Journal of Animal Ecology 2006
75, 130–139

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

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Summary

1. 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.
2. 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 to low altitude conspecifics.
3. Instead, high-altitude grasshoppers evolved faster development, and the correlated change in body size led to an altitudinal size cline mediating a trade-off with female fecundity.
4. An additional juvenile stage occurred in low- but not high-altitude females. This difference is probably due to the evolution of lowered critical size thresholds in high-altitude grasshoppers to accelerate development.
5. 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.
6. Within populations, large individuals developed faster than small individuals, suggesting within-population genetic variation in growth rates.
7. We provide evidence that different time constraints (seasonal, protandry selection) 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, our study illustrates that comparisons of ontogenetic patterns can shed light on the developmental basis underlying phenotypic evolution.

Key-words: body size, development time, intrinsic growth rate, *Omocestus viridulus*, trade-off.

Journal of Animal Ecology (2006) **75**, 130–139
doi: 10.1111/j.1365-2656.2005.01028.x

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 &

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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 *et al.* 2001). 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, unpublished 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 north-eastern Switzerland in one of 3 consecutive years. The sampling sites cover an altitudinal gradient of 2000 m (Table 1; details in Berner *et al.* 2004). At each site, *c.* 14 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 days), 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 into plastic tubes containing moist vermiculite, and incubated at a constant 25 °C for 35 days followed by 4 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 analysed 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 version 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 moulted 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, personal communication) 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 analysed 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.

Table 1. Localities of origin, altitude, geographical 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

GRASSHOPPERS REARED INDIVIDUALLY

In order to examine ontogenetic trajectories in detail using data from all developmental stages (first nymphal to adult), a portion of nymphs from one low- and one high-altitude population (populations 2 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 analysed using GLM with population and sex as fixed factors. Sample size was eight to 11 clutches per population and sex. As above, growth was additionally analysed 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 eight to 13 clutches per population and sex.

For a comparison of growth rates between low-altitude females (population 2) 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 and adult size were analysed 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 put individually into 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 dissected subsequently for egg count under a stereomicroscope. The females were allowed to produce up to three egg pods, but for each individual only the 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 analysed 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 *O. viridulus* populations ($F_{1,15} = 28{\cdot}3$, $P < 0{\cdot}001$, overall correlation $r = 0{\cdot}85$; Fig. 1). Additionally, given a similar developmental time, females attained significantly larger size than males (sex effect $F_{1,15} = 52{\cdot}5$, $P < 0{\cdot}001$), indicating higher growth rates in the female sex. The regression slopes did not differ statistically among the sexes and years (all interactions $P > 0{\cdot}13$).

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 2; Fig. 2). However, high-altitude animals showed shorter nymphal stages and reduced body size. As an exception, nymphal size at first stage did not significantly differ

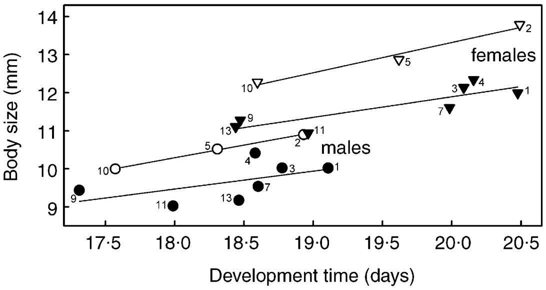

Fig. 1. Relationship between total juvenile development time and adult size in male and female *Omocestus viridulus* progeny from 10 populations sampled in 2002 (filled symbols) or 2003 (open symbols). Shown are population averages based on clutch means. The small numbers refer to the populations from Table 1.

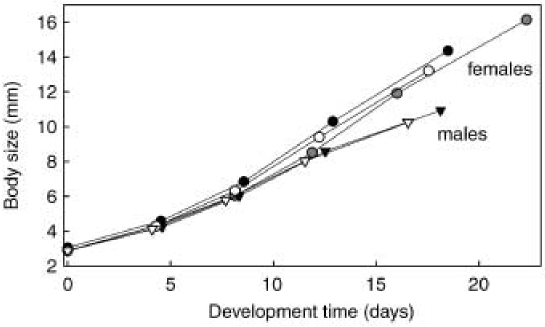

Fig. 2. Growth trajectories of male and female grasshoppers from low altitude (population 2, solid symbols) and high altitude (population 10, open symbols). Grey symbols refer to lowland females with five nymphal stages. Displayed are population averages of clutch means for all developmental stages.

Table 2. Comparison of ontogeny among individually reared male and female grasshoppers from populations 2 (low-altitude) and 10 (high-altitude), based on clutch means*. Results with $P < 0\cdot05$ are shown in bold type. Population × 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.

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.

