Nitrogen limitation and life history adaptation in the grasshopper *Omocestus viridulus*

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Introduction

The choice of the grasshopper *Omocestus viridulus* as a study system offered the opportunity for investigations in two distinct research fields of ecology. In the first chapter, the focus lies on nitrogen limitation and nutrient balancing, aspects of plant-herbivore interaction. An evolutionary ecological perspective is adopted in chapters two and three that deal with life history adaptation.

The basic question underlying my experimental work on plant-herbivore interaction is about the nature of the control of herbivore population densities. It is generally agreed that under most natural conditions herbivores utilize only a relatively small fraction of the biomass produced by terrestrial plant communities, resulting in a 'green world' (Hairston et al. 1960; Polis 1999). This observation has inspired two major lines of reasoning. On the one hand, it has been suggested that predators suppress herbivore populations to relatively low densities, thereby releasing plants from herbivore attacks ('top-down control') (e.g. Hairston et al. 1960, Fretwell 1987, Oksanen and Oksanen 2000). On the other hand, 'bottom-up' approaches imply that plants themselves are relatively well defended against herbivores. Such defence may be structural, due to defence chemicals and/or low nutritional quality (e.g. Ehrlich and Birch 1967, Feeny 1976, Coley et al. 1985, Myers and Bazely 1991, Augner 1995). A specific version of the argument that the availability of nutritional requisites limits herbivore abundance is offered by the **nitrogen limitation** concept (Mattson 1980, White 1993). According to this concept, terrestrial herbivores face a relative shortage of nitrogen (N) that arises from the difference in elemental tissue composition (stoichiometry) between plants and animals. As a result, plant tissue should represent a poor food base causing high mortality in herbivores.

Over the recent years, it has become clear that both top-down and bottom-up forces are important (Cornell and Hawkins 1995, Olff et al. 1999, Polis 1999). However, the degree and consistency to which predators and resources control herbivore abundance in different ecosystems remain little understood (Hunter and Price 1992, Power 1992, Ritchie and Olff 1999, Dyer and Letourneau 2003). This motivated my examination of the applicability of the N limitation framework to grasshopper herbivores. I carried out a series of experiments using food manipulated in its N content and quantified key aspects of grasshopper performance and feeding behaviour. The basic expectation based on N limitation was a high susceptibility of herbivore performance to variation in plant tissue N content - however, I will make the case that this view of population control is overly simplistic. Since the notion of nutrient limitation is relative and meaningful only within the real ecological context of a given species, particular care was taken to choose realistic experimental conditions, e.g. by using natural food plants (instead of artificial diets) and outdoor replication.

As pointed out previously (Jones and Lawton 1995, Polis and Strong 1996, Polis 1999, Schmitz et al. 2000), it is essential to learn more about species specific responses to defined ecological factors before reliable generalizations about trophic interactions at the community level can be made. Since grasshoppers represent the top invertebrate herbivores in many grassland ecosystems (Chapman and Joern 1990, Ingrisch and Köhler 1998), the present investigation of N limitation provides a valuable contribution to this issue. Knowledge about herbivore responses to plant tissue N is also useful in the light of the tremendous human increase in the level of N available to primary producers currently taking place (Vitousek et al. 1997, Kaiser 2001), and has implications for the further development of ecological stoichiometry and nutrient balance theory (Sterner and Elser 2002, Raubenheimer and Simpson 2004).

The starting point of my life history studies (Chapters two and three) was the impressive altitudinal distribution of *Omocestus viridulus*. In Switzerland, this annual grasshopper occurs from roughly 400 m to above 2500 m altitude. This altitudinal gradient coincides with a dramatic decline in temperature, an ecological factor of prime importance to ectotherm life cycles (Johnston and Bennett 1996). As a result, a shorter thermally effective growing season is available to populations occurring at high elevation. In contrast to low elevation conspecifics, high altitude grasshoppers might therefore experience **seasonal time constraints** on the life history in the sense that a short growing season trades off time required to reach maturity with time available to reproduction (Fig. 1). As a response to

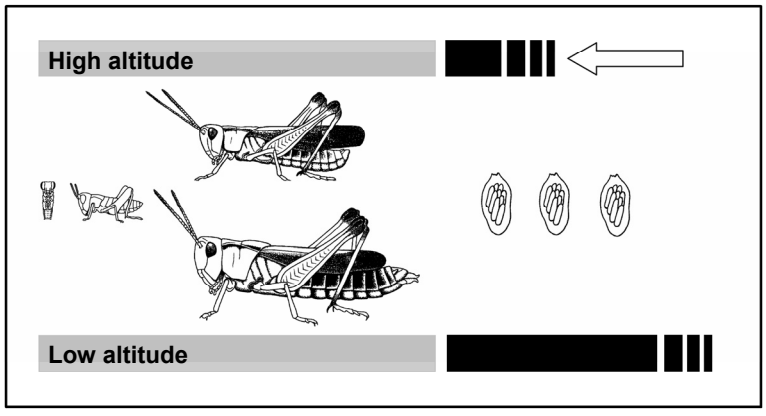


Fig. 1. Annual life cycle of *Omocestus viridulus.* The pre-reproductive life span (grey bars) includes embryonic (egg) development, nymphal development and adult maturation. During the reproductive period (black bars), the females produce multiple egg pods. With increasing altitude, a decrease in season length limits the reproductive period.

selection imposed by such time constraints, life history theory predicts the evolution of accelerated development (Rowe and Ludwig 1991, Stearns 1992, Abrams 1996, Roff 2002). The objectives of the investigation presented in Chapter two were, first, to assess in the field whether seasonal time constraints occur in the species. Secondly, common garden experiments using multiple grasshopper populations from the species' entire altitudinal range served to test the hypothesis that **intrinsic developmental rates** increase with elevation. This investigation thus represents a test of the predictive power of life history theory with natural populations. Likewise, it provides a case of adaptive population divergence in relation

to spatially divergent selection (Endler 1986, Mousseau et al. 2000, Hendry and Kinnison 2001, Kawecki and Ebert 2004), and therefore contributes to understanding ecological forces creating and maintaining genetic biodiversity. Further, the parallel analysis of development rates in the field and laboratory made it possible to disentangle the effects of genetic and environmental responses to local climates. As will be illustrated, these forces can counterbalance each other ('countergradient variation', Conover and Schultz 1995) and lead to cryptic adaptation (see also Merilä et al. 2001). Finally, investigations on adaptive and phenotypic responses of a species to a wide range of climates can shed light on the determinants of species range borders (Hoffmann and Blows 1994, Holt 2005) and how they might be altered by global change (Clarke 1996, Parmesan et al. 2005).

Chapter three focuses on the same set of altitudinal grasshopper populations and largely represents an extension of the analysis detailed in Chapter two. However, here the trait of main interest is the **intrinsic growth rate**. This trait is centrally relevant to life historians because it mediates the relationship between development time (a life history trait) and body size (usually strongly correlated with life history traits, e.g. Roff 2002) across ontogeny. Traditionally, life history theory considers growth rate a constant character within a species, causing a strong positive correlation between development time and body size (Roff 1980, Stearns 1992, Zonneveld 1996; Fig. 2). In contrast, it has sometimes been argued that intrinsic growth rate itself should be viewed as a trait responsive to selection. Organisms facing time constraints should thus evolve faster growth, thereby decoupling development time from body size (Abrams et al. 1996, Arendt 1997; Fig. 2). Surprisingly, the latter view has received little empirical attention despite its importance to life history theory (Roff 2000). As I studied adaptive divergence in development time in relation to seasonal time constraints (see above), I was therefore interested in potential concurrent adaptive change in growth rates, and in the resulting among-population body size pattern. To this end, I analyzed grasshopper ontogenies under laboratory conditions, which additionally provided insights into sex-specific growth, and into the proximate mechanisms involved in altitudinal life history adaptation in *O. viridulus*.

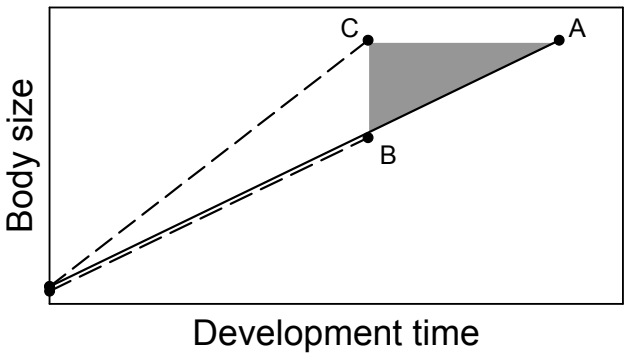


Fig. 2. Ontogenetic responses to time constraints. Organism A represents the ancestral state. Invariant growth rates within the species assumed, selection for earlier maturity (shorter development) leads to a correlated decrease in body size (B). In contrast, organism C evolved faster growth that perfectly buffers body size change.

Chapter 1

Grasshoppers cope with low host plant quality by compensatory feeding and food selection: N limitation challenged

Abstract

The effect of low host plant nitrogen (N) content on herbivore performance has rarely been studied together with the herbivore's feeding behaviour. We explored this relationship with juvenile *Omocestus viridulus* (Orthoptera: Acrididae) grasshoppers using fertilized and unfertilized host grasses. Due to lower growth rates, grasshoppers reared on N-poor grasses exhibited slightly prolonged development and smaller adult size, while mortality was similar among the fertilizer treatments. This was found both in the laboratory and in outdoor cages under natural climatic conditions. A parallel analysis of feeding behaviour revealed that the grasshoppers counterbalance N shortage by compensatory feeding, and are capable of selectively feeding among grasses of contrasting nutritional quality when given a choice. This indicates a striking ability of *O. viridulus* to regulate nutrient intake in the face of imbalanced food sources. Although the species exploits a relatively very poor autotroph nutrient base in the wild, as underpinned by N analysis of natural host grasses and grasshopper tissue, our data suggest that natural food quality imposes no relevant constraint on the herbivore's performance. Our study thus challenges the importance of simple plant-mediated control of herbivore populations, such as N limitation, but supports the view that herbivores balance their intake of N and energy.

Introduction

By virtue of a large mismatch in tissue nitrogen contents between terrestrial autotrophs and consumers (Elser et al. 2000), nitrogen is often considered the key nutrient required by herbivorous arthropods (Mattson 1980, Bernays and Chapman 1994, Schoonhoven et al. 1998). This has led to the nitrogen limitation concept, which holds that the natural nitrogen (N) content of food plant tissues generally impairs consumer performance (White 1993). Although in some cases specific biochemicals rather than bulk N seem to constrain herbivores (Behmer and Joern 1993, Anderson et al. 2004), consumer survival, growth and/or fecundity often respond positively to increased N (or protein) availability (Smith and Northcott 1951, Slansky and Feeny 1977, Lincoln et al. 1982, Ravenscroft 1994, Joern and Behmer 1997, Wheeler and Halpern 1999). The notion of N limitation thus rests on substantial empirical evidence and has lately been extended to the level of carnivorous arthropods (Denno and Fagan 2003). However, studies with crustaceans (Cruz-Rivera and

Hay 2000) and bugs (Di Giulio and Edwards 2003) recently demonstrated that some herbivores are able to overcome N shortage while close relatives are indeed strongly N limited. This suggests that predictions based on N limitation may not account for the diversity of herbivore responses to low N resources.

Over the recent years, an alternative to the strictly one-dimensional view of N limitation has emerged, the nutrient balance (or 'geometric') framework (Raubenheimer and Simpson 1999, 2004, Simpson and Raubenheimer 2000). This concept emphasizes the simultaneous regulation of the intake of nitrogenous nutrients and carbohydrate-derived energy by herbivores. Experimental work on nutrient balance, focused primarily on insect herbivores (but see Mayntz et al. 2005) and artificial diets, has shown that both protein and carbohydrate need to be ingested in a specific amount over a given period to sustain optimal growth and development. This nutritional target can be effectively stabilized over a wide range of food protein and carbohydrate compositions due to the interplay of several behavioural mechanisms. These include selection among unbalanced but complementary resources (Waldbauer and Friedman 1991, Chambers et al. 1995, Behmer et al. 2001, Lee et al. 2002), compensatory feeding by adjusting the total amount of food ingested (Abisgold and Simpson 1987, Raubenheimer and Simpson 1993), and post-ingestive regulation (Zanotto et al. 1993, 1997). Central to studies of nutritional balancing is the premise that animals must trade-off the costs of overeating excess nutrients against undereating those in deficit in the diet, as recently modelled and tested by Simpson et al. (2004).

The objective of the present study is to explore the feeding behaviour and performance of *Omocestus viridulus* (L.) grasshoppers in relation to natural host grasses of contrasting N contents, and to evaluate the results against predictions provided by the nitrogen limitation hypothesis as well as its alternative, the nutrient balance framework. For several reasons, the grasshopper species chosen is particularly suited for this task. First, *O. viridulus* feeds exclusively on grasses. Among insects consuming plant foliage, strict grass-feeders exploit a particularly poor nutritional environment, due to generally lower leaf N contents in grasses compared to herbs (Bernays and Barbehenn 1987). Grass-chewers are found mainly in the major insect orders Lepidoptera, Coleoptera and Orthoptera. Orthopterans, including grasshoppers, display substantially elevated tissue N contents compared to the other, more derived orders (Fagan et al. 2002). Grass-feeding grasshoppers thus represent excellent models to investigate whether organisms with relatively high N demands and naturally confined to poor food sources are N limited, and if and how they balance their nutrient intake. Furthermore, plant secondary compounds, which may confound interpretations based on nutrients, are of little importance in grasses (Bernays and Barbehenn 1987). In addition, the species is a representative of a large group of grass-feeders that often constitute the top arthropod grazers in grassland ecosystems (Blumer and Diemer 1996, Ingrisch and Köhler

1998). Consequently, the significance of bottom-up (plant-mediated) constraints to this grazer is of high interest to food web theory.

