

Larval growth strategies in the genus *Erebia* Dalman, 1816 as analyzed by the size increment of the head capsule: different strategies – same goal (Lepidoptera: Nymphalidae)

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Abstract

Larval growth strategies and trajectories were analyzed in the genus *Erebia* Dalman 1816 in order to understand the interspecies variability in growth increments in connection with Dyar's rule. For this, the number of larval moults and the size increments per moult were comparatively analyzed for the first time in a large number of phylogenetically closely related species, in this case within a single genus. Growth increments and trajectories were determined by measuring head capsule widths of each instar and the subsequent calculation of Dyar's constant as well as by mathematical modeling, applying exponential, logarithmic and second order polynomial functions. Calculated parameters of the exponential and log functions were well suited for inter-species comparison. The frequency distributions of the growth increment parameters derived from 34 data sets are discontinuous, exhibiting four clear cut groups which can be attributed to the number of larval instars. Accordingly, variability of growth increments, expressed as Dyar's constant, and of parameters derived from mathematical functions can be explained by the different number of moults. Developmental polymorphism has been shown for some species. In these cases, the different number of required instars, 4 or 5 of the same species, is associated to an altered growth increment, ultimately leading, however, to the same size in the last instar. The number of moults in an *Erebia* species is related to its life history, namely, to the number of hibernations in the larval stage. There is no association between instar numbers and phylogenetically defined species clusters.

Key Words

Developmental polymorphism, Dyar's rule, growth function, head capsule width, instar number, larval instars, ontogeny, Satyrinae

Introduction

Larval development in different species of butterflies and moths includes varying numbers of moults until the final transformation to the pupal stage. It has been suggested that the number of larval instars is constant for a given insect species (Esperk et al. 2007a; Esperk et al. 2007b). However, an increasing number of studies show that intraspecific variation in the numbers of instars in Lepidoptera is not uncommon. For example, Shreeve (1986) and Garcia-Barros (2006) found four or five instars for *Pararge aegeria* (Linnaeus, 1758) and for

Coenonympha pamphilus (Linnaeus, 1758) (Satyrinae), respectively, Kingsolver (2007) reported 5 or 6 instars for *Manduca sexta* (Linnaeus, 1763) (Sphingidae) and Calvo and Molina (2008) 5 to 8 for *Streblote panda* Hübner, [1820] (Lasiocampidae). In *Melitaea cinxia* (Linnaeus, 1758), a supernumerary instar was induced by environmental conditions (Saastamoinen et al. 2013).

Besides the number of larval instars the size increment per moult determines the final size of the insect in the immature stage as well as in the adult stage (Grunert et al. 2015; Kivelä et al. 2020). Studies by Dyar (1890) showed that the stage-dependent increase in head capsule

width in Lepidoptera larvae follows mathematical rules. Based on the fundamental work of Brooks (1886), on size development in Stomatopoda (Crustacea), Dyar showed that the increase in head capsule width can be described by a constant factor ranging from 1.25 to 1.6 for different Lepidoptera species, known as Dyar's rule or Brooks-Dyar's rule (Hawes 2020). An increase of 1.26 appeared reasonable as this quite accurately corresponds to a doubling in volume ($1.26^3 = 2$) (Przibam and Megusar 1912). Further analyses and interpretation of the variability of the increase factors led Cole (1980) to the conclusion that factors of 1.6 and 1.26 are characteristic for holometabolous and hemimetabolous insects, respectively, but noted there are exceptions. Obviously, this conclusion is not justified and has been rejected (Sukovata 2019). Furthermore, growth increments for successive moults are not necessarily constant in the sense of Dyar's rule. It has been shown, for example, for *Lymantria dispar* (Linnaeus, 1758) that growth factors decrease with the moult number (Jobin et al. 1992). Nevertheless, Brooks-Dyar's rule is often suitable to follow the larval development of a species and to assign larvae/caterpillars to a defined developmental stage. In this respect, determinations of larval head capsule widths have been performed for several Lepidoptera species, particularly those known as crop pests (Matsumoto et al. 1995; Stavridis et al. 2003–2004; Khorasiya et al. 2014; Nur Athiqah et al. 2015; Thakur 2016). As the size increment factor appears constant during development, the growth can be well described mathematically by exponential functions as has been shown for two Satyrinae species, *Erebia montana* De Prunner, 1798 (Roos 1999) and *Heteropsis narcissus* Fabricius, 1798 (Roos 2003), or by second-order polynomial equations as, shown for *Atrophaneura alcinous* Klug 1836 (Papilionidae) (Kim et al. 2016).

To my knowledge, the occurrence and development of the different growth strategies with respect to instar numbers and size increment factors against an evolutionary and life-history context has not been examined to date. Also, the reason for the existence of different size increment factors is not clear. This study attempts to answer these questions by a comparative analysis of growth characteristics determined for a large number of closely related species, as most studies focus only on single species. In the present study the species-rich genus *Erebia* Dalman, 1816, has been examined to characterize the species-specific patterns of larval size development and to test the hypotheses that developmental modes may be related to life histories or to phylogenetically defined species groups.

Materials and methods

Examined species

Species and origin of the females used for the breeding experiment are listed in Table 1.

Rearing

Larvae were kept at about 22 °C in plastic boxes of different sizes, i.e. 5 cm × 3 cm × 2.5 cm for L1 and L2 larvae and 8 cm × 4.5 cm × 3.5 cm for L3 to L5 larvae. Various *Poa* species were used as food plant. All shedded head capsules were collected for further examination.

Measurements

Widths of the head capsules were determined by means of an Olympus VMT-4 stereomicroscope equipped with an ocular micrometer at magnifications of 20× and 80× depending on the size of the head capsule. In some cases, digital images of head capsules in combination with a calibrator were used for measurements by means of IMAGEJ software.

Calculations and graphics

Means and standard deviations of head capsule widths were calculated with EXCEL. GRAPHPAD PRISM was used for calculation of column statistics (t-test) and frequency distributions as well as for linear and nonlinear regression to produce the graphical presentations, i.e. charts, bar diagrams, box plots.

The original data were applied to non-linear regression using an exponential growth equation ($y = a * e^{bx}$) plotting the mean of head capsule widths against the instar number. The log-transformed values were analyzed using linear regression ($y = ax + b$). In addition, a second order polynomial function was applied.

Molecular phylogenetics

The phylogenetic relationships of the studied *Erebia* species were analyzed using published sequence data of the mitochondrial cytochrome oxidase subunit I gene (barcodes). A phylogenetic tree was constructed with MEGA7, version 7.0.18 (Kumar et al. 2016) by the neighbor-joining method (Saitou and Nei 1987). For accession numbers of the used COI-5P sequences and for further details see Fig. 22.

Phylogenetic non-independence of growth parameters was checked using PAST4.17 (Hammer et al. 2001). A parsimony-based tree of COI sequences was constructed and phylogenetic generalized least squares (PGLS) was applied to calculate Pagel's lambda for the growth parameters.

Results

Instar number

The number of moults and the growth characteristics of the larval head capsules of 28 species of the genus *Erebia* were examined. Usually, the different species of the genus *Erebia*

Table 1. List of species used for rearing. Origin and year of the females taken for oviposition are listed by species, including *Erebia* species and six additional selected Satyrinae species belonging to different tribes.

Species	Locality, Year
<i>Erebia aethiopella</i> (Hoffmannsegg, 1806)	Italy, Ligurian Alps, Cuneo, Bocchina dell' Aseo, 1997
<i>Erebia aethiops</i> (Esper, [1777])	France, Hautes Alpes, Agnielle, 1984
<i>Erebia aethiops</i> (Esper, [1777])	France, Isère, Mens, 1984
<i>Erebia aethiops</i> (Esper, [1777])	Germany, Bavaria, Walchensee, 2000
<i>Erebia cassioides</i> (Reiner & Hochenwarth, 1792)	France, Pyrenees, Col du Pourtalet, 1988
<i>Erebia epiphron</i> (Knoch, 1783)	Spain, Galicia, Sierra de Xistral, 1983
<i>Erebia epistygne</i> (Hübner, [1819])	France, Montagne de Lure, St. Etienne, 1981
<i>Erebia epistygne</i> (Hübner, [1819])	Spain, Teruel, vic. Mosqueruela, 2000
<i>Erebia euryale</i> (Esper, 1805)	Italy, Cuneo, Terme di Valdieri, 1991
<i>Erebia gorge</i> (Hübner, [1804])	Italy, Martello Valley, Sälentjoch, 2014
<i>Erebia hispania</i> Butler 1868	France, Pyrenees, Pic du Midi de Bigor, 1985
<i>Erebia lefebvrei</i> (Boisduval, [1828])	Spain, Pyrenees, Huesca, 2200 m, 1988
<i>Erebia manto</i> ([Denis & Schiffermüller], 1775)	Austria, Katschberg Pass, 1979
<i>Erebia medusa</i> ([Denis & Schiffermüller], 1775)	Germany, Naab Valley, 1978
<i>Erebia medusa</i> ([Denis & Schiffermüller], 1775)	France, Vosges, Le Markstein, 1980
<i>Erebia medusa</i> ([Denis & Schiffermüller], 1775)	Germany, 1990
<i>Erebia melampus</i> (Fuesslin, 1775)	France, Savoy, Pralognan, 1500–1700 m, 1989
<i>Erebia melancholica</i> Herrich-Schäffer, [1846]	Turkey, Gümüşhane, Zigana Geçidi, 1987
<i>Erebia melas</i> (Herbst, 1796)	Greece, Parnass-Mountains, 2000 m, 1981
<i>Erebia meolans</i> (De Prunner, 1798)	France, Vosges, vic. Le Markstein, 1980
<i>Erebia mnestra</i> (Hübner, [1804])	Switzerland, Sertig Valley, 1986
<i>Erebia montana</i> (De Prunner, 1798)	Italy, South Tyrol, Pedertal, 1985
<i>Erebia neoridas</i> (Boisduval, [1828])	Italy, Ligurian Alps, Mount Bignone, 1980
<i>Erebia neoridas</i> (Boisduval, [1828])	Italy, Ligurian Alps, Carpe Pass, 1987
<i>Erebia nivalis</i> Lorkovic & de Lesse, 1954	Austria, Mallnitzer Tauern, Jamnigalm, 2000 m, 1987
<i>Erebia oeme</i> (Hübner, [1804])	Switzerland, Valais, Aminona, 1800–1900 m, 1989
<i>Erebia oeme</i> (Hübner, [1804])	Italien, Friuli, Mount Simeone, 1989
<i>Erebia palarica</i> Chapman, 1905	Spain, Picos de Europa, 1200 m, 1993
<i>Erebia pandrose</i> (Borkhausen, 1788)	Switzerland, Faulhorn, vic. Grindelwald, 1989
<i>Erebia pluto</i> (De Prunner, 1798)	Italy, South Tyrol, Ultental, 1985
<i>Erebia scipio</i> Boisduval, [1833]	France, Vaucluse, Mont Ventoux, 1982
<i>Erebia stirius</i> (Godart, [1824])	Italy, Friuli, Mount Simeone, 1989
<i>Erebia triaria</i> (De Prunner, 1798)	Italy, Ligurian Alps, Argentina Valley, Colle Melosa, 1988
<i>Erebia tyndarus</i> (Esper, [1781])	Switzerland, Ticino, Campolungo Pass, 1989
<i>Proterebia afra</i> (Fabricius, 1787)	Croatia, vic. Sibenik, 1983
<i>Melanargia galathea</i> (Linnaeus, 1758)	Austria, Leitha Mountains, 1979
<i>Mycalesis perseus</i> (Fabricius, 1775)	Indonesia, Sulawesi-Tenggara, 2013
<i>Lasiommata maera</i> (Linnaeus, 1758)	Austria, Radstätter Tauern, vic. Flachau, 1999
<i>Hipparchia semele</i> (Linnaeus, 1758)	Italy, Vinschgau, vic. Allitz, 2015
<i>Strabena tamatavae</i> (Boisduval, 1833)	Madagascar, Ambohidratrimo, 1985

