that, within sexes, extra instars serve mainly as a compensatory mechanism for prolonging the growth period in "low-quality" larvae, or larvae growing in suboptimal rearing conditions. The proportion of larvae having an additional instar was notably higher in 2002 trial, which was performed later in the season when the nutritional quality of leaves was lower (e.g., Ayres & MacLean, 1987; Barbehenn et al., 2003). Furthermore, in both *O. antiqua* trials, larvae with an additional instar had longer developmental times than larvae of the same sex having a lower number of instars. However, average pupal weights did not differ significantly between the females having different number of instars. In one case, *O. antiqua* females, with an additional instar even formed significantly lighter pupae than females with a lower instar number (Fig. 2).

In contrast, results from the trials performed with *L. dispar*, support the hypothesis of the extra instar being a compensatory mechanism only partially. Consistent with this hypothesis, 6 instar type females were lighter in the 4th instar than 5 instar type females. Moreover, mass increments of the 4th and the last instar were also lower in 6IT females (Fig. 3). However, females with an additional instar formed, on average, 23% heavier pupae than 5 IT females, whereas the larval period of the former group was approximately 20% longer. These results lead to the

conclusion that having either 5 or 6 larval instars may be related to different growth strategies in *L. dispar* females, for which the relationship between costs and benefits is roughly the same. In particular, 5IT females have a shorter larval period and thereby, a lower risk of mortality before maturity. The 6IT females grow for longer and have a higher risk of mortality but achieve higher pupal weights and fecundities (Leonard, 1981). Moreover, the fact that the durations of single instars in the 6IT females tended to be shorter than in 5IT females further indicates that two different alternative growth strategies, determined earlier in larval life, may exist in *L. dispar*. This is because exactly the opposite (i.e., single instars to be longer) are expected from lower quality larvae. In fact, the present results are qualitatively consistent with those cited in previous studies on *L. dispar*. Leonard (1966, 1968) showed that *L. dispar* larvae that had a higher number of instars were smaller throughout their entire larval period than same instar larvae with eventually lower instar number. However, the pupae of the former group were, on average, 6–7% heavier and the larval period 10% longer.

At a more general level, the results of the present study demonstrate that adding an additional instar constitutes a modification of larval growth schedule, which leads to larger size by means of prolonging the growth period. In particular, a positive correlation of developmental time and size at maturity was found between the sexes in both species. Within the sexes this pattern, which conflicts with the general idea of L-shaped reaction norms, was observed in *L. dispar*, but not in *O. antiqua* (Fig. 1). Thus, at least in some insects, the increase in the number of larval instars provides a developmental option for considerably increasing adult body sizes, and fecundities. If this option is not used, there should be reasons for it. This provides support for an adaptationistic (as opposed to a constraint-based) explanation of the maximal species-specific body sizes in insects (Blanckenhorn, 2000).

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