Specifically, we examined the relative impact of high and low N food on juvenile survival, growth and development. This was done both in the laboratory and under natural outdoor conditions using cages. Given that natural conditions can strongly influence insect responses to food quality (Hunter and McNeil 1997), and that hardly any experimental work dealing with nutrient limitation has been conducted outside the laboratory (Ravenscroft 1994), the outdoor replication clearly increased the scope of our study. We focused on juvenile stages, as high N requirements for tissue growth render juvenile arthropods particularly susceptible to inadequate food quality (Scriber and Slansky 1981, White 1993, Zalucki et al. 2002). Separate experiments were carried out to reveal possible compensation of low food N through adjustment of consumption rates, and to establish if the animals are capable of selecting among food sources of contrasting nutritional quality. Although such behavioural responses have been reported for several arthropods, mostly using artificial diets (Simpson and Simpson 1990; but see Wright et al. 2000), they have rarely been combined with performance experiments (Slansky and Feeny 1977, Cruz-Rivera and Hay 2000, Raubenheimer and Simpson 2004, Simpson et al. 2004). Hence, their ecological significance mostly remains unexplored. Finally, we determined N contents of grasshopper tissue and grass samples from the field. This allowed quantifying the discrepancy in tissue N between resource and consumer, and evaluating experimental N levels against those naturally occurring.

Materials and Methods

Host grass sampling in the field

In order to investigate naturally occurring host N contents, a total of 22 samples from 13 grass species (including one sedge) were taken in 2002 and 2003. We sampled six grassland sites in northeastern Switzerland where *O. viridulus* occurs. Only grass species with relatively high dominance at a site were considered, and all species had been observed to be readily consumed by *O. viridulus* in the wild in a preliminary foraging study. At each site, grass sampling took place during the end of grasshopper juvenile development (June/July), thus taking into account the herbivore's phenology. For a sample, we cut 20 – 40 g fresh weight of leaf blades from several individual plants.

Study organism

All experiments were conducted with the first offspring generation of *Omocestus viridulus* (Acrididae: Gomphocerinae), a grass-feeding grasshopper widespread in central Europe. It

displays one generation a year and has four nymphal (juvenile) stages. Males exhibit slightly faster nymphal development and smaller body size compared to females. We caught ten males and females from each of three locations in northeastern Switzerland in July 2003. The animals were kept in groups in cages in the greenhouse and allowed to reproduce until death. A field-cut grass mixture served as food. Egg pods were put individually in plastic tubes containing moist vermiculite. Subsequently, they were incubated for one month in a climate chamber set to 25 °C, followed by four month at 5 °C for diapause. Further incubation with 14 h days at 27 °C (night 8 °C) for 2 – 3 weeks yielded hatchlings for experiments. To obtain experimental grasshoppers in the last (4th) nymphal stage, hatchlings were transferred to 32 °C (fluctuating diurnally, see below) and raised in groups in cages on a grass mixture from the greenhouse.

Experimental host grass

The four Poaceae *Dactylis glomerata*, *Festuca pratensis*, *Holcus lanatus* and *Trisetum flavescens* were grown from seeds in the greenhouse. A mixture of the four species, all of which belong to the grasshopper's natural diet, was utilized in all trials to mimic natural conditions. Each species was sown separately in pots of 16 cm diameter and 13 cm height. As substrate we used a sand-soil mixture (volume ratio 11:6). Four weeks after germination the grass was assigned one of two treatments: the high N treatment involved an application of 0.1 l of a standard fertilizer solution (Wuxal, 0.4 %; Maag Agro, Dielsdorf, Switzerland), corresponding to 40 mg N, every five days. The low N treatment pots received no N fertilizer, but 0.3 l of a modified Hoagland solution was applied weekly. This solution contained all plant nutrients in balanced proportion except for N and was used to make sure that N was the only element in short supply. Furthermore, all pots were maintained moist by watering every other day. The grasses were grown in five temporal series separated by one week and used 8 – 9 weeks after germination. For a later N analysis, we drew one sample per species and treatment from three series.

Grasshopper performance

Juvenile development on high and low N grass was studied in a climate chamber set to a 16 h light phase at 32 °C. We chose this temperature taking into account the high temperature demands of grasshoppers (Ingrisch and Köhler 1998), which are met in the field by absorption of solar radiation (Begon 1983). Night temperature was 10 °C and relative humidity 40 % throughout. Fresh hatchlings from one population were randomly assigned to an N treatment and individually transferred to plastic containers of 19 cm height and 8 cm diameter. As food, two leaf blades per grass species of the corresponding treatment were cut from the greenhouse pots, offered in small water-filled glass vials, and replaced every third

day. Twice daily, we examined each container, noted new moults, and collected nymphal skins until the grasshoppers reached the adult stage. Hind femur length (henceforth referred to as body size) of nymphal skins and adults was measured using a stereomicroscope. The linear regression slope of body size against development time across the five stages (nymphal 1 – 4, adult) served as an estimate of individual growth rate. Family (= clutch) means of growth rate, adult body size and development time were analysed as general linear models (GLM) with sex and N treatment as fixed factors. Data from 9 – 13 families per sex by treatment combination were available (39 in total). To analyse individual survival to adulthood, we chose Fisher's exact test because expected values in two cells were slightly below five. Here, sample size was 38 and 39 animals for the high and low N treatment, respectively. SPSS 11.5 was used for statistics throughout.

To study juvenile performance under natural climatic conditions, we established grass of contrasting N supply in a similar way as outlined above. Before the experiment started, the soil substrate in each grass pot was cut into quarters. Within each N treatment, soil quarters from each of the four grass species were reassembled in new pots, yielding high and low N grass mixtures. Subsequently, we equipped every pot with a steel frame of 45 cm height and 15 cm diameter that was coated with a nylon stocking. To launch the experiment, six grasshopper hatchlings from one population were introduced in each of these resulting cages. After this, we placed the cages outdoors in an open area at the Swiss Federal Research Station for Agroecology and Agriculture. This was done between May 10 and 14 2004, corresponding to the species' natural hatching period (Berner et al. 2004). Animals of both sexes were used, and their allocation was random. The setup comprised six high N and seven low N cages. During the whole experimental period, the high N pots received 0.1 l of the standard fertilizer solution every 6 – 8 days. The low N treatment involved an application of 0.1 l of the fertilizer solution diluted to 1/3 with the modified Hoagland solution every ten days. In addition, the pots were watered if required. Some nutrient loss owing to rain washout certainly occurred but was not controlled for. For later N analysis, two grass samples were drawn per treatment both in the beginning and after ca. 2/3 of the experimental period. In order to standardize the microclimate in the cages, we cut back the grass to 25 cm height weekly. As indicated by hourly data logger ("StowAway TidbiT", Onset Computer Corporation, Bourne, MA, USA) measurements over several weeks within and outside two additional cages (without grasshoppers), cage temperatures did not systematically deviate from ambient temperatures. For each individual, we determined total nymphal development time with a resolution of 3 – 4 d and adult body size. Cage means of development time and size for each sex were analysed using GLM, with treatment and sex as fixed factors. Survival expressed as the raw number of resulting adults per cage was analysed with a two-sample t test.

Feeding behaviour

Food consumption on high and low N hosts was investigated in the laboratory using females from one population on day two of their final nymphal stage. We used the same chamber conditions and containers as above. The animals were starved for 3 h prior to the experiment to empty their guts. Each grasshopper was offered eight freshly cut leaf blades, two per grass species, of either treatment (non-choice setting). This was enough plant material so that the animals could not deplete it completely during the trial, and its fresh weight was determined immediately after cutting (all eight blades pooled). The grasshoppers were then allowed to feed for an entire light phase, subsequently starved for 3 h, frozen and dried at 45 °C for 72 h. Individual food consumption in terms of dry weight was calculated gravimetrically. For this, we converted initial grass fresh weight to dry weight using a multiply determined constant for each N treatment, and subtracted the dry weight of the grass leftovers. Consumption on a fresh weight basis could be estimated directly. We analysed consumed dry and fresh matter using GLM with treatment as fixed factor and nymphal dry mass as continuous covariate (ANCOVA approach; Raubenheimer and Simpson 1994, Raubenheimer 1995). Sample size was 22 and 23 for the low and high N treatment, respectively. Two random samples, each of 20 dried nymphs, were retained for analysis of grasshopper tissue N content.

The occurrence of food selection behaviour was investigated with nymphal females (age as above) under the laboratory conditions already described. Containers of 11 cm in diameter and 15.5 cm high were utilized. They were provided with two water-filled glass vials, each containing 12 blades (three per species) of high or low N grass, resulting in a choice setting. In the middle of the containers, we inserted a vertical cardboard wall of 10 cm height to separate the vials. This allowed attributing grass leftovers to the corresponding N treatment, but did not impede grasshopper movement. One animal was introduced per container and left to feed for 44 h (28 h light phase). For each of the 16 individuals studied, we gravimetrically assessed the dry mass of high and low N grass consumed. A paired-samples t test was used for analysis.

Nitrogen analysis

Plant material destined for N analysis was oven-dried for 72 h at 50 °C. All samples were ground in a sample mill equipped with a 0.5 mm screen (Cyclotec 1093, Foss Tecator, Sweden). N content was determined by combustion at 900 °C in an elemental analyzer (vario MAX CN, Elementar, Hanau, Germany). Some samples analysed in duplicate showed that measurement error was negligible (mean 1.6 %, maximum 3.9 %).

Results

Nitrogen contents

In grass samples from the field, tissue N on a dry weight basis ranged from 1.76 % to 3.7 % with a median of 2.19 % (Fig 3). There was considerable within-plant species variation

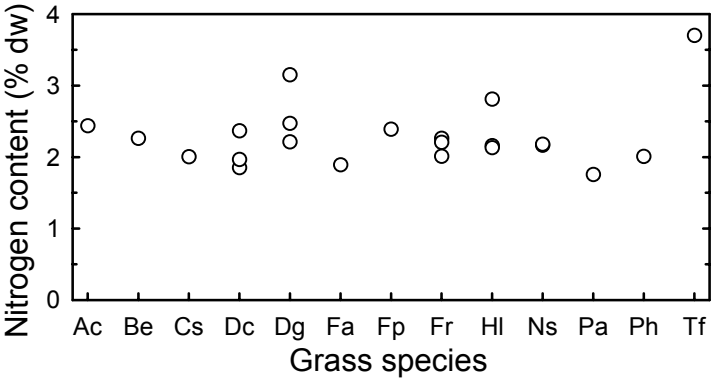
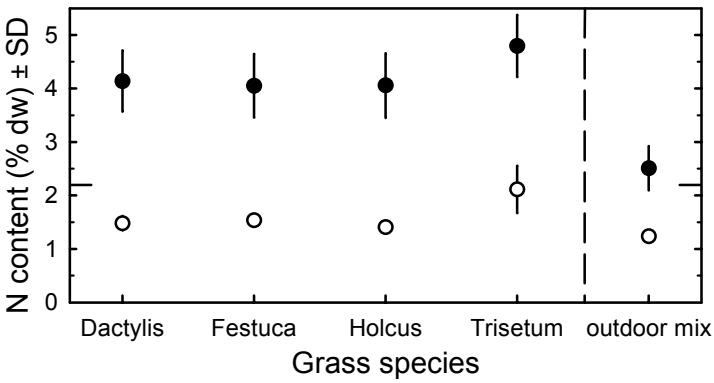


Fig. 3. Nitrogen contents of host grasses collected in the field. The species are from left to right *Agrostis capillaris*, *Bromus erectus*, *Carex sempervirens* (Cyperaceae), *Deschampsia caespitosa*, *Dactylis glomerata*, *Festuca arundinacea*, *F. pratensis*, *F. rubra*, *Holcus lanatus*, *Nardus stricta*, *Poa alpina*, *Phleum alpinum*, *Trisetum flavescens.*

across sites: *Dactylis glomerata* samples, for instance, differed by more than 40 % (2.2 % – 3.2 % N). Such difference partly arises from fertilization, as the three samples with the highest N content stemmed from a manured meadow.

Fertilizer application caused a consistent increase of N content in grass leaves used in the laboratory trials (Fig 4). Averaged across the four grass species, N content in fertilized grass was 2.7-fold higher than in unfertilized grass (4.3 % vs. 1.6 % N respectively). In the outdoor cages, fertilization increased leaf N roughly twofold from 1.2 % to 2.5 % N (Fig 4). Finally, N accounted for 11.3 % of grasshopper tissue dry weight.

Fig. 4. Nitrogen contents of unfertilized (○) and fertilized (●) experimental grasses used in the laboratory (4 species, left) and outdoors (mixture, right). The horizontal bar in the scale indicates the median N content of field samples (from Fig. 3). Some error bars are hidden by their symbol.



Grasshopper performance

In the laboratory, grasshopper survival to adulthood was somewhat higher on unfertilized than on fertilized grass (37 of 39 [95 %] vs. 32 of 38 [84 %], respectively). The difference, however, was non-significant ($p = 0.154$). Juvenile mortality occurred mainly during the first nymphal stage. Growth rates of animals developing on low N grass were reduced as compared to those on high N food ($F_{1,35} = 62.7$, $p < 0.001$; Fig. 5). Consequently, juvenile development of grasshoppers fed low N grass was prolonged by 2.4 d (13 %) on average ($F_{1,35} = 45.5$, $p < 0.001$). Moreover, these animals reached 5 % smaller adult body size ($F_{1,35}$

= 22.8, p < 0.001). The sexes responded similarly, as no sex by treatment interaction was significant (all p > 0.12).

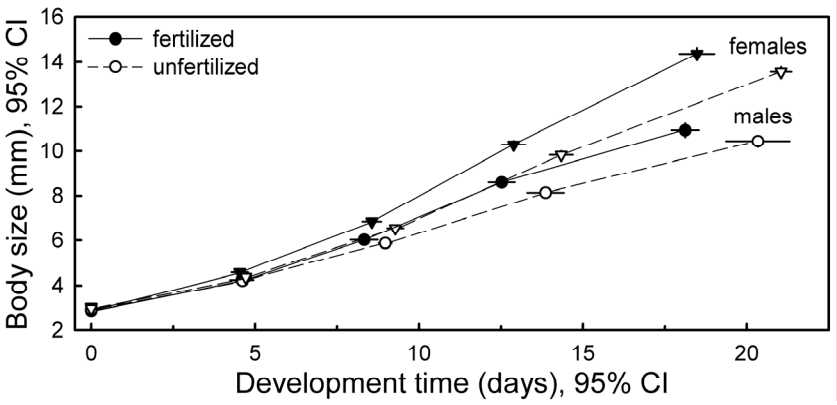


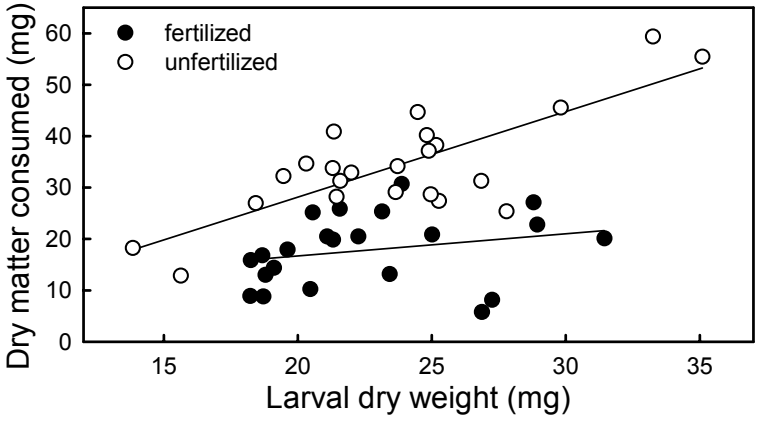
Fig. 5. Growth from the first nymphal stage to adult of male and female grasshoppers reared on unfertilized and fertilized grass in the laboratory, based on family means. Some error bars are hidden by the data point.