undergo metamorphosis either via 4 or via 5 larval instars. As an exception, 6 instars were found in *Erebia triaria* De Prunner, 1798 (Table 3). In three cases, *Erebia aethiops* (Esper, [1777]), *Erebia neoridas* (Boisduval, [1828]) and *Erebia medusa* ([Denis and Schiffermüller], 1775), development either via 4 or via 5 instars has been observed for specimens from different geographical regions.

Defining growth parameters and their variability

For analyses of head capsule growth several parameters were defined and calculated and checked for their suitability for interspecies comparisons. As a basis, means of head capsule widths in μm were determined for all

available capsules of each instar of a species. Plotted values revealed that growth progression is not linear but can be described by nonlinear regression. A good fit can be achieved by application of an exponential growth function of the following simple form:

$$H_x = H_0 * e^{kx} \quad (\text{generally: } y = a * e^{bx}) \quad [1]$$

H_x : head capsule width (μm) of instar x ; H_0 : extrapolated value for H_x at $x = 0$; e : basis of natural logarithms ($e = 2.718$); x : larval instar number; k : growth constant defining the curvature.

After logarithmic transformation of the head capsule values, growth progression can be well described by linear regression resulting in the equation:

$$\log H_x = ax + S \quad (\text{generally: } y = ax + b) \quad [2]$$

a: slope of the line; S: extrapolated value for $\log H_x$ at $x = 0$.

The growth constant k of equation [1] and the slope a of equation [2] turned out to be suitable parameters to describe the size increment of the head capsule. An example of plots and of the equations is shown in Figs 1, 2 for *Erebia gorge* (Hübner, [1804]) with $k = 0.345$ and $a = 0.155$. Goodness of fit values for the two functions amounting to $r^2 = 0.9831$ and $r^2 = 0.9932$, respectively, indicate that the parameters are suitable to describe the head capsule growth. Furthermore, values of the r^2 coefficient approaching 1.0 show that the growth increment follows Dyar's rule.

Another method used by Kim et al. (2016) to mathematically describe the head capsule growth is non-linear regression using a second order polynomial function of the form

$$y = ax^2 + bx + c \quad [3]$$

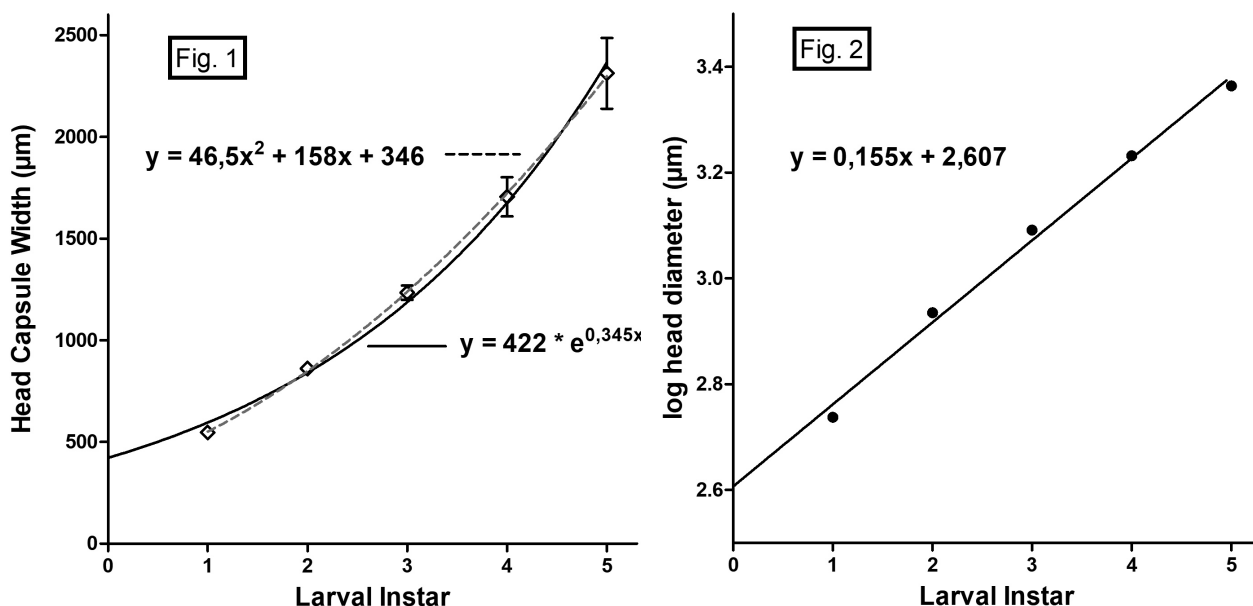
The resultant graph is additionally shown in Fig. 1 for *E. gorge*. Goodness of fit value $r^2 = 0.9885$ is also very high (see also Table 2). However, comparative interspecies analysis using this method is aggravated by the presence of two varying parameters, a and b , which must be considered together for meaningful comparisons. Some examples of the calculated coefficients are presented in Table 2 which illustrates the difficulties for interpretation

$$GP = 1/n_s \cdot \sum [(b^2 - ac)_{s_1} + (b^2 - ac)_{s_2} + (b^2 - ac)_{s_3} + \dots] \quad [5]$$

corresponding to

$$GP = (n - 2)^{-1} \cdot \sum_{x=1}^{n-2} (H_{x+1})^2 - (H_x \cdot H_{x+2}) \quad [6]$$

$n = 4, n = 5$ etc. for the number of larval instars. H: head capsule width (μm).



Figures 1, 2. Growth functions for *Erebia gorge*. 1. Exponential function (black line) and second order polynomial function (grey dotted line). Data points represent means and standard deviations. 2. Logarithmic function.

and comparative analyses in contrast to the growth factor k of the exponential function.

Dyar's constant (r) was also calculated for head capsule widths of each pair of successive instars. To account for "variation of the constant" in a growth sequence the values were finally averaged and termed average per-moult growth rate (Minelli and Fusco 2013), which can be considered the averaged Dyar's coefficient (r_m):

$$r_m = (n - 1)^{-1} \cdot \sum_{x=2}^n (H_{x-1}/H_x) \quad [4]$$

For *E. gorge*, with 5 larval instars, the sequential values are $H_2/H_1 = 0.634$; $H_3/H_2 = 0.698$; $H_4/H_3 = 0.723$; $H_5/H_4 = 0.737$. The values are increasing with higher instar number showing that in this case Dyar's constant is not strictly a constant. To account for deviations of Dyar's constant from constancy the standard deviation (SD) of the averaged data pairs (H_{x+1}/H_x) is calculated. For interspecies comparisons, however, absolute values of SD are less suitable and are therefore transformed into a percentage of the mean. In the case of *E. gorge*, the mean and SD are 0.698 ± 0.046 and the transformed SD amounts to 6.59% [$(0.046 \cdot 100)/0.698$].

Another method to account for deviations from Brooks-Dyar's ratios was proposed by Hawes (2020), who introduced the following equation [5] to evaluate the geometric property of growth progression (GP):

Table 2. Values of the coefficients a and b of the second order polynomial function $y = ax^2 + bx + c$ calculated by nonlinear regression for the size increment of larval head capsule widths of selected *Erebia* species. Goodness of fit (r^2) and the number of larval instars are given. For comparison the constant k and r^2 of the exponential function $H_x = H_0 \cdot e^{kx}$ are shown in columns 6 and 7.