The outdoor cage experiment yielded very similar results. Juvenile survival did not significantly differ between the N treatments (t_{11} = -1.125, p = 0.285) and was close to the laboratory survival rates (high N: 28 of 36 [81 %], low N: 37 of 42 [88 %]). Grasshoppers in unfertilized cages reached adulthood 3.6 d (7 %) later on average ($F_{1,21}$ = 5.566, p = 0.028) and attained ca. 4 % smaller size ($F_{1,21}$ = 20.5, p < 0.001), with similar responses in the sexes (interactions p > 0.26). We consider these differences marginal.

Feeding behaviour

On a dry weight basis, the overall mean food consumption of grasshoppers feeding on low N grass was 82 % higher than on fertilized grass ($F_{1,42}$ = 48.8, p < 0.001; Fig 6). In terms of fresh weight, the consumption was still 40 % higher ($F_{1,42}$ = 15.1, p < 0.001). This indicates that grasshoppers compensated for lower nutritional quality by eating more.

Fig. 6. Dry matter consumption over a 16 h trial period by female *O. viridulus* 4th stage nymphs feeding on low or high N grass. Consumption, adjusted for nymphal weight, is significantly higher on unfertilized grass.



The food selection experiment revealed a striking preference for fertilized grass (paired t_{15} = -8.99, p < 0.001; Fig 7). When there was a choice, individual grasshoppers consumed an average of 39 mg high N grass (dry weight) during the test period, as opposed to only 8 mg low N grass.

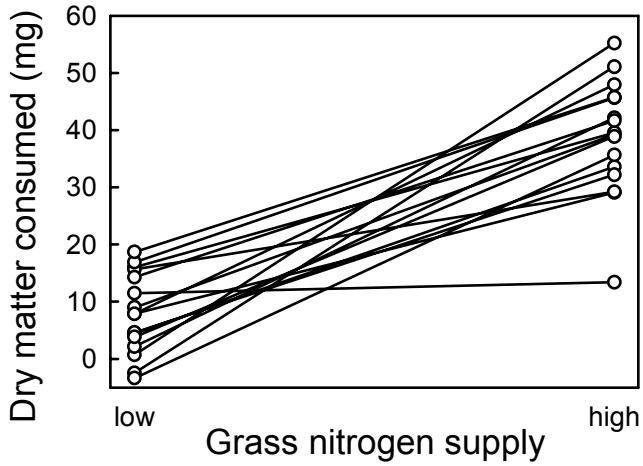


Fig. 7. Dry matter consumption over a 28 h period by female *O. viridulus* nymphs offered unfertilized and fertilized grasses simultaneously. Each line represents one individual. A clear preference for high N grass is evident. The two negative values in the data set point to some estimation error associated with the gravimetrical method applied.

Discussion

Our analyses revealed that *O. viridulus*' natural host grasses contain around 2 % tissue N, a relatively low value typical of grasses (Bernays and Barbehenn 1987). Further, qualitative inspection of the database compiled by Fagan et al. (2002) suggests that with 11.3 % *O. viridulus* does not differ in tissue N content from other grasshopper species that generally exhibit N demands particularly high for herbivores. Along the lines of N limitation, we therefore expected high susceptibility of juvenile performance to experimentally modified host plant quality.

Surprisingly, survival in the laboratory of *O. viridulus* nymphs feeding on low N grass was not lower than on fertilized grass despite a nearly threefold difference in grass tissue N content. Grasshoppers attained almost the same body sizes on both N treatments, although reduced growth rates on low N grass prolonged development to some extent. Grasshopper survival in the wild is known to be severely reduced by wet weather periods (Joern and Gaines 1990, Ingrisch and Köhler 1998), at least partly due to fungal infection (Streett and McGuire 1990). Whether these sources of mortality interact with food quality has to our knowledge never been investigated. We observed no such effect, as the outdoor experiment yielded results highly consistent with those from the benign laboratory environment. This finding is striking for two reasons. First, the low N grass employed outdoors was of extremely poor quality (1.2 % N), containing only about half as much N as grass from the field, whereas the fertilized treatment (2.5 %) matched natural levels. Secondly, rainfall was roughly 20 % above average during the trial period (MeteoSwiss, pers. comm.), indicating rather unfavourable climatic conditions. The weak performance differences in response to the tremendous difference in food plant N, exhibited under strikingly different rearing environments, leads us to conclude that naturally occurring grass N contents impose few constraints upon juvenile survival, growth and development of *O. viridulus*. Our results disagree with the simplistic claim that natural autotroph tissue N inherently limits herbivore performance (Mattson 1980, White 1993).

In several respects, however, our data are consistent with the view that herbivorous insects balance their nutrient budget. One means to stabilize nutrient intake, and thus growth and developmental performance, is through compensatory feeding. Indeed, dry weight food consumption of *O. viridulus* nymphs exposed to low N grass was almost twice as high as on fertilized grass. However, differing water contents of food plants may result in differential dry weight consumption even if fresh weight intake is similar (Wheeler and Halpern 1999), and thus may lead to the erroneous inference of compensatory feeding. The fact that our experiments also revealed a 40 % increase of fresh matter consumption on low quality grass indicates that elevated food intake is a real behavioural response of *O. viridulus* to low grass N content. Similar intake adjustment in response to nutrient dilution or imbalance has been documented for crustaceans (Cruz-Rivera & Hay 2000), slugs (Rueda et al. 1991), and other insects (e.g. Slansky and Feeny 1977, Karowe and Martin 1989, Raubenheimer and Simpson 1993, Stockhoff 1993, Obermaier and Zwölfer 1999, Jones and Raubenheimer 2001). This suggests that compensation is a common phenomenon in invertebrates. To compensate for protein dilution of artificial diets, the grasshoppers *Locusta migratoria* (Simpson and Abisgold 1985) and *Melanoplus differentialis* (Yang and Joern 1994) increased feeding frequency while meal size remained constant. Although we did not observe feeding behaviour directly, it is very probable that the same behavioural response holds for *O. viridulus* on the natural hosts used.

Two further issues arising from the compensation experiment deserve discussion. On the one hand, it is evident that compensation was not fully successful in the grasshoppers fed low N grass, as these animals exhibited somewhat prolonged nymphal development and slightly decreased body size. Similar developmental responses when exposed to highly N deficient artificial foods have been reported previously for locusts (Raubenheimer and Simpson 1993) and caterpillars (Lee et al. 2002). Most probably, the degree of N dilution in the low N grass exceeded the digestive capacity of the grasshoppers; hence, nitrogen could not be derived from the food at a rate needed to maintain nutritional homeostasis and thus optimal growth. On the other hand, we can assume that growth occurred at a close to optimal rate in grasshoppers fed high N grass. Nevertheless, our data suggest that here mortality was higher compared to the low N treatment, although the trend was not significant. A possible explanation accounting for this pattern lies in the fact that compensatory feeding implies a trade-off in that one nutrient is always ingested in excess of the physiological requirements unless the nutrients are perfectly balanced (Zanotto et al. 1993, 1997, Simpson et al. 2004). Using the conversion constant of 6.25 to estimate crude protein from total N (Allen 1989), we obtain an average protein content of 27 % for the high N grass. Although we did not measure carbohydrate levels, it is highly probable that our high N grass was N-biased relative to the grasshopper's nutritional needs, since *L. migratoria* grasshopper nymphs performed best on artificial food containing only 19 % dw protein and 23 % carbohydrate

(Chambers et al. 1995). *O. viridulus* nymphs fed fertilized grass thus ingested N in excess of physiological demands to satisfy their carbohydrate requirements, which likely entailed N loading and a survival cost. This view is supported by the finding that high growth rates associated with high N supply came at the expense of juvenile survival in butterflies (Fischer and Fiedler 2000) and locusts (Raubenheimer and Simpson 2004). However, further detailed experiments are needed to clarify these relationships in our species, and to exclude alternative explanations such as an intrinsic trade-off between growth rate and survival (Arendt 1997).

The above results show that compensatory feeding enables grasshoppers reaching or approaching their nutrient target when exposed to highly unbalanced natural food. However, compensation not only implies surplus consumption and processing of non-limiting food components, but also greater feeding effort that can increase predation risk (Loader and Damman 1991, Werner and Anholt 1993, Eklöv and Halvarsson 2000). Hence, given an appropriate choice of foods, it is generally a better response to stabilize nutrient intake by food selection. Indeed, some experiments have demonstrated the ability of herbivores to achieve an optimal nutrient balance by switching between unbalanced but complementary synthetic foods (Chambers et al. 1995, Behmer et al. 2001, Lee et al. 2002). As argued above, our high N grass was N-biased to some degree relative to the grasshoppers' nutritional needs. In contrast, the low N grass was strikingly N-deficient but likely provided an ample carbohydrate source, given that sugar and starch contents of grass leaves increase with decreasing plant N supply (Bernays and Chapman 1994, Marschner 1999). If the grasshoppers optimized N and carbohydrate intake simultaneously, we would therefore expect the animals to feed predominantly on high N grass, but complement their diet with energy-rich low N grass. The results from our food selection experiment are entirely consistent with this nutrient balancing view. However, did the animals really select for nitrogen and carbohydrates, or is the observed selection pattern due to other plant characters changing with N supply, such as water content or leaf toughness? In general, turgescent plant tissues exceed grasshopper water demands (Bernays and Barbehenn 1987). Furthermore, *O. viridulus* did not discriminate against grasses like *Bromus erectus* or *Festuca arundinacea* in a preliminary choice trial. The leaves of these species proved particularly tough in a subsequent analysis (Berner, unpubl. data), confirming that physical leaf properties are unlikely to influence grasshopper feeding behaviour (Bernays and Chapman 1970, Heidorn and Joern 1984, Chapman 1990). Therefore, we believe that our inference of food selection balancing the intake of key nutrients is valid.

The selection pattern observed raises the question about the proximate control of food choice behaviour in *O. viridulus*. Two different, mutually not exclusive mechanisms likely provide the answer. The first mechanism involves the modulation of taste receptor responsiveness by blood nutrient concentration. As shown for locusts (Simpson and

Simpson 1990, Simpson et al. 1995; see also Cook et al. 2000), increasing levels of amino acids in the blood reduce the stimulatory responsiveness of the corresponding taste receptors on the mouthparts and legs. Hence, a protein-deficient individual (exhibiting a low blood amino acid level) will readily start feeding on a protein-rich food source. The same food, however, is less attractive and may not stimulate feeding when the animal's blood amino acid level is high. Since the same mechanism applies to carbohydrates, nutrient regulation based on the herbivore's nutritional status may cause selective feeding. On the other hand, a learned association of food quality with an environmental cue via nutritional feedback may underlie the documented food selection (Simpson and White 1990, Waldbauer and Friedman 1991, Bernays 1995). Food selection based on associative learning in insects commonly involves olfactory stimuli, but visual cues are used as well (Raubenheimer and Tucker 1997). It is possible that the *O. viridulus* individuals learned the spatial location of the food sources rich in N and carbohydrate, as reported for locusts (Dukas and Bernays 2000).

Overall, the present study illustrates that natural food imposes no relevant nutritional constraint on the performance of *O. viridulus* grasshoppers. The species is capable of behaviourally stabilizing its nutrient intake over an impressive range of food qualities. Our study using real plants thus corroborates a body of work that has documented nutrient balancing in herbivores based on synthetic diets. However, our data challenge the general utility of simple bottom-up approaches to consumer population dynamics, such as N limitation.

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Chapter 2

Grasshopper populations across 2000 m of altitude: is there life history adaptation?

Abstract

Life history differentiation along climatic gradients may have allowed a species to extend its geographic range. To explore this hypothesis, we compared eleven *Omocestus viridulus* (Orthoptera: Acrididae) populations along an altitudinal gradient from 410 m to 2440 m in Switzerland, both in the field and laboratory. In situ temperature records indicated a striking decline in available heat sums along the gradient, and field populations at high altitudes reached egg hatching and adulthood much later in the year than at low elevation. The reproductive period at high altitude is thus severely limited by season length, especially during a cool year. However, controlled environment experiments revealed that intrinsic rates of embryonic and juvenile development increased with the populations' altitude of origin. This countergradient variation is largely genetic and conforms to predictions of life history theory. No corresponding differentiation in the overwintering egg stage, a pivotal determinant of phenology, was found. This trait seems conserved within the gomphocerine grasshopper subfamily. Although we found evidence for altitudinal adaptation in development, the potential of *O. viridulus* to adapt to cool alpine climates appears restricted by a phylogenetic constraint.

Introduction

The seasonal recurrence of adverse climatic conditions is a principal force shaping ectotherm life cycles in temperate regions. Growth, development, reproduction and dormancy need to be coordinated and timed in relation to the available growing season (Taylor and Karban 1986, Danks 1994). The set of adaptations, which synchronizes the life cycle with the growing season, is reflected in the organisms' phenology (Tauber et al. 1986). The length of the growing season generally declines with increasing latitude and altitude. Thus, geographically widespread species have to cope with a variety of climatic conditions, which can basically be achieved in two – not mutually exclusive – ways. Firstly, a generalist genotype may display plastic responses in relation to environmental conditions (Gotthard and Nylin 1995, Schlichting and Pigliucci 1998). Phenotypic plasticity in traits relevant to seasonal timing has been documented and interpreted in adaptive terms in several insect species (Tanaka and Brookes 1983, Nylin 1994, Blanckenhorn 1997, Kingsolver and Huey 1998). Secondly, spatial variation in selection pressures may give rise to genetic differentiation

between populations due to natural selection. Prerequisites are heritable genetic variation and restricted gene flow between local populations (Slatkin 1987). Both responses, local adaptation and phenotypic plasticity, may allow a species to extend its distribution across a range of altitudes and latitudes. Several studies of ectotherms report genetic life history differentiation in relation to systematic geographic variation in climate (Masaki 1967, Berven and Gill 1983, Dingle et al. 1990, Ayres and Scriber 1994, Blanckenhorn and Fairbairn 1995, Telfer and Hassall 1999, Merilä et al. 2000; but see Lamb et al. 1987). However, almost all studies focus on latitude, whereas evidence for altitudinal life history adaptation in animals is exceedingly scarce. This distinction matters indeed: altitudinal changes in climate typically occur on a particularly small spatial scale, where continuous gene flow is likely to impede genetic differentiation unless strong local selection is acting.

An insect species with a remarkable altitudinal distribution is the grasshopper *Omocestus viridulus* (L.) (Acrididae: Gomphocerinae). In Switzerland, it occurs in grasslands from below 400 m to above 2500 m (Thorens and Nadig 1997), making it a suited system for the study of altitudinal adaptation. Over the whole range, it displays an annual life cycle, which includes egg hatch in spring, four larval instars followed by adult molt in summer, and reproduction until autumn. The species overwinters as an egg in embryonic diapause (Ingrisch and Köhler 1998). This dormant phase is characterized by suppressed development, reduced metabolism, and high tolerance to harsh environmental conditions (Danks 1987, Leather et al. 1993). The developmental stage during diapause strongly influences later phenology, as it determines how many developmental steps an embryo has to pass through before hatch in spring. In addition, seasonal timing can be achieved through adjustment of development rates (Danks 1987). Higher rates of embryonic and larval development allow reaching adulthood, and thus the subsequent reproductive phase, sooner. The diapause stage, as well as embryonic and larval development rates, can be identified as chief traits determining phenology, and are therefore of high significance to geographic life history adaptation.

Along the altitudinal gradient, *O. viridulus* faces a decline in the length of the growing season, which is likely to require phenological adjustment. Moreover, the species is sedentary (Ingrisch and Köhler 1998) and most populations are separated to some extent by migration barriers, suggesting rather low levels of gene flow. For these reasons, we hypothesize that local life history adaptation, rather than phenotypic plasticity, allowed the grasshopper to extend its distribution to the wide range of altitudes. In this case, the species would represent a fine-grained patchwork of local demes, which are differentiated in traits relevant to seasonal timing. We address the hypothesis by both field and laboratory approaches. In a first step, the natural temperature regimes and their effect on field phenologies along the gradient are explored. In a second step, we compare populations with respect to developmental rates and diapause characteristics in common laboratory

environments. The latter approach serves to remove environmental variation and reveal genetic differences in life histories, if they occur.

Materials and methods

Study populations

The present investigation includes a total of eleven *Omocestus viridulus* populations. The study sites were chosen to form a transect from the Swiss lowland into the Alps, covering an altitudinal gradient of 2000 m (Fig. 8). Distribution data were provided by the Swiss center of cartography of the fauna (CSCF). We considered only sites where large populations had been reported over several years. Some 110 km separate the furthermost sites. Although this spatial scale is relatively small, the study populations can be viewed as reasonably independent, as most populations of this widespread species are isolated to some degree by natural and human dispersal barriers (e.g. forests or farmland).

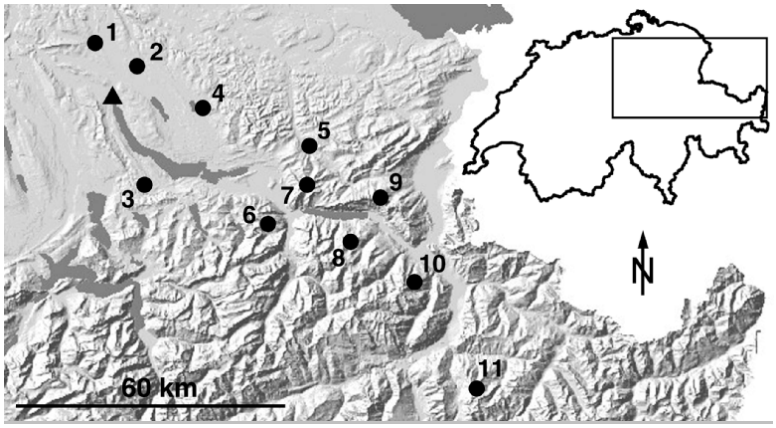


Fig. 8. The rectangle in the outline map shows the location of the study area in Switzerland. The sampling sites and corresponding altitudes are 1) Neerach 410 m, 2) Birchwil 540 m, 3) Schönenberg 670 m, 4) Bäretswil 830 m, 5) Bendel 1055 m, 6) Näfels 1350 m, 7) Speer 1610 m, 8) Flumserberg 1850 m, 9) Gamserrugg 2060 m, 10) Pizol 2215 m, 11) Hörnli 2440 m. The triangle denotes the city of Zürich (47°22' N / 8°31' E).

Field studies

To estimate the length of the growing season in the field, temperatures were recorded hourly during the 2002 season at sites 1, 5, 7 and 10 (see Fig. 8) by means of data loggers ("StowAway TidbiT", Onset Computer Corporation, Bourne, MA, USA). We were primarily interested in the conditions the embryos (eggs) experience. Since *O. viridulus* lays its clutches into the top soil layer or at the base of grass tussocks (Ingrisch 1983; Berner, personal observation), we positioned the loggers' sensors at 1 cm soil depth under natural vegetation cover. Two loggers were used per site, and their measurements averaged for all calculations. Two different indices of season length were computed: one index uses the date at which 11 °C was exceeded for the first time. This date roughly corresponds to the initiation of postdiapause embryonic development, which is inhibited at temperatures below ca. 11 °C (Wingerden et al. 1991). The second index uses the cumulative degree hours above 14 °C between the first appearance of larvae at each site and the end of the year. This

approximates the season length for larvae and adults. 14 °C was chosen based on a study by Hilbert and Logan (1983), because postembryonic development thresholds were unavailable for the species. However, grasshoppers are known to increase body temperature by basking (Begon 1983, Chappell 1983). Hence, the latter index must be viewed as a relatively crude, but still informative, estimate of the thermally effective season length.

Field phenologies were studied at the same sites and in the same year as the temperature records. Each site was visited in regular intervals of six to eleven days over the growing season. We censused by direct observation along transects, noting the stage (larval instars 1-4, adult) of each grasshopper. (The insertion of an additional larval instar reported from other gomphocerine grasshoppers (references in Ingrisch and Köhler 1998), occurred neither in the field nor laboratory.) Although males reach adulthood slightly earlier than females in this species, the sexes were pooled *post hoc* for simplicity. This did not influence the results substantially. As 2002 was a rather cool and cloudy year and 2003 was particularly sunny, we also checked the stage composition at site 1 in late June and site 10 in mid July 2003. These snapshots during the second year allowed a comparison of phenologies between climatically rather different seasons.

Breeding techniques and laboratory experiments

To establish breeding populations, ca. 14 individuals of each sex were caught at the beginning of the reproductive period at each of the eleven sites. The populations 2, 5, 7 and 10 were sampled in 2001, all others in 2002. The animals were kept in groups in cages in a greenhouse under natural photoperiods until death. Field-cut grass (largely of *Dactylis glomerata* and *Agropyron repens*) was provided as food. Egg pods were collected twice a week as they were laid, put in plastic vials containing moist vermiculite, and incubated at 25 °C for 35 days which allows the embryos to reach the diapause stage (Wingerden et al. 1991). After this, the clutches were stored at 5 °C.

Postdiapause embryonic development time was studied in a climate chamber set to a photoperiod of 14 h at 27 °C. Night temperature was 8 °C. All eggs had spent at least three month at 5 °C, which is enough to break diapause (Ingrisch and Köhler 1998). The vials were inspected twice daily for newly hatched larvae, until no further hatch occurred. Individual hatch dates were noted and converted to degree hours with 11 °C as threshold. We tested clutch medians in a general linear model (GLM), with study year as a fixed factor and altitude of origin (= populations) as a continuous covariate. Effective sample size varied between 18 and 79 clutches per population. To verify the robustness of our 27 °C results, a subset of the clutches laid in 2002 was incubated at 19 °C, but otherwise treated and analyzed in the same way. Here, sample size varied between 14 and 48.

To investigate larval development time, hatchlings were immediately transferred to another climate chamber with a 16 h photoperiod at 32 °C and a night temperature of 10 °C. This

experiment was conducted with the seven 2002 populations only. Larvae were kept clutchwise in plastic containers (19 cm high, 8 cm in diameter) in groups of six at most. Small pots with a grass mixture provided food. Adult emergence was checked twice daily, and individual degree hours for larval development were determined using 14 °C as threshold. We analyzed clutch medians using GLM. Altitude was entered as a covariate, and sex as a fixed factor, since the sexes differed in development time.

To assess diapause stages, ten random clutches from each of six populations (1, 2, 4, 7, 8, 9) were removed from the cold. The outer layer (chorion) of every single egg in the clutch was scraped off with a fine blade under a stereomicroscope so that the embryo could be seen and assigned a developmental stage. We used the classification system of Cherrill (1987), which divides the continuous process of differentiation to the fully developed embryo into twenty discrete morphological steps. Based on individual eggs, the clutch median stage was determined and treated as one data point. Differences between populations were tested using one-way ANOVA and a distribution-free Kruskal-Wallis test. All statistics were performed with SPSS 11.1.

Results

Temperature regimes

Daily mean temperatures of the top soil layer display a sharp decline with altitude (Fig. 9). Over the summer months, mean temperatures at 2215 m remain approximately 7 °C below those at 410 m. Moreover, snow cover maintains spring temperatures around zero at the high elevation sites, most dramatically at 2215 m. Indeed, the very first hourly temperature record above the estimated embryonic threshold of 11 °C occurs as late as on the 31st of May at 2215 m (Table 1). At the low elevation sites this threshold is exceeded almost three month earlier. Season length estimated as degree hours above 14 °C shows a more than tenfold reduction from 410 m to 2215 m altitude (Table 1). Hardly any hourly records above 14 °C were made after the end of August at the highest site. Roughly speaking,

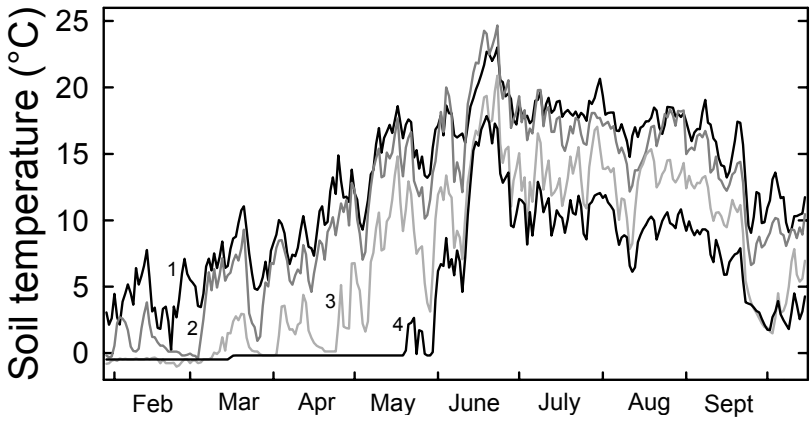


Fig. 9. Daily mean temperatures during the 2002 season at altitudes of 410 m (1), 1055 m (2), 1610 m (3) and 2215 m (4).

postembryonic development was possible during seven months at low altitude, whereas only three months were available at the highest site in 2002.

Table 1. Indices of the 2002 season length at four altitudes, based on hourly temperature records at 1 cm soil depth

Altitude (m)	Date of first record > 11 °C	Degree hours > 14 °C*
410	8 March	12'536
1055	8 March	10'019
1610	5 April	3'182
2215	31 May	904

* From the onset of larval hatch to the end of the year

Field phenologies

The phenology curves in Fig. 10 indicate a marked delay in the emergence of first instar larvae at the high elevation sites, where the first hatchlings appeared three (1610 m) and seven (2215 m) weeks later than at the lowest location. The delay carries over to the adults: the graphically estimated dates at which each population reaches an adult frequency of 75 % are June 26 (410 m), June 31 (1055 m), July 23 (1610 m) and August 19 (2215 m). Consequently, adult emergence at the highest site is delayed by almost two months compared to the lowland site. During the 2002 season lowland grasshoppers had already started reproducing when first instar larvae just started hatching at high altitude. In accordance with the temperature regimes, the difference in phenology between the sites at 410 m and 1055 m is small.

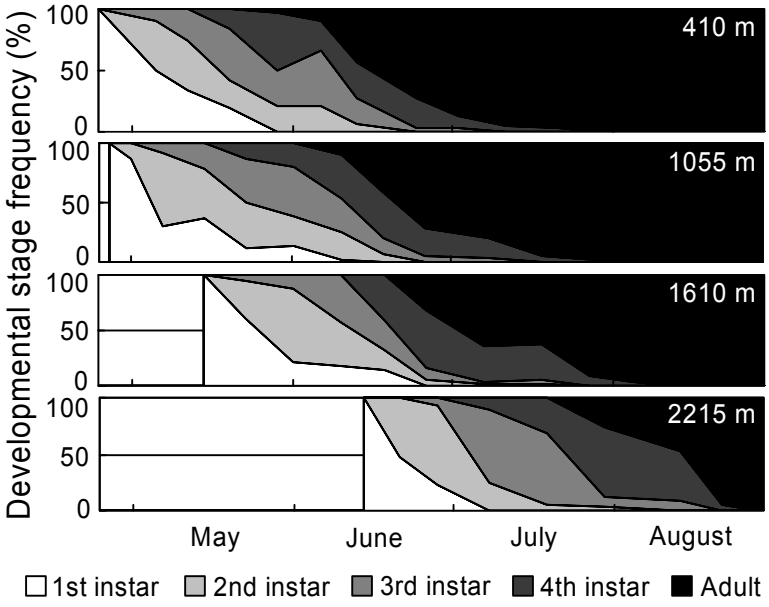


Fig. 10. Field phenologies of *O. viridulus* at four altitudes during the season of 2002. The vertical line represents the first observation of hatchlings.

The comparison of 2002 (cool year) and 2003 (warm year) reveals a small difference in the low elevation phenology (Table 2). The greatest majority of the 410 m population reaches adulthood by late June in both years. At 2215 m, however, the phenological difference between the two seasons is much larger. Clearly, the high altitude grasshoppers are delayed in both 2002 and 2003 relative to the lowland, but the phenological delay is more pronounced in the cooler year of 2002.