Species	Instars	Second order polynomial function			Exponential function	
		A	b	r^2	k	r^2
<i>E. hispania</i>	4	131.1	-127.0	0.9997	0.413	0.9984
<i>E. aethiops</i>	4	15.2	383.0	0.9966	0.402	0.9828
<i>E. lefebvrei</i>	4	124.8	77.9	0.9998	0.442	0.9989
<i>E. melampus</i>	4	79.1	37.9	0.9997	0.398	0.9989
<i>E. oeme</i>	4	152.1	-161.1	0.9996	0.434	0.9986
<i>E. aethiopella</i>	5	52.6	125.0	0.9986	0.321	0.9952
<i>E. euryale</i>	5	94.8	-32.3	0.9990	0.382	0.9999
<i>E. gorge</i>	5	97.2	-48.0	0.9997	0.336	0.9962
<i>E. melas</i>	5	63.6	163.6	0.9936	0.325	0.9924
<i>E. pandrose</i>	5	103.9	-128.6	0.9981	0.383	0.9990
<i>E. epiphron</i>	5	24.2	226.5	0.9980	0.295	0.9887
<i>E. triaria</i>	6	8.2	396.3	0.9955	0.232	0.9822

The optimal fit, when the growth constant is absolutely constant, results in GP = 0 which is achieved and when the terms $(H_{x+1})^2 - (H_x \cdot H_{x+2}) = 0$, i.e., when $(H_{x+1})^2 = (H_x \cdot H_{x+2})$ or $b^2 = ac$ for equation [5] of Hawes (2020), respectively, for all instars. However, a GP of 0 may also occur when growth deviates from Brooks-Dyar’s rule. This is the case when individual terms derive from zero but are positive ($b^2 > ac$) or negative ($b^2 < ac$) and cancel each other in the sum. It is

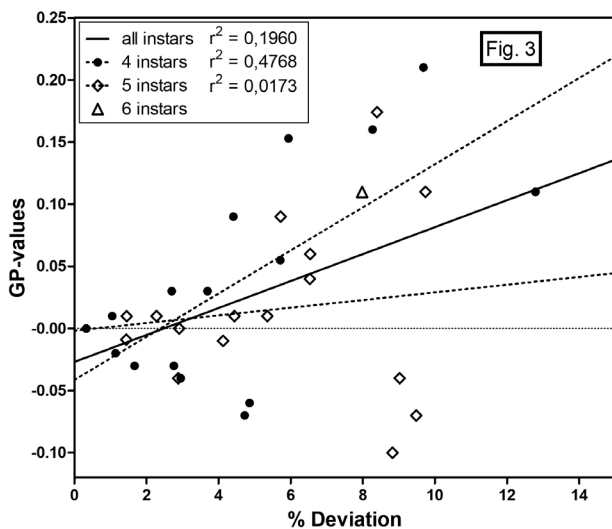


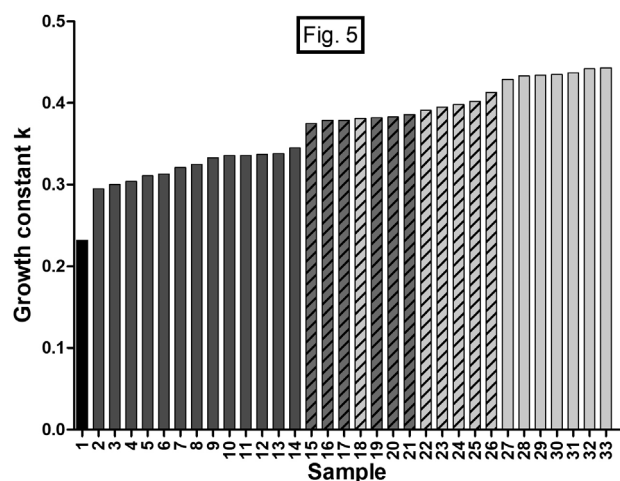
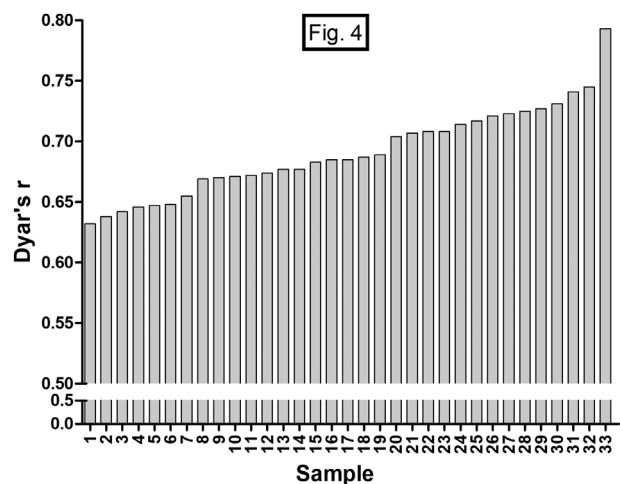
Figure 3. Linear regression analysis for a possible correlation between GP-values and percentage transformed SDs from averaged Dyar’s r (% Deviation). Data from 12 species (16 data sets) developing via 4 instars (*Erebia manto*, *E. melampus*, *E. medusa*, *E. cassioides*, *E. hispania*, *E. neoridas*, *E. oeme*, *E. aethiops*, *E. alberganus*, *E. lefebvrei*, *E. stiri*, *E. styx*), 16 species (16 data sets) via 5 instars (*E. melancholica*, *E. melas*, *E. aethiopella*, *E. euryale*, *E. epiphron*, *E. gorge*, *E. mnestra*, *E. neoridas*, *E. pronoe*, *E. aethiops*, *E. pluto*, *E. tyndarus*, *E. nivalis*, *E. epistygne*, *E. palarica*, *E. pandrose*), and 1 species via 6 instars (*E. triaria*) was used.

shown in Fig. 3 that GP values and percentage-transformed SDs of averaged Dyar’s r are not correlated. Thus, GP values may be misleading in interpretation.

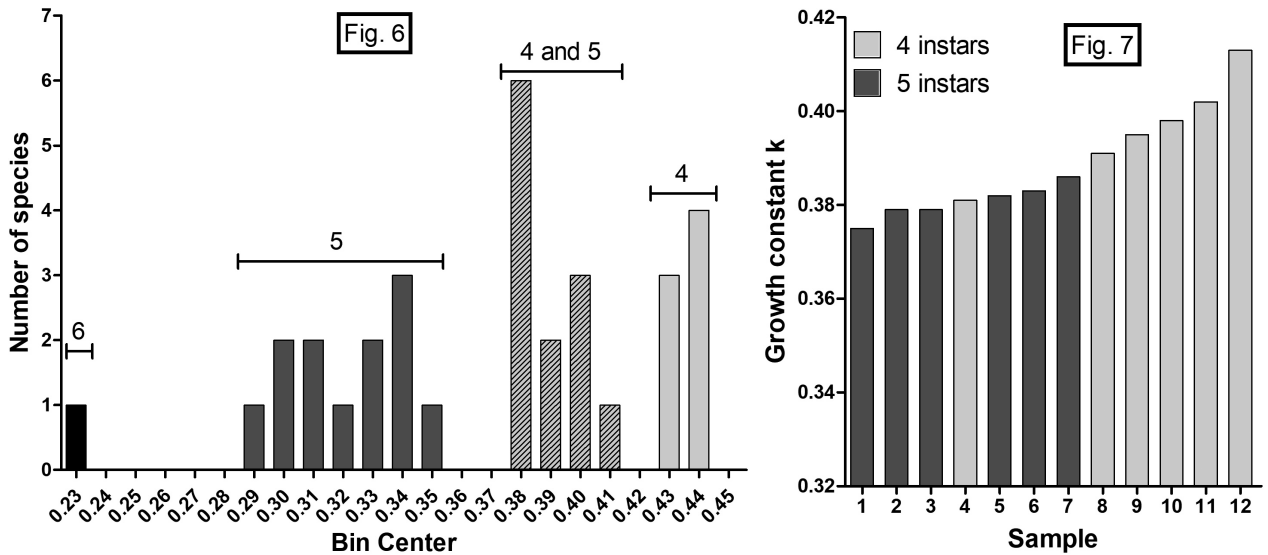
Interspecies comparison

Parameters describing head capsule growth such as averaged Dyar’s r (r_m , see Methods) and the growth constant of the exponential growth function k vary over a large range for different *Erebia* species. Dyar’s r ranges between 0.63 and 0.79 and the growth constant k ranges between 0.23 and 0.44 (Figs 4, 5).

The observed variation in growth parameters appears to be related to the number of moults in different *Erebia* species. The frequency distribution of the growth constant k reveals 3 distinct clusters comprised of species which develop via 4, 5 or 6 larval instars. A fourth cluster includes species with 4 or 5 instars, which cannot be clearly



Figures 4, 5. 4. Variation of Dyar’s r of head capsule growth among 33 samples of *Erebia* species arranged by increasing values. 5. Variation of the growth constant k for head capsule growth among 33 samples of *Erebia* species arranged in order of increasing values. Bar types indicate species samples with different numbers of larval instars according to the groups defined in Tables 2, 3. Black: 6 instars. Dark grey: 5 instars. Light grey: 4 instars. Shaded bars refer to subgroups 4B and 5A as defined in the text (see Tables 3, 4).



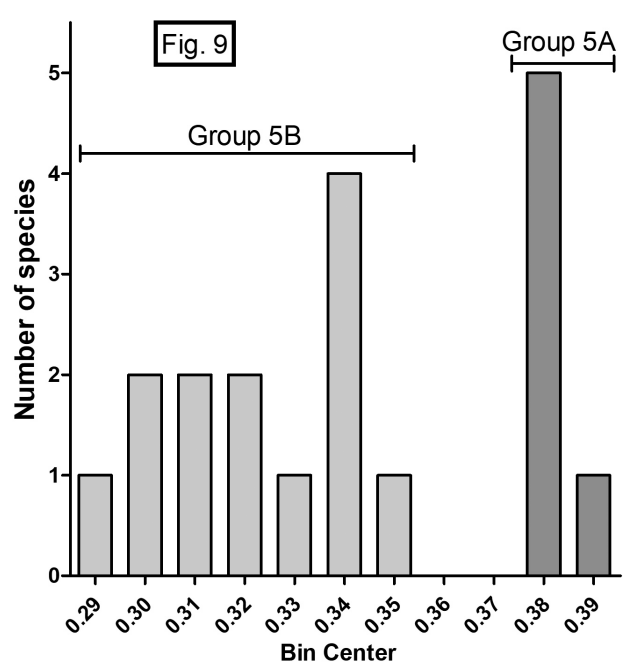
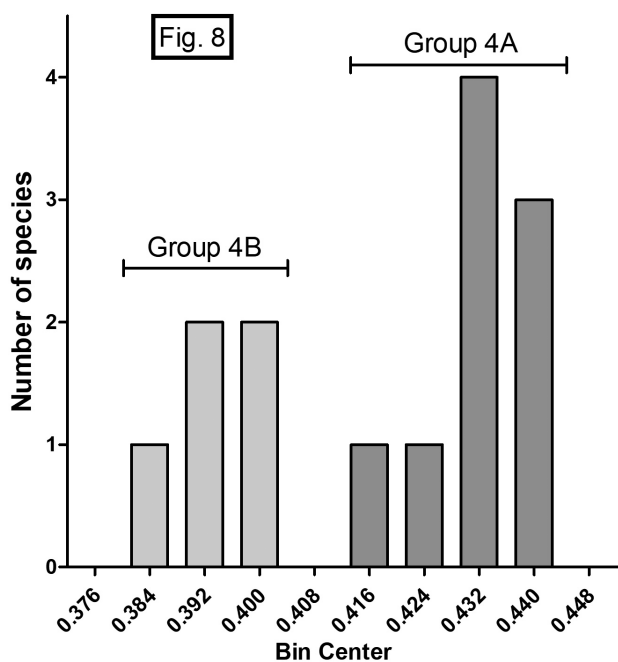
Figures 6, 7. 6. Frequency distribution of the growth constant *k* among *Erebia* species. Three clusters can be distinguished which contain species with 4 (light grey), 5 (dark grey) or 6 (black) larval instars. An additional cluster comprises species with 4 or 5 instars (hatched grey). 7. Detailed presentation as a bar diagram of the cluster from Fig. 6 comprising 12 species with 4 or 5 instars. Samples are arranged by increasing values of the growth constant *k*. Dark grey bars: species with 5 instars. Light grey bars: species with 4 instars.

separated (Fig. 6). The detailed composition of this cluster is depicted in Fig. 7 in which samples are arranged according to increasing *k*-values and are additionally distinguished by their instar number. Samples with 4 or 5 instars tend to be grouped close to their respective clusters of the frequency distribution. Means and standard deviations of the growth constant *k* for species with 4, 5, or 6 instars amount to 0.419 ± 0.022 ($n = 13$), 0.341 ± 0.031 ($n = 19$), and 0.232 ($n = 1$), respectively (Tables 3, 4). Corresponding values of the slope (*a*) derived from the logarithmic functions are 0.181 ± 0.010 ($n = 13$), 0.151 ± 0.012 ($n = 19$) and 0.108 ($n = 1$) (see also Tables 3, 4 below).

The frequency distributions of *k*-values for species developing via 4 or 5 instars reveal two distinct clusters

within each group that differ significantly from each other with $p < 0.0001$. Means and standard deviations of the growth constant *k* for species of group 4A, 4B, 5A and 5B are 0.435 ± 0.006 , 0.397 ± 0.011 , 0.381 ± 0.004 and 0.323 ± 0.016 , respectively.

The determined growth parameters are summarized for the different *Erebia* species in Tables 3, 4 in which the species are arranged according to the clusters defined above. For comparison data for some Satyrinae species belonging to tribes other than Erebiini are shown in Table 5. In addition, Table 6 presents calculated parameters of published data including results from three North American *Erebia* species and from two non-Satyrinae species.



Figures 8, 9. Frequency distribution of the growth constant *k* among *Erebia* species developing via 4 (8) or 5 (9) larval instars.

Table 3. Head capsule growth parameters for *Erebia* species with 4 larval instars.

Species	Instars	Exponential function		log-function		Dyar's $r r_m$	Ratio H_i/H_1
	Number n	Constant k	Start H_0	Slope a	Start S		
Group 4A							
<i>Erebia aethiops</i>	4	0.427	491	0.185	2.694	0.652	3.62
<i>Erebia stirus</i>	4	0.437	510	0.189	2.708	0.646	3.67
<i>Erebia meolans</i>	4	0.429	428	0.184	2.638	0.655	3.55
<i>Erebia montanus</i>	4	0.433	429	0.191	2.622	0.647	3.68
<i>Erebia oeme</i>	4	0.434	444	0.182	2.667	0.648	3.50
<i>Erebia lefebvrei</i>	4	0.442	486	0.198	2.667	0.632	3.95
<i>Erebia cassioides</i>	4	0.443	410	0.192	2.614	0.642	3.57
<i>Erebia neoridas</i>	4	0.435	466	0.197	2.644	0.638	3.83
Mean 4A		0.435	458	0.190	2.657	0.645	3.67
Standard Deviation 4A		0.006	36	0.006	0.033	0.007	0.15
Group 4B							
<i>Erebia hispania</i>	4	0.413	437	0.173	2.659	0.671	3.29
<i>Erebia aethiops</i>	4	0.402	501	0.173	2.706	0.669	3.29
<i>Erebia medusa</i>	4	0.395	464	0.171	2.667	0.674	3.29
<i>Erebia medusa</i>	4	0.381	547	0.166	2.737	0.683	3.13
<i>Erebia melampus</i>	4	0.398	380	0.175	2.574	0.672	3.30
<i>Erebia oeme</i>	4	0.391	515	0.170	2.711	0.677	3.23
Mean 4B		0.397	474	0.171	2.676	0.674	3.25
Standard Deviation 4B		0.011	60	0.003	0.058	0.005	0.07
Group 4 total (A and B)							
Mean 4A+4B		0.419	465	0.182	2.665	0.658	3.49
Standard Deviation 4A+4B		0.021	46	0.011	0.044	0.016	0.24

Table 4. Head capsule growth parameters for *Erebia* species with 5 or 6 larval instars. * L1 to L3 only; the value for L5 was extrapolated to calculate the ratio H_i/H_1 .

Species	Instars	Exponential function		Log-function		Dyar's $r r_m$	Ratio H_i/H_1
	Number n	Constant k	Start H_0	Slope a	Start S		
Group 5A							
<i>Erebia euryale</i>	5	0.382	411	0.168	2.606	0.677	4.73
<i>Erebia pandrose</i>	5	0.383	382	0.161	2.602	0.689	4.43
<i>Erebia nivalis</i>	5	0.386	393	0.167	2.598	0.670	4.69
<i>Erebia pluto</i>	5	0.375	479	0.168	2.661	0.687	4.80
<i>Erebia manto</i>	5	0.379	403	0.168	2.592	0.685	4.70
<i>Erebia melancholica</i>	5	0.379	454	0.164	2.659	0.685	4.56
Mean 5A		0.381	420	0.166	2.620	0.682	4.65
Standard Deviation 5A		0.004	38	0.003	0.032	0.007	0.13
Group 5B							
<i>Erebia melas</i>	5	0.325	585	0.148	2.743	0.723	3.96
<i>Erebia scipio</i>	5	0.338	523	0.153	2.695	0.704	4.06
<i>Erebia epistygne</i> *	5	0.333	481	0.145	2.682	0.717	3.79
<i>Erebia epistygne</i>	5	0.336	457	0.146	2.662	0.708	3.95
<i>Erebia tyndarus</i>	5	0.311	471	0.139	2.659	0.721	3.70
<i>Erebia aethiopella</i>	5	0.321	454	0.151	2.616	0.725	3.92
<i>Erebia mnestra</i>	5	0.313	436	0.142	2.618	0.731	3.64
<i>Erebia pararica</i>	5	0.337	709	0.138	2.687	0.714	3.51
<i>Erebia aethiops</i>	5	0.300	587	0.133	2.757	0.741	3.62
<i>Erebia epiphron</i>	5	0.295	481	0.139	2.643	0.745	3.64
<i>Erebia gorge</i>	5	0.345	422	0.155	2.607	0.708	4.24
<i>Erebia medusa</i>	5	0.336	448	0.147	2.648	0.707	3.99
<i>Erebia neoridas</i>	5	0.304	538	0.139	2.699	0.727	3.54
Mean 5B		0.323	507	0.144	2.670	0.721	3.81
Standard Deviation 5B		0.016	84	0.007	0.048	0.013	0.22
Group 5 total (A and B)							
Mean 5A+5B		0.341	480	0.151	2.654	0.709	4.08
Standard Deviation 5A+5B		0.031	80	0.012	0.048	0.022	0.45
Group 6							
<i>Erebia triaria</i>	6	0.232	738	0.108	2.834	0.793	3.14

Table 5. Head capsule growth parameters for some non-*Erebia* species (Erebiina) belonging to different subtribes of the Satyrini.

Species Subtribus	Instars	Exponential function		Log-function		Dyar's $r r_m$	Ratio H_t/H_1
	Number n	Constant k	Start H_0	Slope a	Start S		
<i>Melanargia galathea</i> Melanargiina	4	0.475	393	0.194	2.632	0.637	3.84
<i>Strabena tamatavae</i> Ypthimina	4	0.408	447	0.173	2.664	0.672	3.29
<i>Protorebia afer</i> Callerebiina	5	0.301	640	0.132	2.801	0.730	3.44
<i>Mycalesis perseus</i> Mycalesina	5	0.378	396	0.153	2.637	0.708	4.02
<i>Lasiommata maera</i> Parargina	5	0.320	534	0.140	2.723	0.714	3.82
<i>Hipparchia semele</i> Satyrina	5	0.449	377	0.194	2.580	0.650	5.60

Table 6. Head capsule growth parameters calculated from published data for three North American *Erebia* species, for *Erionota thrax* (Hesperiidae) and for *Mechanitis polymnia* (Danainae). Original data of head capsule widths used for calculation were published by * Hilchie (1990), ** Matsumoto et al. (1995) and *** Carvalho et al. (2019).