Table 2. Frequency (%) of *O. viridulus* instars in the years 2002 and 2003 at low and high elevation. Note that the two populations were censused on different dates

	410 m, late June		2215 m, mid July	
	2002	2003	2002	2003
2nd instar	-	-	21	-
3rd instar	3	-	64	9
4th instar	18	3	15	31
Adult	79	97	-	60

Laboratory experiments

There is a clear relationship between embryonic development time in the laboratory at 27 °C and a population's altitude of origin (Fig. 11): high altitude embryos complete development faster, resulting in earlier hatching of the first instar larvae ($F_{1,431} = 50.9$, $p < 0.001$). However, the maximal difference between populations in development time amounts to some ten percent only. Expressed in real time, the population averages declined from 14.1 to 12.3 days. The year factor is also significant because temperature conditions differed slightly between the years (different climate chamber types; $F_{1,431} = 40.5$, $p < 0.001$). Faster development of the high altitude embryos was also found at the lower experimental temperature of 19 °C. The correlation of population averages of embryonic development time at the two incubation temperatures yields coefficients of 0.85 (Pearson's r, $p = 0.015$) and 0.93 (Spearman's rank, $p = 0.003$).

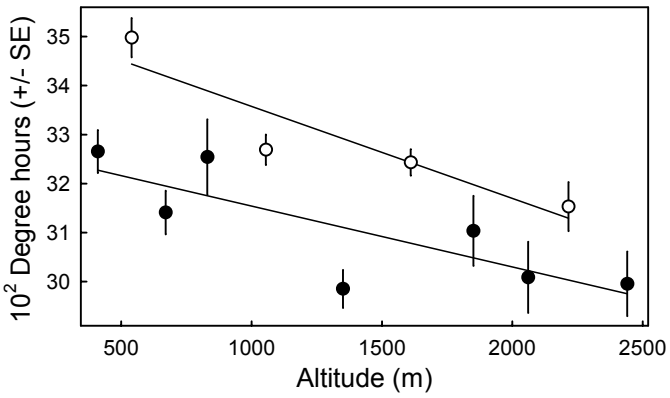


Fig. 11. Physiological time required by *O. viridulus* populations from different altitudes for postdiapause embryonic development. Data from 2002 (○) and 2003 (●). Degree hours were calculated using 11 °C as threshold.

The duration of development through all larval instars to adults clearly gets shorter with altitudinal origin (Fig. 12; $F_{1,223} = 51.9$, $p < 0.001$), similar to embryonic development. As in

the field, males always reach adulthood earlier than females ($F_{1,223}$ = 16.1, p < 0.001), but the altitudinal response is similar in the sexes, as indicated by a nonsignificant interaction ($F_{1,223}$ = 1.3, p = 0.26). Again, the difference between the fastest and slowest population is only about ten percent. In real time, the population averages for larval development ranged from 23.6 to 20.9 days in males and from 25.2 to 22.3 days in females for low and high altitude, respectively.

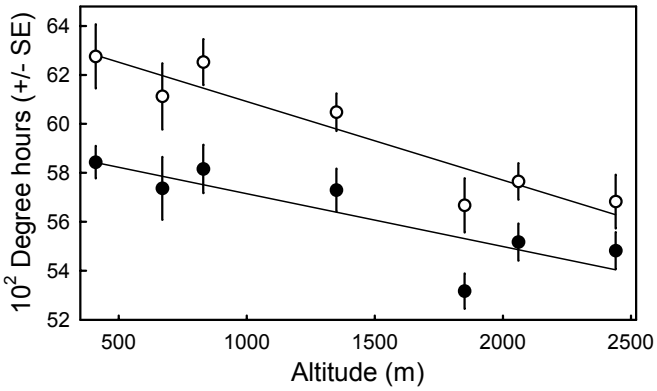
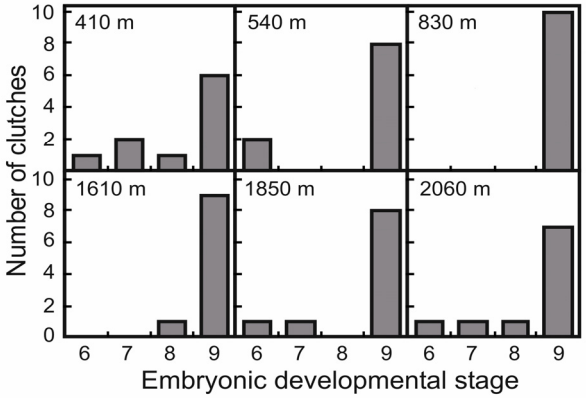


Fig. 12. Physiological time required by female (○) and male (●) grasshoppers from different altitudes for larval development. A threshold of 14 °C was used for calculation.

The stage of embryonic diapause does not significantly differ between the populations, and no altitudinal trend is evident (Fig. 13; ANOVA $F_{5,54}$ = 1.34, p = 0.26; Kruskal-Wallis χ^2_5 = 8.09, p = 0.15). In all *O. viridulus* populations studied, the vast majority of embryos diapauses at developmental stage nine, which corresponds to stage IVd of Cherrill (1987). The embryos are then arrested just before the onset of embryonic rotation. Most clutches contained some retarded eggs, but no single embryo developed further than stage nine.

Fig. 13. Embryonic developmental stage at diapause in *O. viridulus* populations from six altitudes. Plotted are clutch medians (N=10 per population).



Discussion

Our laboratory study documents increasing rates of embryonic and juvenile development in *O. viridulus* with increasing altitude. As a consequence, high altitude grasshoppers attain adulthood in shorter time than their low altitude counterparts when grown in a common environment. In contrast, the diapause stage, another key determinant of phenology, shows no difference among the populations. The field work indicates a time constraint on the life cycle of high altitude animals. The cooler high elevation temperature regimes substantially

delay larval hatch and adulthood. In a cloudy year like 2002, the reproductive life span of alpine grasshoppers is thus severely truncated and reproductive success very poor. Moreover, a considerable fraction of the produced eggs may fail to reach the overwintering stage due to insufficient late season heat. This was shown to entail delayed hatching in *Chorthippus brunneus* (Cherrill and Begon 1991) and survival costs in *Camnula pellucida* (Pickford 1966). Certainly, cool and cloudy years are severe selection events at the species' upper range margin. Only during particularly sunny seasons like 2003 is the reproductive period sometimes terminated by intrinsic senescence at both low and high elevation. Thus the variance in the available season length increases with altitude.

Under such a seasonal time constraint, annual organisms face the problem of optimally allocating time to development and reproduction. Life history models predict that decreased season length will favor faster development and hence decreased time to maturity (Cohen 1976, Roff 1980, 2002, Rowe and Ludwig 1991, Abrams et al. 1996). In *Omocestus viridulus* with its wide altitudinal distribution, therefore, we expected differences in traits determining postdiapause development time. The higher rates of development exhibited under laboratory conditions by the alpine populations thus conform well to the theoretical prediction. A genetic basis to the acceleration of development is strongly suggested because, firstly, maternal influence on offspring embryonic development appears negligible in the related *Chorthippus parallelus* (Köhler 1983). Secondly, our field records indicate that the temperatures used in the laboratory may be experienced by all populations in the field. Absorption of solar radiation may allow even high altitude larvae to rise body temperature well above 32 °C. Furthermore, embryonic development rates were found to increase with altitude at incubation temperatures of both 19 °C and 27 °C. Strong genotype by environment interactions are thus excluded. For these reasons we suggest that the observed developmental differences are robust and reflect an adaptive strategy. Increased embryonic and larval development rates allow high altitude animals to reach maturity relatively faster, and hence prolong reproductive life span when time is short. The hypothesis of within species life history differentiation on a small spatial scale is thus confirmed. Apparently the level of gene flow between the *O. viridulus* populations is too low to counteract local adaptation.

O. viridulus agrees well with some other ectotherms in which differentiation of development along gradients in season length has been documented (Masaki 1967, Berven and Gill 1983, Dingle et al. 1990, Ayres and Scriber 1994, Telfer and Hassall 1999). The shortening of development time proves a common adaptive response to seasonal time constraints. Intraspecific differentiation in traits related to phenology may be quite frequent in annual ectotherms with relatively long development times covering wide geographic ranges. However, our field surveys make it clear that the cool climates at high elevation retard grasshopper phenologies despite higher intrinsic rates of development in those populations. Thus, the high elevation grasshoppers are only partly able to compensate the delaying

environmental influence on time to adulthood. This agrees with the relatively modest level of differentiation found in the laboratory. As the genetic response along the altitudinal gradient is opposed to the phenotypic response to the environmental conditions, *O. viridulus* provides an example of countergradient variation (Conover and Schultz 1995). A merely phenotypic comparison of development times within the species would have failed to demonstrate altitudinal differentiation.

At the proximate level, increased development rates may be associated with metabolic temperature compensation (Danks 1987). Hadley and Massion (1985) for example report increased metabolic rates in high altitude populations of the grasshopper *Aeropedellus clavatus*. Likewise, latitudinal differences in metabolism were found in the butterfly *Papilio canadensis* (Ayres and Scriber 1994). However, physiological traits of *O. viridulus* populations have not been compared so far.

A question arising from the observed patterns is why higher intrinsic rates of development did not evolve in the lowland populations. What could be the disadvantage of a similarly rapid development as at high elevation? On the one hand, adverse climatic conditions early in the season are likely to select against precocious larval emergence. Carrière et al. (1996), for example, demonstrate a mortality cost associated with precocious larval hatch in *Gryllus pennsylvanicus*, due to unfavorable temperature conditions. Furthermore, trade-offs with other fitness components could maintain developmental rates below the physiological potential exhibited by the alpine populations (Schluter et al. 1991, Stearns 1992, Roff 2002). For instance, Tatar et al. (1997) found increased senescence in *Melanoplus sanguinipes* grasshoppers from high elevation sites compared to the slower developing low altitude animals. Likewise, given that juvenile development time, adult size, and fecundity are often correlated positively (Roff 1980, 2002, Rowe and Ludwig 1991, Honek 1993), a shortened juvenile development will negatively affect fecundity. According to Orr (1996) this is the case in *M. sanguinipes*. Most probably, elevated rates of development bear fitness costs and are selected against in the absence of a seasonal time constraint on the life cycle, as is the case at low elevation. However, low altitude seasons appear still too short for two generations, as the species exhibits an annual life cycle throughout its range.

Besides embryonic and larval development rates, the stage of overwintering strongly determines time to adulthood. Central European grasshoppers of the gomphocerine subfamily are believed to show an obligatory diapause during embryonic development. According to some studies, the dormant stage is inserted shortly before embryonic rotation (Köhler 1991, Ingrisch and Köhler 1998). This stage has been designated IVd by Cherrill (1987). However, geographic variation in diapause stage within an insect species is possible in principle (references in Tauber et al. 1986), but has not been investigated to date in any European grasshopper. In *O. viridulus* we found no such altitudinal differentiation: in all populations, most clutches mainly contained embryos arrested at the aforementioned stage,

and no embryo developed further. Thus, the species displays an obligatory diapause and is uniform with respect to the stage of dormancy, conforming to other members of the subfamily. This finding stands in striking contrast to other orthopteran studies, which document intraspecific variation in embryonic diapause stage and/or expression along gradients in season length (Mousseau and Roff 1989, Groeters and Shaw 1992, Dingle and Mousseau 1994, Tanaka 1994, Bradford and Roff 1995). The catantopine grasshopper *Melanoplus sanguinipes*, for example, occurs from sea level to above 3800 m (Chappell 1983) and displays enormous variation in embryonic diapause stage within its North American range. High elevation populations overwinter almost completely developed and attain the hatching state at low heat sums. This is interpreted as an effective means to decrease postdiapause development time under short seasons (Dingle et al. 1990, Dingle and Mousseau 1994). In *M. sanguinipes*, adult emergence at above 2600 m and at sea level happens roughly at the same time! Not surprisingly, another catantopine, *M. frigidus*, is the highest reaching species in the Alps (Carron 1996). This grasshopper subfamily illustrates the importance of flexibility in the overwintering stage for altitudinal adaptation. In this light, the lack of variation in the stage of diapause within *O. viridulus* likely represents a phylogenetic constraint (Gould 1989, Stearns 1992, Schlichting and Pigliucci 1998) to altitudinal range expansion. The stage of dormancy as a conserved trait within the gomphocerine lineage precludes tuning of development in a way expected to be optimal under seasonal time constraints. However, this has to be confirmed by investigating other, closely related species.

To summarize, *O. viridulus* exhibits altitudinal differentiation in development as an adaptive response to selection imposed by local climates. However, the potential for altitudinal adaptation is limited by the invariant stage of overwintering diapause, probably indicating a phylogenetic constraint within the gomphocerine grasshopper lineage. As a consequence, the degree of climatic compensation displayed by field populations along the altitudinal gradient is rather low as compared to other ectotherms.

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Chapter 3

Grasshopper ontogeny in relation to time constraints: adaptive divergence and stasis

Abstract

Life history theory generally predicts a trade-off between short juvenile development and large adult size, assuming invariant growth rates within species. This pivotal assumption has been explicitly tested in few organisms. We studied ontogeny in 13 populations of *Omocestus viridulus* grasshoppers under common garden conditions. High altitude populations, facing short growing seasons and thus seasonal time constraints, were found to grow at a similar rate as low altitude conspecifics. Instead, high altitude grasshoppers evolved faster development, and the correlated change in body size led to an altitudinal size cline that mediated a trade-off with female fecundity. Moreover, an additional juvenile stage occurred in low but not high altitude females. This difference is likely due to the evolution of lowered critical size thresholds in high altitude grasshoppers to accelerate development. In contrast, we found a strikingly lower growth rate in males than females that we interpret as the outcome of concurrent selection for protandry and small male size. Further, our data revealed that within populations, large grasshopper individuals developed faster than small individuals, suggesting within-population genetic variation in growth rates. We provide evidence that different time constraints (seasonal, protandry) can lead to different evolutionary responses in intrinsic growth, and that correlations among ontogenetic traits within populations cannot generally be used to predict life history adaptation among populations. Moreover, detailed comparisons of ontogenetic patterns can shed light on the developmental basis underlying phenotypic evolution.