Species	Instars	Exponential function		Log-function		Dyar's $r r_m$	Ratio H_t/H_1
	Number n	Constant k	Start H_0	Slope a	Start S		
<i>Erebia magdalena</i> *	5	0.325	591	0.141	2.776	0.720	3.70
<i>Erebia mackinleyensis</i> *	5	0.351	523	0.152	2.718	0.705	4.05
<i>Erebia fasciata</i> *	5	0.350	493	0.152	2.690	0.705	4.04
Mean		0.342	536	0.148	2.728	0.710	3.93
<i>Erionota thrax</i> **	5	0.346	641	0.147	2.820	0.710	3.97
<i>Mechanitis polymnia</i> ***	5	0.350	400	0.162	2.566	0.690	4.41

The averaged growth parameters for the recognized groups (4, 5, 6) and subgroups (4A, 4B, 5A, 5B) as listed in Tables 3, 4 are presented as box plots in Figs 10–12 which demonstrate that the groups are convincingly distinguishable by the growth parameters k and a of the mathematical functions. They differ significantly ($P < 0.0001$), except for the subgroup pair 4B/5A with lower significance ($P = 0.0052$ and $P = 0.0121$ for the growth parameters k and a, respectively; Table 7). Dyar's r also differs significantly between all groups except for the subgroup pair 4B/5A. A box plot for the calculated start values H_0 of the exponential growth function for the different clusters is presented in Fig. 13. A corresponding significance analysis does not reveal any relevant differences between the groups or subgroups (Table 7).

There is a good correlation between Dyar's r and the growth constant k ($r^2 = 0.9674$) depicted in Fig. 14 in which the different subgroups are also distinguished. All the differences in growth parameters between the groups are highly significant ($P < 0.0001$). This is also true for Dyar's r of the groups with 4 and 5 instars.

Discrimination between instars

The head capsule size of a larval stage varies among individuals. However, the ranges of variation between successive larval stages rarely overlap. Therefore, in most cases, it is possible to assign a larva to a defined stage based on head size. This is exemplified in Figs 15, 16, which show the size distribution of larval head capsules as box plots for *Erebia gorge* and *Erebia aethiops*. Similar results were obtained for the other *Erebia* species studied.

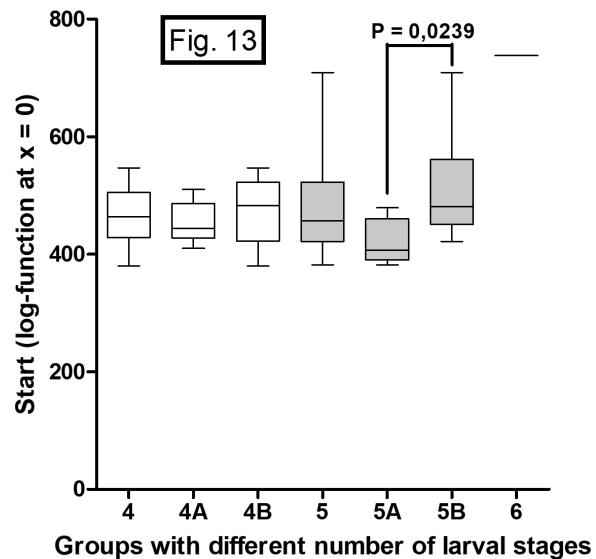
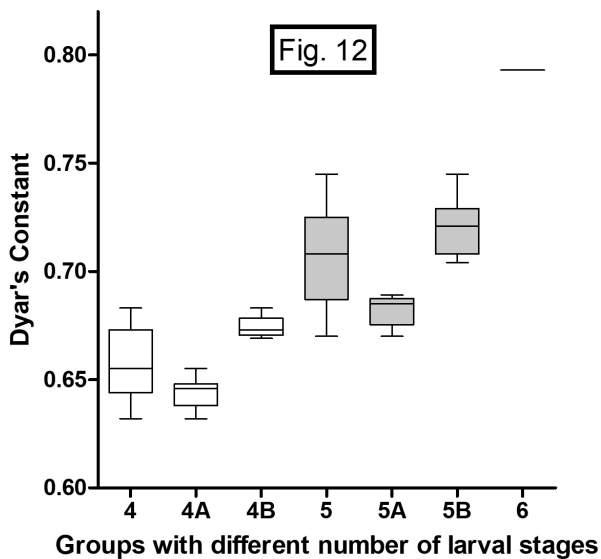
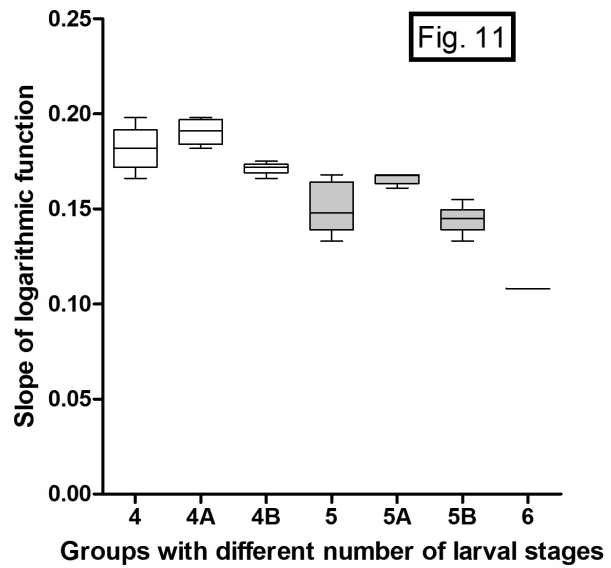
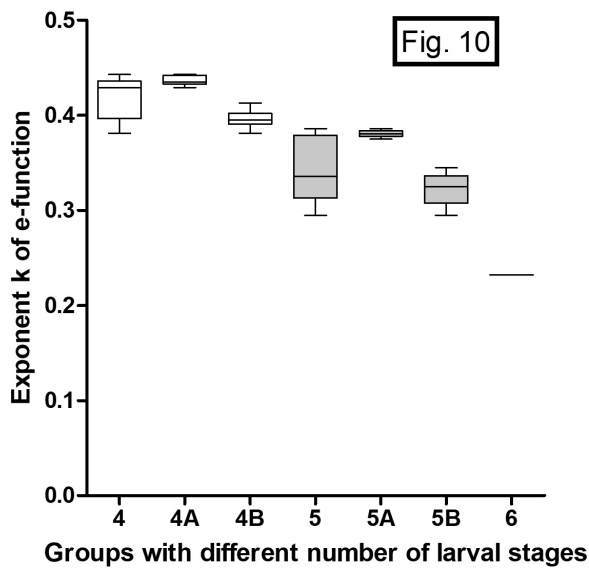
Table 7. Unpaired t-Test to verify significant differences in head capsule growth parameters between defined groups of *Erebia* species with 4 or 5 instars (see Figs 10–13). Student's t-test was applied. t-values (t), degrees of freedom (df) and P-values (P) are shown. For column statistics see Tables 3, 4. * not significant.

Compared Groups	Constant k e-function			Slope a log-function		
	t	df	P	T	df	P
4 vs 5	7.734	30	<0.0001	7.388	30	<0.0001
4A vs 4B	9.510	12	<0.0001	6.966	11	<0.0001
4A vs 5A	22	11	<0.0001	9.036	11	<0.0001
4A vs 5B	17.58	18	<0.0001	15.43	18	<0.0001
4B vs 5A	3.477	11	0.0052	3.057	10	0.0121
4B vs 5B	10.65	18	<0.0001	9.520	17	<0.0001
5A vs 5B	8.391	17	<0.0001	7.697	17	<0.0001

Compared Groups	Dyar's $r r_m$			Start (H_0) e-function		
	t	df	P	T	df	P
4 vs 5	7.076	30	<0.0001	0.6768	30	0.5037*
4A vs 4B	8.419	11	<0.0001	0.7706	11	0.4572*
4A vs 5A	9.329	11	<0.0001	1.614	11	0.1349*
4A vs 5B	14.33	18	<0.0001	1.661	18	0.1139*
4B vs 5A	2.178	10	0.0545*	1.851	10	0.0939*
4B vs 5B	8.386	17	<0.0001	0.8907	17	0.3855*
5A vs 5B	6.766	17	<0.0001	2.480	17	0.0239

Intra-species variability of instar numbers

For a few species, variability in growth parameters was examined for samples from different localities or populations, including cases in which the same species can develop via 4 or 5 larval instars. In Figs 17, 18 the growth functions of head capsule widths for *Erebia aethiops* from Hautes Alpes, Isère, and Bavaria are shown. Growth characteristics of larva from Isère and Bavaria, which develop via 4 instars are similar and clearly differ from the Hautes Alpes specimens (5 instars). Regardless of the



Figures 10–13. Box plots of different growth parameters for the defined species groups listed in Tables 2, 3. Values are presented as median, upper and lower quartiles, maximum, and minimum.

developmental type, however, the head capsule widths of the final instars (L4 or L5), were nearly identical (Figs 17, 18). As the initial head capsule widths in L1 are similar, the ratios of the head capsule widths of the final and the first instar are also identical: namely 3.62 (Hautes Alpes), 3.62 (Isère) and 3.61 (Bavaria). Thus, the same final result is achieved regardless of developmental type. Similar results were obtained for *Erebia medusa* (not shown) and also for *Erebia neoridas* (Figs 19, 20) with H_i/H_0 ratios of 3.83 and 3.54, respectively.

Growth strategies and life history

For most of the studied species their life cycle is known. Respective data were well summarized by Lafranchis (2000) and by Sonderegger (2005) for the French and the Swiss *Erebia* species, respectively. The arrangement of Sonderegger (2005) was adopted here and related to

the development groups defined above. The arrangement considers larval developmental time (1 or 2 years) as well as the stage of hibernation, and has been coded using numerical values according to Table 8. The assigned values were then plotted against the growth constant k of the exponential function (Fig. 21).

The plot shows that species with 4 larval instars (4A and 4B) develop within a 1-year life cycle with one exception only (*Erebia lefebvrei*), while species with 5 instars of group 5B may develop over one or two years. All five species of group 5A demand a 2-year life cycle.

Growth strategies and phylogenetics

To test the hypothesis that the various growth strategies are associated with phylogenetically based species clusters of the *Erebia* radiation several analyses were performed. First, a neighbor-joining tree and a maximum

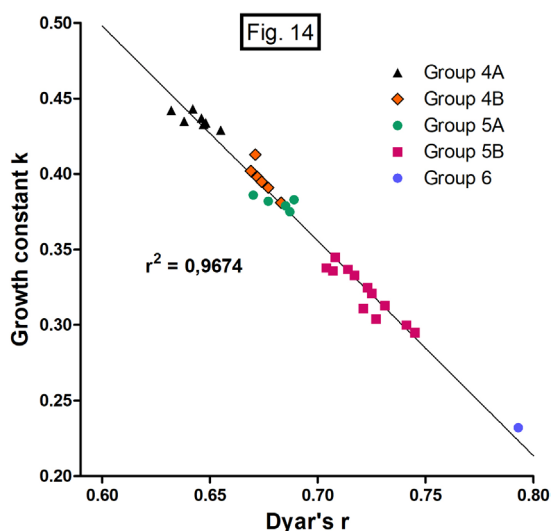
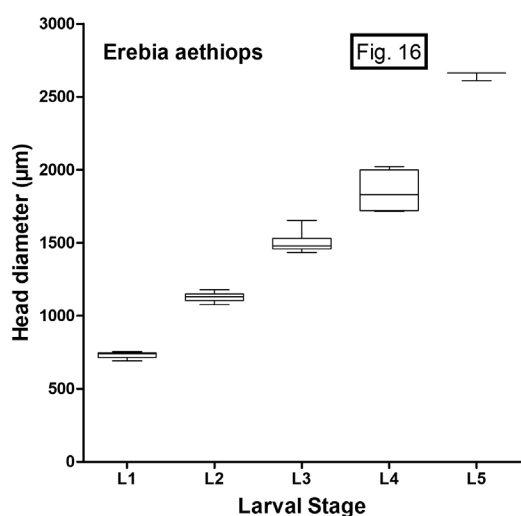
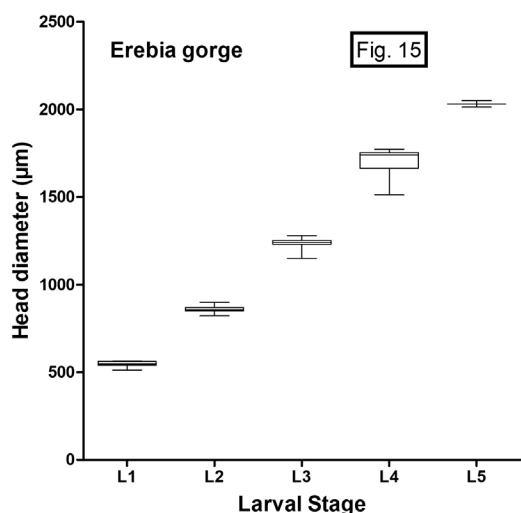


Figure 14. Correlation between Dyar's *r* and the growth constant *k* of the exponential function for head capsule growth among 33 samples of *Erebia* species. Species samples are assigned to the defined growth types as indicated by color and shape of the symbols.



Figures 15, 16. Size variability of head capsule width of defined larval instars of *Erebia gorge* and *Erebia aethiops*. Data are presented as box-plots showing median, upper and lower quartiles, maximum, and minimum.

Table 8. Larval development patterns of *Erebia* species. The complete life cycle may span one or two years and accordingly includes one or two hibernations in different developmental stages as indicated. Life cycle data were adopted from Lafranchis (2000) and Sonderegger (2005). The numerical code is used for the graphical presentation in Fig. 21.

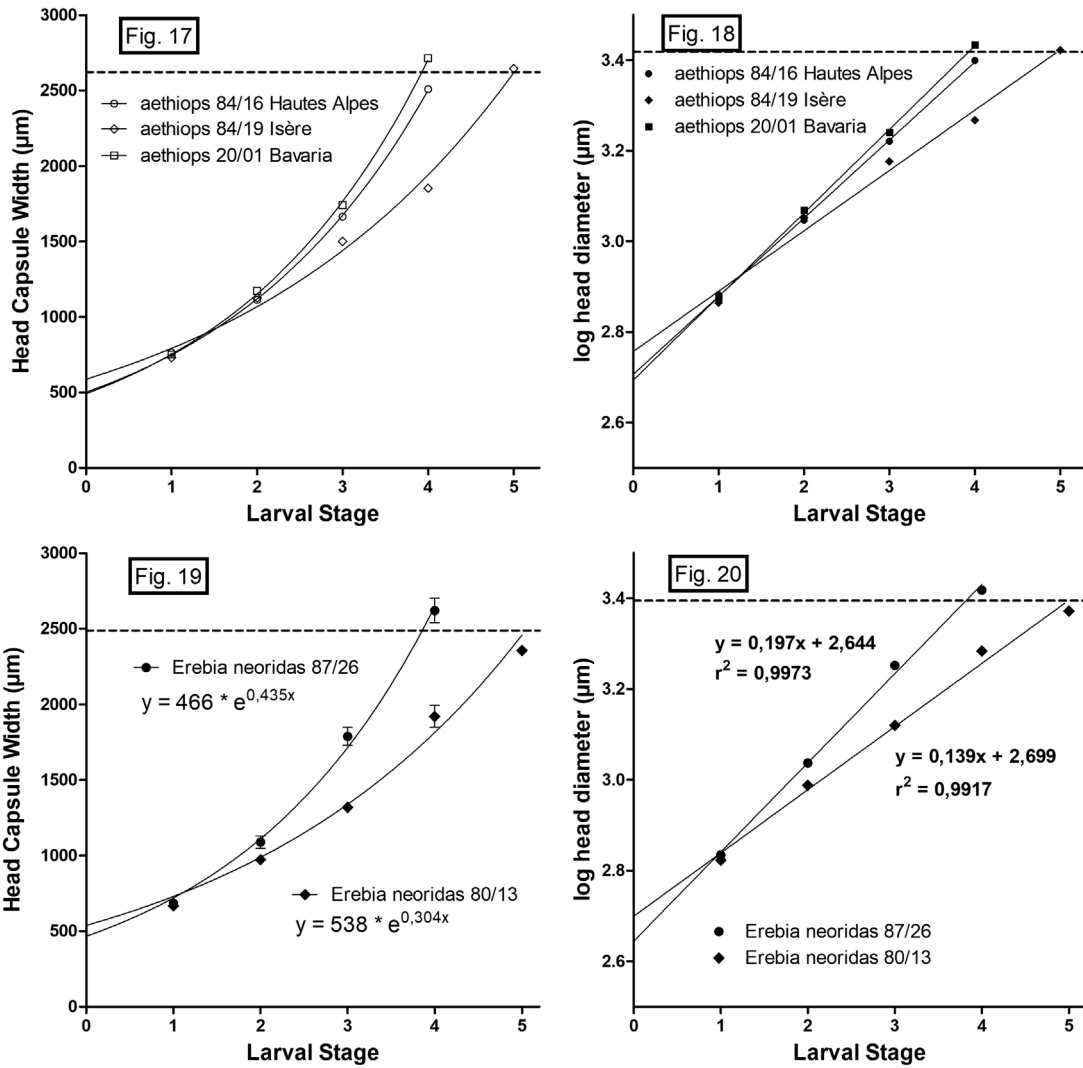
Code	Duration years	Hibernation 1 stage	Hibernation 2 stage	Species
11	1	L ₁ or L ₂	-	<i>E. aethiops</i> , <i>E. cassioides</i> , <i>E. melas</i> , <i>E. montanus</i> , <i>E. neoridas</i> , <i>E. stirius</i> , <i>E. tyndarus</i>
12	1	L ₂	-	<i>E. melampus</i>
13	1	L _{t-1}	-	-
14	1	L _t	-	<i>E. epistygne</i> , <i>E. medusa</i> , <i>E. meolans</i> , <i>E. oeme</i> , <i>E. triaria</i>
21	2	Egg	L _{t-1}	<i>E. euryale</i>
22	2	L ₁	L _{t-1}	-
23	2	L ₁ or L ₂	L _{t-1}	<i>E. epiphron</i> , <i>E. gorge</i> , <i>E. lefebvrei</i> , <i>E. manto</i> , <i>E. mnestra</i> , <i>E. nivalis</i> , <i>E. scipio</i>
24	2	L ₁ or L ₂	L _t	<i>E. pandrose</i> , <i>E. pluto</i>

parsimony tree were constructed for the species examined in this study. They are based on COI DNA-sequence sections of the 5'-region with a length of 658 base pairs accessible from GenBank (National Center for Biotechnology Information). For *Erebia melancholica* and the outgroup species *Callerebia polyphemus* (Oberthür, [1876]), slightly shorter sequence sections were available only.

Fig. 22 shows the resultant neighbor joining tree in which the species have been marked by colored circles and diamonds according to the growth types described in Tables 3–6. Additionally, four species clusters were marked with A – D, including the species of the *pronoe*-group (A) as defined by Warren (1936) and the species of the *tyndarus*-group (C), see Lorkovic (1957, 1958). They are considered monophyletic for the following reasons. They consistently appear in trees based on sequence data of further mitochondrial and nuclear genes, namely ND1, ND5, Wingless, GAPDH, RpS5, and 16SrRNA as well as on restriction data of ND5 (Roos, unpublished). The 4 clusters are also identified in the recently published multi-gene phylogeny of European butterflies (Wiemers et al. 2020). In addition, their monophyly is supported by imaginal and preimaginal morphological characters (Roos, unpublished).