Introduction

Populations within a species experience seasonal time constraints on their life history if a relatively short available growing season restricts the reproductive life span. Life history models predict that populations facing seasonal time constraints should evolve accelerated development (earlier maturity) to ensure an appropriate reproductive period (Roff 1980, 2002; Rowe & Ludwig 1991; Abrams et al. 1996). Indeed, a number of empirical studies document faster development in high latitude or altitude ectotherm populations that experience relatively short growing seasons (e.g. Berven & Gill 1983; Dingle, Mousseau & Scott 1990; Blanckenhorn & Fairbairn 1995; Laugen et al. 2003; Berner, Körner & Blanckenhorn 2004). A common assumption of life history models dealing with time

constraints is a constant, maximal or at least optimal intrinsic growth rate within species. As a consequence, accelerated development (and thus a shorter growth period) results in smaller size. The decline in body size along gradients of declining season length ('the converse to Bermann's Rule'; Park 1949; Mousseau 1997; Blanckenhorn & Demont 2004) found in some insects lends support to this view (Masaki 1967; Mousseau & Roff 1989; Orr 1996; Telfer & Hassall 1999).

An analogous argument applies to protandry, the faster development of males relative to females. In arthropods, protandry is generally viewed as the outcome of sexual selection on males to mature first to maximize matings (reviewed by Morbey & Ydenberg 2001). Similar growth rates among the sexes assumed (Thornhill & Alcock 1983; Zonneveld 1996), protandry implies a shorter male growth period that leads to sexual size dimorphism with males smaller than females, a common pattern in arthropods (e.g. in many butterflies: Wiklund & Forsberg 1991). Hence, both seasonality and sexual selection might impose time constraints on the life history that lead to adaptive divergence in developmental timing between populations of a species as well as between the sexes. These changes in development time typically entail correlated changes in body size (time-size trade-off).

However, the general assumption of constant growth rates within species that underlies the above reasoning might not be true. For instance, northern butterfly populations facing relatively short growing seasons exhibit faster development, but buffer body size change through faster intrinsic growth (Ayres & Scriber 1994; Nylin 1994). Similarly, accelerated growth in males sometimes allows protandry without concurrent female-biased size dimorphism (e.g. Wiklund & Forsberg 1991; Nylin et al. 1993; Lounibos et al. 1996). Furthermore, growth rate has been found to respond to artificial selection in laboratory populations (Bradshaw & Holzapfel 1996; Prasad et al. 2000; D'Amico, Davidovitz & Nijhout 2003). An important issue emerging from these studies is that intrinsic growth rate, because it mediates the relationship between development time (or time to maturity, a fitness component) and body size (a pivotal morphological character generally correlated with many fitness components; Peters 1983; Blanckenhorn 2000; Roff 2002), should itself represent an adaptively flexible trait optimized by natural selection (Abrams et al. 1996; Arendt 1997). Despite its importance to life history theory (Stearns 1994; Higgins & Rankin 1996; Cueva del Castillo & Nuñez-Farfan 1999; Roff 2000), this hypothesis (or assumption) has received very limited attention to date, and thorough empirical tests are restricted to some temperate butterflies (Nylin et al. 1993; Nylin 1994 and references therein; Fischer & Fiedler 2001). Moreover, these studies emphasize that the role of growth rate for life histories should be explored by adopting an explicitly ontogenetic approach that integrates development time, growth rate, and body size (Higgins & Rankin 1996).

The prime objective of our study was to explore the evolution of growth rates and body size in relation to time constraints in the grasshopper *Omocestus viridulus* (L.). In particular, we

evaluated the conflicting views that growth rates are constant within species (causing a time-size trade-off), or that growth rates are flexibly adjusted by selection. The chosen species is particularly suited for this task because two different types of time constraints occur. On one hand, a recent study has documented adaptive divergence in development time in response to seasonality (Berner et al. 2004). Populations occurring at high altitude face a substantially truncated growing season and consequently display accelerated intrinsic development rates relative to low altitude populations. It is unknown, however, whether the strongly time-constrained high altitude grasshoppers also evolved relatively faster growth that would, at least in part, buffer concomitant body size reductions. On the other hand, *O. viridulus* males develop faster than females (Berner et al. 2004). This protandry is very probably the direct result of sexual selection: in this species, female reproductive quality declines relatively rapidly with age (Berner, unpubl. data), a condition favouring protandry (Morbey & Ydenberg 2001). Nevertheless, the relationship between development time and body size among males and females remains unclear because sex-specific growth has not yet been explored in this or in any related species. A further convenient property of our study system is that it develops through discrete juvenile stages typical of hemimetabolous insects. This permits the accurate determination of the three-dimensional relationship between time, growth rate and size over the whole developmental period, a precondition for meaningful ontogenetic comparisons (Klingenberg & Spence 1993).

Our investigation is based on a suite of common garden experiments. Specifically, we included multiple grasshopper populations from different altitudes to study ontogenetic responses to seasonal time constraints and sex-specific growth. This approach enabled us to address a further important issue. There has been long standing interest and controversy as to whether among-population (or higher level) evolutionary divergence reflects corresponding within-population trait correlations (Sokal 1978; Houle 1991; Armbruster & Schwaegerle 1996; Phelan et al. 2003; Bégin & Roff 2004). For this reason, we not only compared developmental variation among *O. viridulus* populations (and the sexes), but also among individuals within populations, expecting congruence in ontogenetic patterns. For instance, a positive correlation between development time and body size (due to fixed growth) among populations should mirror a positive time-size correlation among individual grasshoppers within populations.

As described above, life history adaptation in response to seasonality often involves divergence in body size between populations. This might cause another life history trade-off if body size itself is correlated with fecundity (Schluter, Price & Rowe 1991; Roff 2002). In relation to seasonal time constraints, this trade-off has rarely been scrutinized (but see Blanckenhorn & Fairbairn 1995). We therefore additionally examined the relationship between body size and fecundity characters (clutch size, offspring size) in grasshopper females.

Materials and methods

Study organism and source populations

Omocestus viridulus (Orthoptera: Acrididae) is an annual grasshopper widespread in central Europe. The literature indicates four nymphal (juvenile) stages (Ingrisch & Köhler 1998; Berner et al. 2004), but five stages can occur in females (see Results). All our experiments were carried out with F_1 progeny of grasshoppers sampled from 13 field populations in northeastern Switzerland in one of three consecutive years. The sampling sites cover an altitudinal gradient of 2000 m (Table 3; details in Berner et al. 2004). At each site, ca. 14

Table 3. Localities of origin, altitude, geographic situation, and sampling year of the studied grasshopper populations

Number	Locality	Altitude (m)	Latitude (N)	Longitude (E)	Year
1	Neerach	410	47° 29' 51"	8° 28' 38"	2002
2	Birchwil	540	47° 27' 34"	8° 37' 23"	2003
3	Schönenberg	670	47° 11' 51"	8° 38' 08"	2002
4	Bäretswil	830	47° 20' 57"	8° 50' 58"	2002
5	Rothenthurm	910	47° 06' 58"	8° 40' 11"	2003
6	Bendel	1055	47° 16' 10"	9° 10' 21"	2001
7	Näfels	1350	47° 06' 42"	8° 59' 30"	2002
8	Speer	1610	47° 11' 30"	9° 07' 06"	2001
9	Flumserberg	1850	47° 04' 21"	9° 16' 17"	2002
10	Elm	1860	46° 55' 32"	9° 08' 16"	2003
11	Gamserrugg	2060	47° 09' 27"	9° 20' 02"	2002
12	Pizol	2215	46° 58' 43"	9° 25' 20"	2001
13	Hörnli	2440	46° 46' 15"	9° 37' 17"	2002

adults per sex were caught at the onset of the reproductive period. They were kept in groups in cages in the greenhouse (three cages per population), fed field-cut grass and allowed to reproduce during 4 – 5 weeks. Because the grasshoppers were kept in groups, it was not possible to attribute the produced egg pods to the corresponding females. Nevertheless, because few females were kept in a single cage, and due to relatively long clutch laying intervals in the species (2 – 4 d), it was possible to verify that all females produced roughly equal number of clutches. Egg pods were collected twice a week as they were laid, put individually in plastic tubes containing moist vermiculite, and incubated at constant 25 °C for 35 d followed by four months at 5 °C (egg diapause). Subsequent incubation in a climate chamber with a 14 h light period at 27 °C and 8 °C night temperature yielded grasshopper nymphs for experiments. Hind femur length, henceforth body size, of field animals from all 13 populations was measured under a stereomicroscope and analyzed as a general linear model (GLM) with sex as a fixed factor and altitude of origin (= population) as continuous covariate. All statistics were performed with SPSS 11.5.

Grasshoppers reared in clutch groups

Newly hatched nymphs were immediately put into rearing containers of 19 cm height and 8 cm diameter and transferred to a climate chamber set to a light period of 16 h at 32 °C and night temperature of 10 °C. Day temperature was chosen taking into account the high temperature requirements of grasshoppers (Begon 1983; Ingrisch & Köhler 1998). The nymphs were kept in clutch groups of up to six individuals. Water-filled glass vials containing a standardized grass mixture grown in the greenhouse provided food. They were replaced every third day. First-stage nymphal exuviae (shed skins) were collected once the nymphs had molted to the second stage, dried for 24 h at 45 °C and weighed to the nearest microgram using a microbalance (MX5, Mettler Toledo, Greifensee, Switzerland). First-stage exuviae consist entirely of maternally invested tissue. They thus represent an appropriate measure of offspring (or egg) size (Köhler, pers. comm.) and are hereafter referred to as such. To study its relationship with female size among the 13 populations, mean offspring size based on clutch medians (N = 21 – 36 per population) was regressed against average body size of parental females.

At the end of nymphal development, the rearing containers were inspected twice daily. Adults were removed from the experiment continuously, their total development time noted and adult body size determined. (Unfortunately, development time was not recorded for the 2001 populations 6, 8 and 12.) The relationship between laboratory (F_1) adult size and altitude was investigated as for the field animals, although here clutch averages (N = 10 - 27 per population and sex) were used instead of individual values. Moreover, study year was introduced as an additional blocking factor to account for potential differences in climate chamber conditions. Growth rates, expressed as adult size divided by total juvenile development time, were analyzed both *among* populations and among individuals *within* each population. In the former case, population mean adult size was treated as response variable in a GLM with sex and study year as fixed factors and average development time as covariate. In the latter case, clutch average body size was entered as response variable, sex as a fixed factor, population as a random factor, and development time as covariate. Here, sample size per population and sex was 10 - 22.

Grasshoppers reared individually

In order to examine ontogenetic trajectories in detail using data from all developmental stages (1st nymphal to adult), a portion of nymphs from one low and one high altitude population (populations two and 10, respectively) were reared individually under otherwise similar conditions. This experiment also allowed us to explore the effect of rearing density (in groups vs. singly) on individual ontogeny. Here, containers were inspected twice daily throughout the experiment, moults noted and all nymphal exuviae collected for size (i.e. hind femur) measurement. For each animal with four nymphal stages, stage-specific growth rates were calculated by dividing size increment by stage duration. Growth rate, stage duration

and body size of each stage were analyzed using GLM with population and sex as fixed factors. Sample size was 8 - 11 clutches per population and sex. As above, growth was additionally analyzed within populations. Here, clutch mean adult size was treated as response variable, population and sex as fixed factors and total development time as covariate. Sample size was 8 – 13 clutches per population and sex.
For a comparison of growth rates between low altitude females (population two) undergoing four and five nymphal stages, the linear regression slope of body size against development time across all developmental stages served as an estimate of an individual's growth rate. This cruder procedure was chosen because here the nymphal stages were not homologous across the two developmental pathways. Direct comparison of stage-specific data was thus inappropriate. Clutch means (N = 4 for females with five stages) of growth rate, development time, adult size were analyzed with sex and nymphal stage number as fixed factors.

Female fecundity

The influence of female body size on clutch size was examined using individuals from the clutch group growth experiment. Upon reaching adulthood, virgin females selected at random from populations 1, 3, 4, 9, 11 and 13 were individually put in rearing containers together with a random male from the same population. Only one female from any clutch was used. Under similar laboratory conditions as above, the grasshoppers were allowed to reproduce. All containers were inspected every other day and new egg pods (clutches) removed. They were subsequently dissected for egg count under a stereomicroscope. The females were allowed to produce up to three egg pods, but for each individual only the one clutch containing the highest number of eggs entered the analysis (usually the first clutch). Clutch size certainly represents an informative index of fecundity in this system, as during years with unfavourable climatic conditions high elevation grasshopper females produce a few clutches at best (Berner et al. 2004). Female body size was determined at the end of the experiment. Clutch size was analyzed using GLM with the corresponding female's size as covariate and population as random factor. The sample comprised 47 females.

Results

Comparison among populations

We found a strong positive association between juvenile development time and adult size across *Omocestus viridulus* populations ($F_{1,15}$ = 28.3, P < 0.001, overall correlation r = 0.85; Fig. 14). Additionally, given similar developmental time, females attained significantly larger size than males (sex effect $F_{1,15}$ = 52.5, P < 0.001), indicating higher growth rates in the

female sex. The regression slopes did not statistically differ among the sexes and years (all interactions P > 0.13).

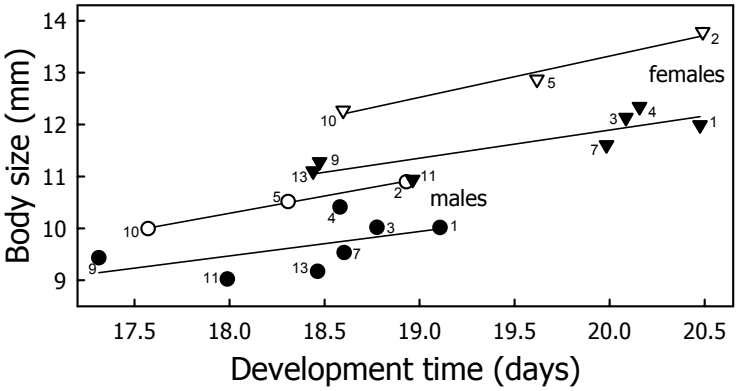


Fig. 14. Relationship between total juvenile development time and adult size in male and female *Omocestus viridulus* progeny from 10 populations sampled in 2002 (filled symbols) or 2003 (open symbols). Shown are population averages based on clutch means. The small numbers refer to the populations from Table 3.

Table 4. Comparison of ontogeny among individually reared male and female grasshoppers from populations two (low altitude) and 10 (high altitude), based on clutch means*. Results with $P < 0.05$ are bold-faced. Population by sex interactions are not shown, as all P were > 0.075.