Obviously, there is no association between growth strategies and phylogenetically defined species clusters, in particular pointed up by the monophyletic *melas*-group (A) and *tyndarus*-group (C).

In a second approach, phylogenetic non-independence of all growth parameters of Tables 3–6 was checked by



Figures 17–20. Growth functions for two species with larval development via 4 or 5 instars. **17.** *Erebia aethiops*, exponential function. **18.** *Erebia aethiops*, logarithmic function. **19.** *Erebia neoridas*, exponential function. Data points represent means and standard deviations. **20.** *Erebia neoridas*, logarithmic function. Hatched lines indicate the means of maximal values.

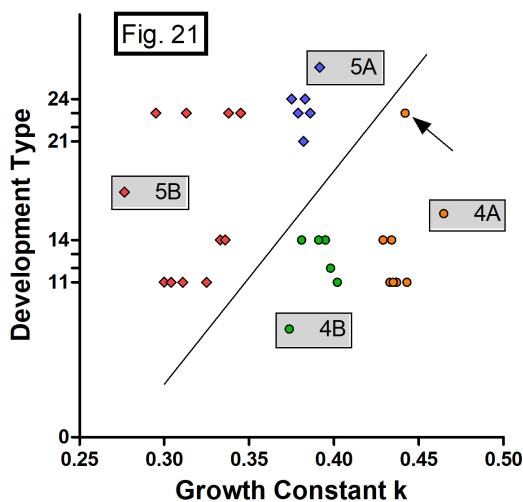


Figure 21. Relationship between life cycle type as defined in Table 8 and growth constant k for larval head capsule development of various *Erebia* species. Samples were color-coded for the distinguished growth types (see Tables 3, 4). The arrow marks the position of *Erebia lefebvrei*.

PGLS (phylogenetic generalized least squares). A parsimony-based tree was used with *Proterebia afra* as outgroup. Pagel’s lambda was calculated for all growth parameters. The low lambda-values, most are zero, show that growth parameters are independent of the phylogenetic background (Table 9).

Dyar’s rule

As is evident from Table 10 the ratio of head capsule widths (H) from successive instars (Dyar’s r) of a species is not constant in most cases and thus does not follow Dyar’s rule. The deviation from constancy can be expressed by the calculated standard deviation (SD) of averaged Dyar’s r which is further transformed into % deviation for better inter-species comparability. For some data sets the deviation is below 2% such as for *E. medusa*, *E. cassioides* and *E. melancholica* so that constancy of the size increment, i.e. consistency with Dyar’s rule, can be assumed.

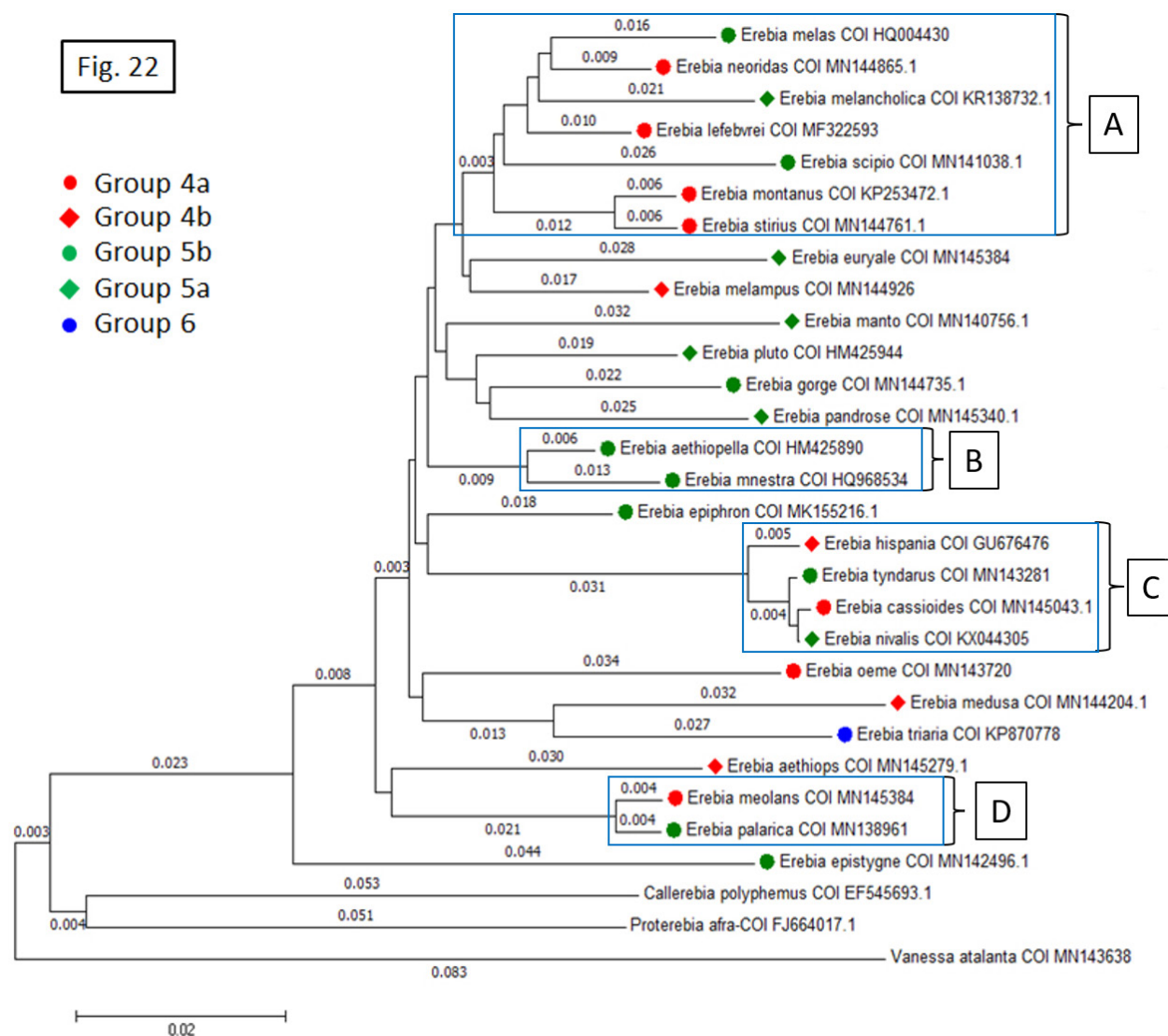


Figure 22. Evolutionary relationships of the studied *Erebia* species using published barcode sequences, i.e. partial DNA-sequences of the mitochondrial gene for cytochrome oxidase subunit I (COI). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The optimal tree with the sum of branch lengths = 0.79628874 is shown. The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. The analysis involved 30 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 609 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016). Species are marked with colored circles and diamonds corresponding to the growth strategy via 4 (red), 5 (green) or 6 (blue) larval instars. Groups are indicated as follows: red circles (group 4A), red diamonds (group 4B), green diamonds (group 5A), green circles (group 5B). Four monophyletic species clusters are framed and marked with A–D. Accession numbers from GenBank for the sequences used are given.

High deviations from the rule, however, were observed for *E. hispania*, *E. alberganus*, developing via 4 instars, and *E. melas*, *E. neoridas*, *E. aethiops* developing via 5 instars.

Discussion

To understand variability in larval growth trajectories of butterflies with respect to growth increments and instar numbers as well as in deviations from Dyar's rule numerous phylogenetically closely related species of a radiation

concerning a single genus have been studied in this respect. To my knowledge such an analysis has not been performed so far. Altogether, 27 species of the genus *Erebia* were included in the study, some of them from several different geographical locations.

For interspecies comparisons, suitable parameters for growth characteristics had to be defined which can be easily determined and which allow easy interpretation. As such the constants of an exponential growth function, i.e. the growth constant k , and of the corresponding logarithmic function, i.e. the slope a , proved to be the most

Table 9. Pagel’s lambda assessed for the various growth parameters by PGLS. The data set of Tables 3, 4 is used. For the calculations and the tree construction with PAST4.17 *Proterebia afra* (Table 5) was chosen as outgroup.

	Instars	Exponential function		Log-function		Dyar’s $r r_m$	Ratio H_i/H_1
	Number n	Constant k	Start H_0	Slope a	Start S		
Pagel’s Lambda	0	0	0	0	0.711	0	0.409

Table 10. Dyar’s r for head capsule widths (H) of successive instars of various *Erebia* species which develop via 4 and 5 instars. Means (= averaged Dyar’s r) and standard deviations (SD) were calculated. For better interspecies comparison, the percentage deviation (% Dev) is shown in addition. GP values, as defined by Hawes (2020), are listed in the last column.