Trait	Factor	Development stage	$F_{1,32}$	P
Growth rate	Population	1st	0.02	0.887
		2nd	1.36	0.253
		3rd	3.62	0.066
		4th	0.18	0.678
	Sex	1st	6.71	**0.014**
		2nd	11.57	**0.002**
		3rd	78.94	**< 0.001**
		4th	296.93	**< 0.001**
Stage duration	Population	1st	5.76	**0.022**
		2nd	10.95	**0.002**
		3rd	16.74	**< 0.001**
		4th	27.14	**< 0.001**
	Sex	1st	0.22	0.646
		2nd	4.61	**0.039**
		3rd	6.85	**0.013**
		4th	7.96	**0.008**
Body size	Population	1st	3.80	0.06
		2nd	11.90	**0.002**
		3rd	21.81	**< 0.001**
		4th	57.74	**< 0.001**
		Adult	44.86	**< 0.001**
	Sex	1st	3.80	0.06
		2nd	19.31	**< 0.001**
		3rd	70.85	**< 0.001**
		4th	250.08	**< 0.001**
		Adult	542.68	**< 0.001**

* all data from individuals with four nymphal stages

A more detailed analysis of ontogenies based on individual stage-specific data from two populations confirmed these findings: within the sexes, high and low altitude grasshoppers exhibited similar growth rates in all stages (non-significant population effects, Table 4; Fig. 15). However, high altitude animals showed shorter nymphal stages and reduced body size. As an exception, nymphal size at first stage did not significantly differ between the two populations and sexes, indicating comparable size at the onset of ontogeny. Females displayed elevated growth rates in all nymphal stages compared to males.

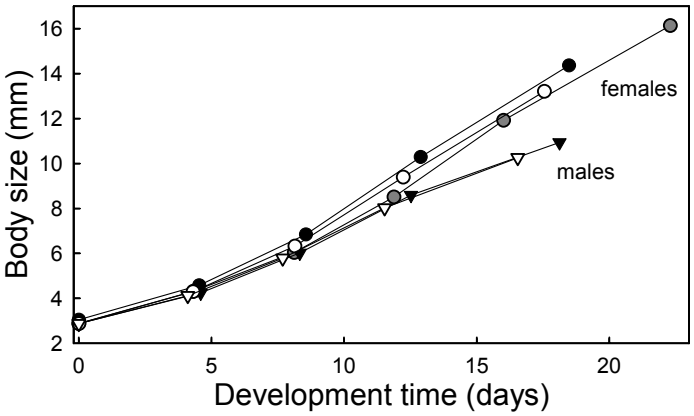


Fig. 15. Growth trajectories of male and female grasshoppers from low altitude (population 2, solid symbols) and high altitude (population 10, open symbols). Grey symbols refer to lowland females with five nymphal stages. Displayed are population averages of clutch means for all developmental stages.

Unexpectedly, some 30 % (five out of 17) of low altitude females included an additional (fifth) nymphal stage in their juvenile development. These animals exhibited growth rates similar to lowland females undergoing the usual four stages ($F_{1,9}$ = 2.4, P = 0.155, Fig. 15). As a consequence, the insertion of an additional nymphal stage prolonged development by 3.9 d (20 %) on average ($F_{1,9}$ = 77.7, P < 0.001) and led to 12 % larger adult size ($F_{1,9}$ = 39.9, P < 0.001). These differences are substantial. The occurrence of a fifth nymphal stage was unknown in this species, but has been reported for related grasshoppers (Ingrisch & Köhler 1998). As in these species, *O. viridulus* females with five stages inserted a pure growth stage (during which no visible differentiation occurred) before the penultimate developmental stage.

Resulting from uniform growth rates but differential development time, an altitudinal body size cline was evident for both field grasshoppers ($F_{1,384}$ = 389.5, P < 0.001) and their laboratory reared offspring ($F_{1,454}$ = 203.4, P < 0.001; Fig. 16). The smallest high altitude field females were, on population average, 16 % smaller that the largest low altitude females. In field males and in both sexes in the laboratory, this difference was 12 %. Moreover, field grasshoppers attained larger size than those raised in the laboratory, which can be attributed to the direct developmental influence of somewhat cooler average temperatures experienced by field animals ('temperature-size rule', van der Have & de Jong 1996; Atkinson & Sibly 1997).

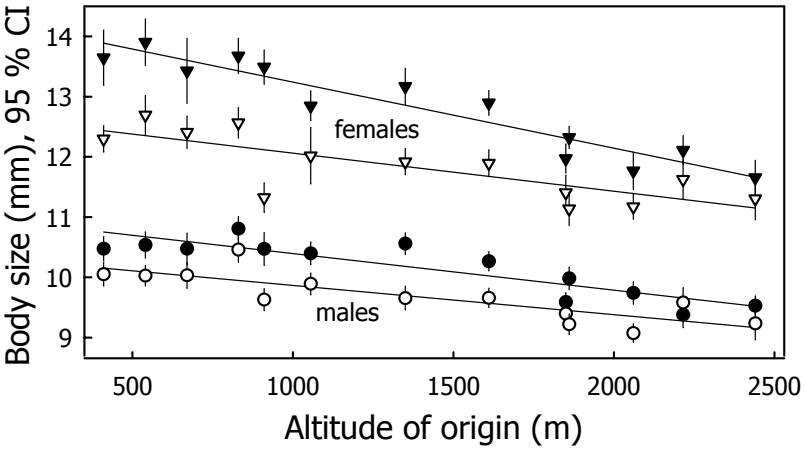


Fig. 16. Altitudinal body size cline in 13 *O. viridulus* populations from the field (filled symbols, based on individual values) and their laboratory-reared offspring (open symbols, based on clutch averages adjusted for study year).

Growth rates within populations

Contrary to the positive correlation of development time and adult size observed *among* populations, relatively large male and female grasshoppers reached adulthood faster than small conspecifics *within* the 10 populations considered (effect of development time on body size $F_{1,268} = 64.5$, $P < 0.001$, overall mean correlation coefficient $r = -0.454$). The slopes did not significantly differ between the sexes and populations (all interactions $P > 0.28$). Data from two populations (two and 10) suggested that this ontogenetic pattern was not dependent on rearing density, as a negative correlation between development time and body size was also expressed in grasshoppers reared singly ($F_{1,39} = 6.95$, $P = 0.012$). For the sake of clarity, Fig. 17 displays individual growth patterns of females from populations two and 10 only, reared both singly and in groups.

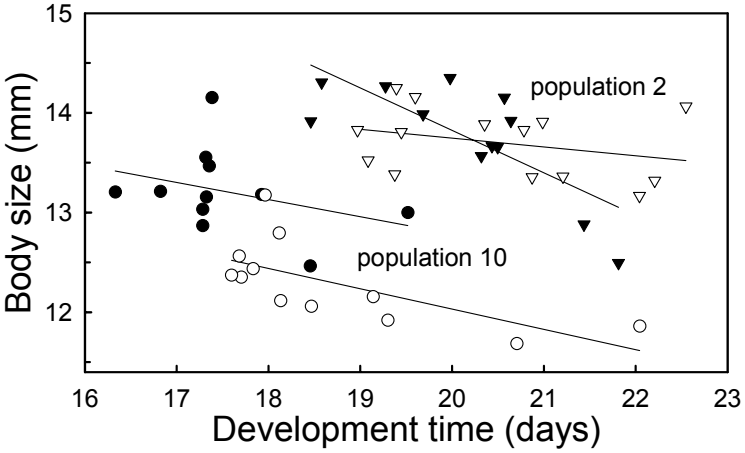
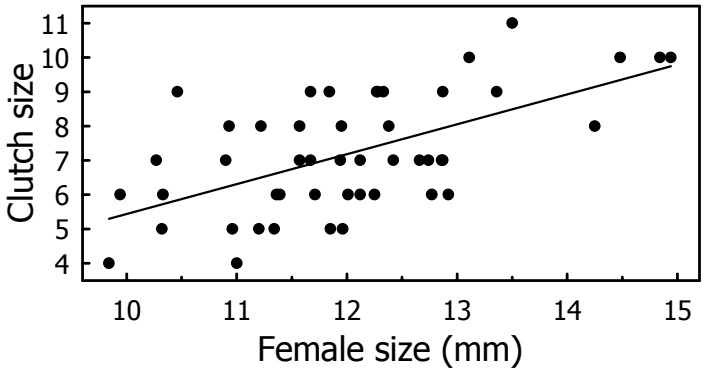


Fig. 17. Adult size in relation to development time in females from two populations, reared in groups (open symbols) and singly (solid symbols). Shown are clutch averages from animals with four instars.

Clutch size and offspring size

Clutch size in the laboratory increased as a function of body size of grasshopper females ($F_{1,39} = 9.01$, $P = 0.005$; Fig. 18). Clutches produced by the smallest individuals from high altitude sites contained only half as many eggs on average as those from the largest lowland conspecifics. (As both the population effect and the population by size interaction were non-significant [all $P > 0.36$], the data are pooled in Fig. 18) In contrast, no phenotypic correlation could be detected between average female size and offspring size among the 13 study

Fig. 18. Clutch size in relation to body size in *O. viridulus* females reared in the laboratory.



populations ($F_{1,11}$ = 0.26, P = 0.62; Fig. 19). (Regression against a population's altitude instead of mean female size also yielded no correlation [$F_{1,11}$ = 0.096, P = 0.76]). Nevertheless, mean offspring size differed by up to 15 % between populations. Moreover, there was considerable scatter within populations, resulting in large standard errors.

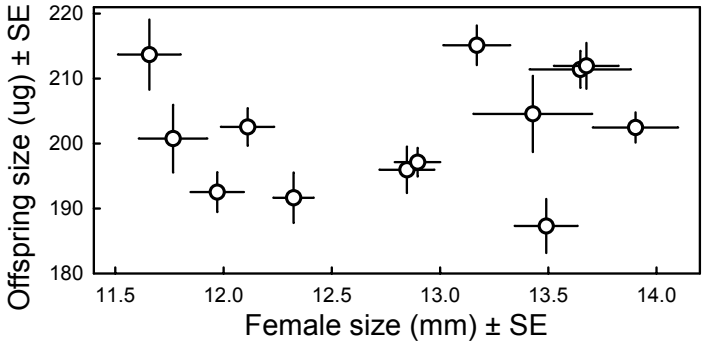


Fig. 19. Offspring size (expressed as first-stage exuvia weight) in relation to maternal body size across 13 *O. viridulus* field populations. Shown are population averages based on clutch medians (N = 20 - 36 per population).

Discussion

Growth rates among and within populations

A previous study demonstrated accelerated juvenile development in high altitude populations of *Omocestus viridulus* as an adaptive response to seasonal time constraints (Berner et al. 2004). Is this fast development due to fast intrinsic growth? Our results here clearly demonstrate that this is not the case, as growth rates are largely invariant among the studied grasshopper populations; instead, the grasshoppers can grow larger only by means of prolonging their development time. This species thus exhibits a trade-off between development time and body size. As a consequence, body size among populations decreases with altitude, providing an example of a converse altitudinal Bergmann size cline (Mousseau 1997; Blanckenhorn & Demont 2004). Our findings disagree qualitatively with the prediction that higher growth rates should evolve in populations facing seasonal time constraints (Abrams et al. 1996). Rather, the documented stasis in growth rate confirms a fundamental assumption of life history theory (e.g. Roff 1980; Rowe & Ludwig 1991). However, studies with temperate butterflies showed that in this group adaptive divergence in growth rates in relation to seasonality is frequent (Ayres & Scriber 1994; Nylin 1994 and references therein). Whether the potential for intraspecific evolution of growth rate is contingent on phylogenetic affiliation thus seems an interesting question (Stearns 1994) that would, however, require comparable data from many other taxa.

Based on the conserved growth rate found among populations, we expected a similarly strong positive association between development time and body size among individuals within each population. Surprisingly, we found an opposite pattern with large males and females completing juvenile development faster than small individuals of the same population. However, this correlation is phenotypic and evolutionarily relevant only if it

mirrors an underlying genetic correlation of the same sign (Reznick 1985; Roff 2000). Is there indication that this is the case in *O. viridulus*? Or is the trend attributable to environmental or maternal effects? On one hand, rearing density seems unlikely as a confounding environmental factor because a negative time-size correlation was evident in individuals reared both in groups and singly (cf. Wall & Begon 1987). On the other hand, relatively large offspring often grow faster (Fox & Czesak 2000) and the pattern might thus be ascribed to differences in offspring size, a maternal effect (Wall & Begon 1987; Mousseau & Dingle 1991). In order to test this possibility, we *ad hoc* analyzed linearly estimated individual growth rates from populations two and 10 with population and sex as fixed factors and offspring size as covariate. This analysis revealed no relationship between offspring size and growth rate ($F_{1,52} = 1.64$, $P = 0.21$). Maternal influence on growth rate via offspring size is therefore unlikely.

In addition and most importantly, phenotypic correlations between development time and adult size have been shown to generally represent reliable (albeit conservative) estimates of underlying genetic correlations (Roff 1996, 2000). For these reasons, our data suggest genetic variation in growth rates among individuals of *O. viridulus*, as seen in some other systems (Ueno 1994; Klingenberg & Spence 1997; Simons, Carrière & Roff 1998). This result is intriguing in view of the stasis in growth rate found among grasshopper populations, because we would expect the rapid fixation of alleles associated with fast growth in time-constrained high elevation populations. However, genetic variation in growth detected in the laboratory might disappear under more variable natural conditions (Simons & Roff 1996), and therefore not be available to selection. This issue clearly deserves further empirical treatment. In any event, our data caution against the prediction of among-population ontogenetic evolution based on the association between time, size and growth at the within-population level.

Developmental pathways and proximate cause

An unexpected difference between high and low elevation grasshopper ontogenies was the tendency of some females to develop through five nymphal stages. This alternative developmental pathway occurred in low altitude *O. viridulus* females but was absent at high elevation. Although proved directly only for two populations where individual ontogenies were carefully tracked, inspection of body size patterns suggests that this difference between low and high altitude females might be general: adult females with five nymphal stages are much larger than four-stage conspecifics. Variance in female size should thus be greater in low altitude populations where both pathways exist. Such a tendency is suggested in some field populations (but absent in laboratory females where sample sizes were variable; see Fig. 16). In further support, Telfer and Hassall (1999) report an analogous latitudinal decline in the occurrence of an additional nymphal stage in a related grasshopper.

Variation within populations in the number of developmental stages is generally interpreted as a plastic ontogenetic response to the available season length mediated by temperature and/or photoperiodic cues (Tanaka & Brookes 1983; Bellinger & Pienkowski 1987; Telfer & Hassall 1999; Fischer & Fiedler 2001). Nevertheless, we tested *ad hoc* for a possible relationship between developmental pathway and offspring size among females of the low-land population two and obtained an interesting result: females undergoing five stages were significantly smaller at hatching (5 % on average) than four-stage individuals (t test: t_{15} = 4.453, P < 0.001, two-tailed). This strongly suggests that in *O. viridulus*, the insertion of an additional nymphal stage is mediated by small offspring size. The developmental pathway thus seems at least partly determined by maternal influence.

Our finding that the five-stage pathway is probably induced by small offspring size in low but not high altitude populations is important. It may provide the key to understanding the proximate mechanism that underlies both the evolution of fast development at high altitude and the positive time-size correlation among populations. Generally, arthropod molting is bound to stage-specific critical size (or weight) thresholds during sensitive phases (Nijhout 1981; Tanaka 1981; Woodring 1983; Davidovitz, D'Amico & Nijhout 2003). These thresholds regulate the relationship between development time and body size during ontogeny in a pleiotropic fashion: lower critical size causes earlier moulting at smaller size. Failure to reach a certain critical size can sometimes induce an additional juvenile stage that permits further growth but prolongs development (Nijhout 1981; Tanaka 1981). Consistent with this notion, our data indicate that some low altitude nymphs exhibited subcritical size during their second stage and subsequently inserted an additional growth stage. Given that similarly small hatchlings also occurred at high altitude, the absence of the five-stage pathway from high altitude populations likely represents a side effect of reduced critical size thresholds. It is very probable that such a decrease in critical size arose in response to selection for fast development under seasonal time constraints at high elevation. Owing to the pleiotropic nature of size thresholds, body size thus declines along with development time over the altitudinal gradient, which explains the positive time-size correlation among populations. Consistent with our data, adaptive divergence in development time through shifts in critical size thresholds does not require changes in growth rates.

However, comparable evidence from other systems, against which the proposed scenario of adaptive evolution in critical size could be evaluated, is sadly lacking. At least in partial support of our view, body size evolution among laboratory populations of *Manduca sexta* moth involved a shift in critical weight (D'Amico et al. 2003). Our analysis illustrates that comparisons of ontogenetic patterns can provide insights into developmental mechanisms underlying adaptive divergence, and thus link development, ecology and evolution (Amundson 2001; Brakefield, French & Zwaan 2003).

Protandry and sex-specific growth

Protandry is necessarily associated with female-biased size dimorphism if the sexes grow at similar rates (Zonneveld 1996). However, there is often conflicting selection for protandry and large size in males (Thornhill & Alcock 1983; Wiklund & Forsberg 1991; Cueva del Castillo & Nuñez-Farfan 1999) promoting the evolution of faster growth in males than females. This has been found in a number of species including spiders (Gunnarson & Johnsson 1990; Uhl et al. 2004), flies (Lounibos et al. 1996; Blanckenhorn 1998) and butterflies (Wiklund, Nylin & Forsberg 1991; Nylin et al. 1993; Fischer & Fiedler 2001). In striking contrast, *O. viridulus* males grow considerably slower than females, especially during the last stage, which causes pronounced sexual size dimorphism. Slower relative growth in males is uncommon (Fairbairn 1990; Bradshaw & Holzapfel 1996) and of particular interest because it suggest selection against large male size, an issue that has received relatively little attention so far (but see Blanckenhorn 2000). A likely explanation for slow male growth in our species builds on the fact that small males have lower metabolic demands than large males and may therefore spend less time foraging (Reiss 1989; Blanckenhorn, Preziosi & Fairbairn 1995). This allows increased mate pursuit and courting activity. Our species is characterized by scramble competition for females (Ingrisch & Köhler 1998; Berner, pers. observ.). Higher mate search and courting effort by males thus likely correlates with increased mating success. This suggests that male *O. viridulus* experience *both* protandry selection and selection for small size. Probably, slow relative growth in males is the outcome of simultaneous optimization of development time and body size.

It is evident that extensive experimental work would be needed to disentangle and quantify the different selection pressures shaping male and female ontogenies in this species. Nevertheless, our study lends support to the notion that sex-specific growth trajectories enable the independent optimization of development time and body size in both sexes. This finding contrasts to the uniform growth documented at the population level.

Clutch size and egg size

Development time and body size are correlated positively and decline with elevation across grasshopper populations. Since small female size can limit reproductive output, this raises the important question of whether body size mediates a life history trade-off between development time and fecundity (Schluter et al. 1991; Roff 2002). Indeed, we found a positive phenotypic correlation between female size and clutch size in *O. viridulus*. Our result is not surprising because this is the rule in insects (Honek 1993). Similar positive *genetic* correlations between female size and fecundity are frequent and reliably estimated using phenotypic data (Roff 2000). Hence, small body size incurs a substantial fecundity loss to

high altitude grasshopper females, and altitudinal adaptation therefore involves a real life history trade-off.

As regards offspring size, general predictions are rendered difficult by the fact that this trait is both a maternal and offspring phenotype (Bernardo 1996). On one hand, small female size might limit offspring size. However, consistent with theoretical work (Parker & Begon 1986), our data provide no indication of such a tendency. On the other hand, based on the assumption that large offspring grow faster and attain maturity earlier than small offspring (Fox & Czesak 2000), theory predicts the evolution of larger progeny in populations experiencing seasonal time constraints (Parker & Begon 1986; Sibly & Monk 1987; Roff 2002). This idea is supported by studies showing a positive correlation between egg size and latitude among populations (Ayres & Scriber 1994; Telfer & Hassall 1999). In *O. viridulus*, we found no evidence for larger offspring in time-constrained high altitude grasshopper populations. A confounding influence of female age (offspring size commonly increases with maternal age in Orthoptera; Fox & Czesak 2000; Cherrill 2002) is very unlikely to affect our data set because we sampled grasshoppers at similar phenological stages at all sites. We therefore conclude that the fast development displayed by high altitude grasshoppers is certainly not attributable to larger offspring.

Implications

We presented a detailed analysis of grasshopper ontogenies at the level of multiple altitudinal populations, the sexes, and individuals. Three important issues emerged. First, the evolutionary response of growth rate to different types of time constraints can greatly differ. We found stasis in growth rate among grasshopper populations in the face of selection imposed by seasonality, ultimately causing a life history trade-off. In contrast, males and females evolved strikingly divergent growth rates in relation to putative selection for protandry, facilitating independent adjustment of development time and body size. Second, ontogenetic evolution at the among-population level cannot necessarily be extrapolated from within-population trait associations. The sign of the time-size correlation was positive among populations, but negative among individuals within populations. Third, our work illustrates that detailed comparisons of ontogenetic pathways can provide valuable insights into the developmental basis of phenotypic evolution. Adaptive ontogenetic divergence among *O. viridulus* populations is probably due to shifts in critical size thresholds. Clearly, our understanding of life history evolution will greatly benefit from similar studies in diverse taxa integrating time, size and intrinsic growth at different levels.

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Summary

The role of plant-mediated constraints to herbivore populations in terrestrial ecosystems remains relatively poorly understood. One aim of this study was therefore to explore the effects of low host plant nitrogen (N) content on herbivore performance and feeding behaviour, and thereby to evaluate the utility of the N limitation and nutrient balance concepts. The grass-feeding grasshopper *Omocestus viridulus* (Orthoptera: Acrididae) provided a well-suited model system as the species exhibits high tissue N demands (11.3 % N dw) compared to other herbivores, and uses a relatively poor food source in the wild (median N content: 2.2 % dw). Using N-poor soil and fertilizer, natural host grasses of contrasting N contents (high vs. low N) were grown in pots in the greenhouse. Juvenile performance experiments were then carried out with first generation (F_1) offspring of grasshoppers caught in the field at three sites in Switzerland. The experiments were conducted both in a climate chamber and in outdoor cages under natural climatic conditions. I consistently found lower growth rates in grasshoppers reared on low N grasses, leading to somewhat prolonged development (13 % in the laboratory, 7 % outdoors) and slightly (4 – 5 %) smaller adult size. Juvenile mortality was low (always < 20 %) and similar among the food N treatments. As N contents differed strongly between the experimental grass treatments (e.g. 1.6 % vs. 4.3 % N dw in the climate chamber trial), my results suggest that natural food quality imposes no relevant nutritional constraint on grasshopper performance. Separate laboratory experiments on feeding behaviour served to investigate how grasshoppers cope with low quality food. First, female last-instar nymphs were allowed to feed singly on either high or low N grass (non-choice setting) over a 16 h period. The food's initial fresh weight was known and converted to dry weight using constants. Individual food consumption was subsequently calculated by subtracting the dry weight of the food leftovers from the initial dry mass (gravimetrical method). This experiment showed that grasshoppers facing N-poor grass displayed substantially elevated food consumption (82 % higher on average) compared to conspecifics fed N-rich food. The animals thus compensated for lower nutritional quality by eating more. In a choice experiment, single grasshopper nymphs were offered high and low N grass simultaneously during a 28 h period. Individual consumption of both foods was calculated gravimetrically and revealed a striking preference for fertilized grass. On average, N-poor grass only accounted for some 20 % of the total ingested plant dry mass. It is argued that the proportion of consumed high N (protein-rich) and low N (energy-rich) grass likely represents the outcome of nutrient balancing. Proximately, the observed food selection can be explained by the modulation of taste receptor responsiveness via metabolic feedback, and/or associative learning of the spatial location of the different food sources. Overall, my results disagree with simple bottom-up predictions of herbivore population control, such as N limitation. Rather, this investigation provides

evidence for a high ability of arthropod herbivores to balance their nutrient intake, and thus corroborates a body of work performed with synthetic diets.

Another set of experiments using the same study organism was aimed at exploring the hypothesis that life history differentiation along a climatic gradient may have allowed a species to extend its geographic range. To this end, a total of eleven *O. viridulus* populations from eastern Switzerland were selected to span the species' entire altitudinal range (410 – 2440 m). Temperature measurements and phenological surveys repeated in regular intervals at four field sites allowed quantifying the decline of available heat sums with increasing elevation, and its immediate effect on development rates. During the relatively cool season of 2002, embryonic development at 2215 m altitude started nearly three month later than at 410 m, and nymphal hatching and adult emergence were delayed by roughly two month. This implied a strong truncation of the reproductive period in high altitude animals, a situation predicted by life history theory to promote the evolution of shortened time to maturity (faster juvenile development). During the following, climatically favourable year, the phenological delay in the field of high altitude relative to lowland populations was less pronounced. This suggested that the variance in available season length increases with altitude, and that the strength of natural selection owing to time constraints at high elevation fluctuates considerably. In order to investigate adaptive (genetic) divergence in grasshopper life history, several traits determining development time (or time to maturity) were compared across the eleven study populations under common garden conditions, using offspring of field-caught animals. Embryonic development rates were found to increase according to the population's altitude of provenance, with embryos from highest altitudes completing development some ten percent faster than lowland animals. This was found both at 27 °C and 19 °C incubation temperature, genotype by temperature interactions thus proved unimportant. Similarly, nymphal development accelerated with elevation of origin, the maximal difference between high and low altitude populations again amounting to roughly ten percent. In contrast, the stage of embryonic overwintering diapause, a pivotal determinant of developmental timing in annual organisms, was constant across six inspected populations from different altitudes. Embryonic development was always arrested just before the onset of embryonic rotation. This result agrees with data from other members of the same subfamily (Gomphocerinae), but disagrees with life history responses to seasonal time constraints reported for other Orthoptera. The documented lack of flexibility (genetic variation) in the diapause stage is therefore best understood as a phylogenetic constraint. This investigation offers strong support for the hypothesis of life history adaptation to local climates, although the degree of differentiation in intrinsic development rate among the populations is relatively modest and essentially limited by the conserved diapause stage. As a result, the immediate influence in the wild of decreasing temperatures along the altitudinal gradient overwhelms the genetic response observed in the laboratory, thus providing an example of cryptic evolution.

Life history theory generally predicts a trade-off between short juvenile development and large adult size, assuming invariant growth rates within species. This basic assumption has been explicitly tested in few organisms. The relationship between growth rate, juvenile development time and body size was examined in *O. viridulus* grasshoppers from 13 populations from different altitudes (including the populations mentioned above). All experiments were performed with first generation offspring at 32 °C in the climate chamber. I hypothesized that time constraints imposed by the altitudinal decline in season length, and arising from the advantage of earlier emergence of males relative to females (protandry), should have favoured increased intrinsic growth rates in high altitude animals and in the male sex. However, growth rates were similar across the populations. Instead, accelerated development with increasing elevation resulted in an altitudinal body size cline, with animals from the highest sites exhibiting roughly 12 % smaller adult size on average compared to lowland conspecifics. This size pattern was also observed in field animals. As I found a positive correlation between female body size and the number of eggs per clutch (but not offspring size), adaptation of *O. viridulus* to alpine climates involves a life history trade-off between time to maturity (development time) and fecundity, mediated by body size. An additional (fifth) nymphal stage, inserted before the penultimate nymphal stage and leading to an extended (by 20 %) developmental period and 12 % larger adult size, occurred in some females from low altitude. Individuals with five as opposed to four nymphal stages displayed significantly (5 %) smaller hatchling size, indicating that the insertion of the additional stage is contingent on maternal investment in the egg. It is argued that the absence of the five-stage developmental pathway from high elevation populations likely represents a side effect of the evolution of lower critical size thresholds in high altitude grasshoppers to accelerate development. Contrary to expectations, males were found to grow at substantially lower rates than females. This finding suggests that *O. viridulus* males not only experience protandry selection, but simultaneous selection for small size associated with reduced energetic requirements during mate search. Relatively slow growth in males probably allows the independent optimization of development time and body size among the sexes. My data further revealed that within populations, large individuals consistently developed faster than small individuals, resulting in a negative correlation between development time and size. This pattern could neither be ascribed to sib competition, as it was expressed in individuals both reared in groups and individually, nor to small hatchling size, a maternal effect. Hence, genetic variation in growth rate among individuals within populations seems probable. This study illustrates that the evolutionary response of intrinsic growth rate to different types of time constraints can greatly differ. I found stasis in growth rate among grasshopper populations in relation to seasonality, but highly divergent growth between the sexes owing to protandry selection. Further, ontogenetic evolution at the among-population level cannot necessarily be predicted based on within-population trait associations. The sign of the time-

size correlation was positive among populations, but negative among individuals within populations. Finally, I illustrate that detailed ontogenetic comparisons can shed light on the developmental cause (here a shift in critical size thresholds) underlying phenotypic evolution.

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Curriculum Vitae

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