Species	Parameter	L1	L2	L3	L4	L5	Mean	SD	% Dev	GP
<i>E. medusa</i>	H (µm)	685	1034	1505	2253	-	-	-	-	-0.013
	Dyar’s r	-	0.662	0.687	0.668	-	0.672	0.013	1.94	
<i>E. cassioides</i>	H (µm)	643	987	1554	2410	-	-	-	-	0.006
	Dyar’s r	-	0.651	0.635	0.645	-	0.644	0.008	1.27	
<i>E. hispania</i>	H (µm)	681	1027	1851	2253	-	-	-	-	0.453
	Dyar’s r	-	0.663	0.555	0.822	-	0.680	0.134	19.73	
<i>E. neoridas</i>	H (µm)	684	1089	1788	2620	-	-	-	-	0.153
	Dyar’s r	-	0.628	0.609	0.682	-	0.640	0.038	5.94	
<i>E. oeme</i>	H (µm)	736	1091	1566	2292	-	-	-	-	-0.005
	Dyar’s r	-	0.674	0.697	0.683	-	0.685	0.011	1.65	
<i>E. aethiops</i>	H (µm)	751	1173	1741	2714	-	-	-	-	-0.042
	Dyar’s r	-	0.640	0.674	0.642	-	0.651	0.019	2.92	
<i>E. alberganus</i>	H (µm)	726	1054	1573	2016	-	-	-	-	0.159
	Dyar’s r	-	0.688	0.670	0.780	-	0.710	0.059	8.31	
<i>E. styx</i>	H (µm)	883	1261	2016	2985	-	-	-	-	0.055
	Dyar’s r	-	0.701	0.625	0.675	-	0.666	0.038	5.71	
<i>E. melancholica</i>	H (µm)	662	982	1406	2057	3021	-	-	-	-0.009
	Dyar’s r	-	0.675	0.698	0.684	0.681	0.684	0.010	1.44	
<i>E. melas</i>	H (µm)	749	1083	1673	2074	2790	-	-	-	0.035
	Dyar’s r	-	0.691	0.647	0.807	0.743	0.722	0.069	9.49	
<i>E. aethiopella</i>	H (µm)	565	813	1266	1698	2324	-	-	-	0.036
	Dyar’s r	-	0.696	0.642	0.745	0.731	0.703	0.046	6.52	
<i>E. gorge</i>	H (µm)	546	861	1233	1705	2312	-	-	-	0.059
	Dyar’s r	-	0.634	0.698	0.723	0.737	0.698	0.046	6.55	
<i>E. neoridas</i>	H (µm)	666	973	1318	1920	2355	-	-	-	0.174
	Dyar’s r	-	0.684	0.738	0.687	0.815	0.731	0.061	8.39	
<i>E. pronoe</i>	H (µm)	626	934	1380	1999	3030	-	-	-	-0.047
	Dyar’s r	-	0.670	0.677	0.690	0.660	0.674	0.031	1.90	
<i>E. aethiops</i>	H (µm)	731	1127	1501	1853	2645	-	-	-	-0.067
	Dyar’s r	-	0.649	0.751	0.810	0.701	0.728	0.069	9.49	

suitable parameters. Although the second order polynomial functions reveal even a slightly better fit to the data sets compared to the exponential growth functions, the resultant set of two varying coefficients, a and b, is not suitable for inter-species comparisons.

The stepwise increase in head capsule size during larval development of butterflies allows the identification of the different instars, a feature which has been often used to follow growth progression of species known as crop pests (Matsumoto et al. 1995; Stavridis et al. 2003–2004; Khorasiya et al. 2014; Nur Athiqah et al. 2015; Thakur 2016). According to Dyar’s rule, the size increment is proposed to remain constant from moult to moult for a given species (Dyar 1890) but may differ between species. As shown by the data presented here and also by published data (Calvo and Molina 2008; Goettel and

Philogène 1979; Hutchinson et al. 1997; Springolo et al. 2021), constancy of the growth increment according to Dyar’s rule, however, is an exception but not at all the rule. This is also true for the suggestion that the number of moults is constant for a given species as has been refuted by literature data (Morita and Tojo 1985; Shreeve 1986; Garcia-Barros 2006; Kingsolver 2007; Calvo and Molina 2008; Abarca et al. 2020) and the findings presented here (Tables 3, 4).

The growth parameters derived from the exponential and logarithmic functions vary substantially between different *Erebia* species. The parameter values do not follow a Gaussian distribution, as one may expect, but form discrete clusters in the frequency distribution. These clusters are linked to the life-histories of the included species. As is shown in Tables 3, 4, the total number of instars,

usually 4 or 5 in *Erebia*, can be derived from the growth parameters. The constant k of the exponential function amounts to 0.419 ± 0.021 and 0.341 ± 0.031 for species developing via 4 or 5 instars, respectively. Corresponding values of the slope a of the logarithmic function are 0.182 ± 0.011 and 0.151 ± 0.012 .

Growth parameters calculated from published data of Hilchie (1990) on larval head capsule growth of three North American *Erebia* species (Table 6) fit well into the subgroup model presented here. The mean of the constant k of 0.342 (for *E. magdalena*, *E. mackinleyensis* and *E. fasciata*) is close to a value of 0.323 for subgroup 5B with 5 instars (Table 4). Whether the determined growth parameters can be absolutely applied to butterfly groups other than *Erebia* has to be examined. The data of Table 5 suggest that *Proterebia*, *Mycalesis* and *Lasiommata* show growth parameters similar to *Erebia*. Aside from the Satyrinae, the skipper *Erionota thrax* and the danaine *Mechanitis polymnia* also fit well into the growth scheme.

The different larval growth increments of *Erebia* species obviously allow to predict the number of moults the distinct species have to pass through. Furthermore, they allow a rough assignment to the life history of a species with respect to the number of hibernations, 1 or 2, in the larval stage (Fig. 21).

Erebia species for which varying instar numbers were observed, such as *E. aethiops*, *E. neoridas* and *E. medusa*, interestingly attain the same final larval head capsule size irrespective of the instar number. This is achieved by a counterbalanced growth increment. To formulate it shortly: different strategies but same goal! This has been also shown for the skipper butterfly *Epargyreus clarus* (Cramer, 1775) or the arctiid moth *Phoenicoprocta capistrata* (Fabricius, 1775) for which no differences in the ultimate head capsule size achieved via 5 to 6 or 6 to 8 instars, respectively, were detected (Rodríguez-Loechesa and Barro 2008; Abarca et al. 2020). The authors also showed that growth data obtained from the first 3 instars will allow to predict the final number of moults. This will probably also hold for the *Erebia* species analyzed here, but has to be proven yet. The suggestion is supported by the fact that head capsules of a defined instar show only a small variation in size (Figs 15, 16).

In some cases the increase in head capsule size shows strong deviations from Dyar's rule, such as for *E. hispania*, *E. aethiops*, *E. melas* and *E. neoridas* (Table 9). However, these deviations do not affect the assignment of the species to the different growth increment clusters. Obviously, the deviation induced by one larval stage is compensated by the development of the following stages. Deviation from Dyar's rule is best calculated via the standard deviation of averaged Dyar's r . Values obtained by the method of Hawes (2020) to assess the deviation can be misleading as has been explained in the Methods section.

The underlying biochemical/physiological mechanisms of moulting in particular the final moulting to the chrysalis are well known (Nijhout 2003, 2013). However, the genetic background and the mechanisms which determine the number of moults and the size increment per moult

are not well understood although these parameters are important as they determine the size of the resulting adult (Kivelä et al. 2020). Esperk et al. (2007a) and Abarca et al. (2020) argue that variability in size increments such as a decrease due to nutrient or other environmental stress will result in supernumerary instars resulting in a 'normal sized' adult. Etilé and Despland, (2008) showed for *Malacosoma disstria* (Hübner, 1820) that a final threshold of the head capsule size or of the larval body weight has to be reached to determine moulting to the pupa. According to their study, restrictions in nutrient supply may lead to an increase in larval instar numbers and slower growth rate and consequently to a prolonged development time. The mechanism, however, by which threshold size is assessed remains unknown (Nijhout and Callier 2015)

The present study shows, however, that different instar numbers and size increments are obviously programmed and will be realized a priori, i.e. with hatching of the larva. Examples are the head capsule growth characteristics of *Erebia aethiops*, *Erebia medusa* and *Erebia neoridas* which can develop dependent on their origin either via 4 or 5 instars and attain identical head capsule sizes in the final instars irrespective of the number of moults. As the larvae developing via 4 or 5 instars in each case descend from different females, an explanation for the different instar numbers by the compensation hypothesis (Esperk and Tammaru 2004, 2010; Esperk et al. 2007a; Barraclough et al. 2014) appears improbable. However, this variation can be better interpreted as an indication for a genetically based fixation of the instar numbers and represents a kind of developmental polymorphism within the species. Daimon et al. (2021) identified three alleles of the Hox gene *Scr* (Sex combs reduced) as responsible for the determination of the larval instar number, either 4, 5 or 6, in *Bombyx mori* (Linnaeus, 1758). *Scr* is specifically expressed in the prothoracic gland of larvae and influences the synthesis of moulting hormones. Genetic fixation of instar numbers does not exclude further modulatory effects on numbers by environmental factors during development which then lead to stronger deviation from Dyar's rule. As already mentioned above, Esperk et al. (2007a) conclude from available data that supernumerary instars may occur to compensate for growth deficiencies and Abarca et al. (2020) and Kingsolver (2007) have shown that they can be induced by thermal or host plant stress. Also restricted oxygen supply caused by increasing body size but fixed configuration of the tracheal system leads to a moulting signal (Callier and Nijhout 2011). This mechanism does not explain the variability in instar numbers of the above-mentioned *Erebia* species.

Obviously, there is no clear association between phylogenetically based species clusters and the mode of growth strategies, i.e. number of moults and different growth parameters. Thus the different growth modes have been independently developed several times in different species and species clusters. This becomes particular clear for the species of the *Erebia tyndarus* cluster which includes species belonging to groups 4A, 4B, 5A and 5B, namely

E. cassioides, *E. hispania*, *E. nivalis* and *E. tyndarus*, respectively. Taking genetic fixation of the instar number as a basis, one can assume that only slight modifications in respective regulatory proteins or their expression may lead to an evolutionary switch in growth modes.

Conclusions

Larval growth trajectories differ between species of the genus *Erebia* with respect to number of moults and growth increments. Variation of the growth increments and of Dyar's constant is related to the number of instars. For some *Erebia* species, developmental polymorphism has been detected. For these species, development via 4 or 5 instars results in the same size as the last instar due to a respective change in growth increments. The number of instars is linked to the life cycle of a species. Species which develop via 4 instars hibernate only once in the larval stage while those with 5 instars may hibernate one or two times. There is no association between growth strategies and phylogenetically defined species clusters.

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