Unexpectedly, *c.* 30% (five 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 ($F_{1,9} = 2\cdot4$, $P = 0\cdot155$, Fig. 2). As a consequence, the insertion of an additional nymphal stage prolonged development by 3·9 days (20%) on average ($F_{1,9} = 77\cdot7$, $P < 0\cdot001$) and led to a 12% larger adult size ($F_{1,9} = 39\cdot9$, $P < 0\cdot001$). 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 ($F_{1,384} = 389\cdot5$, $P < 0\cdot001$) and their laboratory-reared offspring ($F_{1,454} = 203\cdot4$, $P < 0\cdot001$; Fig. 3). 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).

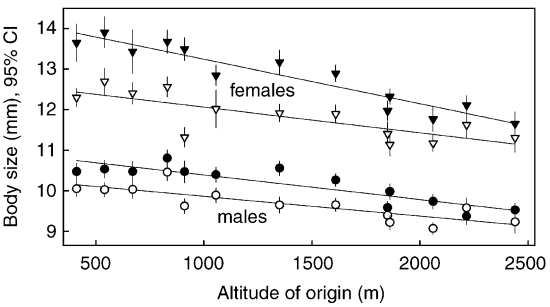

Fig. 3. Altitudinal body size cline in 13 *O. viridulus* populations from the field (filled symbols, based on individual values) and their laboratory-reared offspring (open symbols, based on clutch averages adjusted for study year).

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 $F_{1,268} = 64\cdot5$, $P < 0\cdot001$, overall mean

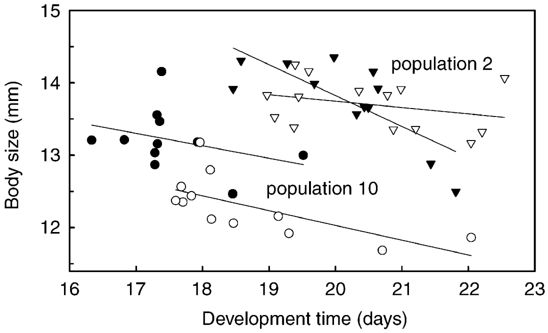

Fig. 4. Adult size in relation to development time in females from two populations, reared in groups (open symbols) and singly (solid symbols). Shown are clutch averages for animals with four nymphal stages.

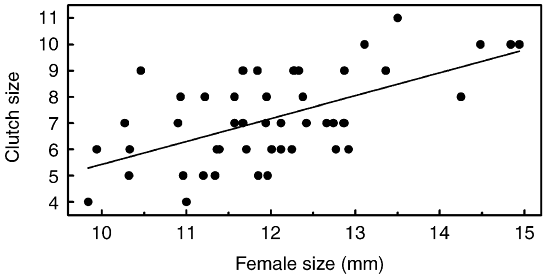

Fig. 5. Clutch size in relation to body size in *O. viridulus* females reared in the laboratory.

correlation coefficient $r = -0{\cdot}454$). The slopes did not differ significantly between the sexes and populations (all interactions $P > 0{\cdot}28$). Data from two populations (2 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 ($F_{1,39} = 6{\cdot}95$, $P = 0{\cdot}012$). For the sake of clarity, Fig. 4 displays individual growth patterns of females from populations 2 and 10 only, reared both singly and in groups.

CLUTCH SIZE AND OFFSPRING SIZE

Clutch size in the laboratory increased as a function of body size of grasshopper females ($F_{1,39} = 9{\cdot}01$, $P = 0{\cdot}005$; Fig. 5). 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 × size interaction were non-significant (all $P > 0{\cdot}36$), the data are pooled in Fig. 5.] In contrast, no phenotypic correlation could be detected between average female size and offspring size among the 13 study populations ($F_{1,11} = 0{\cdot}26$, $P = 0{\cdot}62$; Fig. 6). [Regression against a population's altitude instead of mean female size also yielded no correlation ($F_{1,11} = 0{\cdot}096$, $P = 0{\cdot}76$)]. Nevertheless, mean offspring size differed by up to 15% between populations. Moreover, there was considerable scatter within populations, resulting in large standard errors.

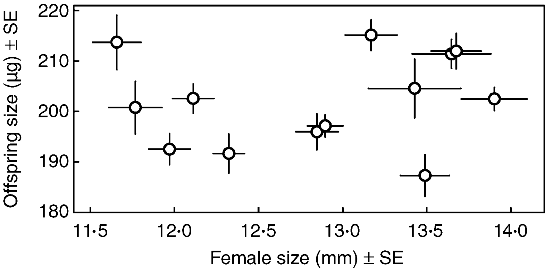

Fig. 6. Offspring size (expressed as first-stage exuvia weight) in relation to maternal body size across 13 *O. viridulus* field populations. Shown are population averages based on clutch medians ($n = 20$–36 per population).

Discussion

GROWTH RATES AMONG AND WITHIN POPULATIONS

A previous study demonstrated accelerated juvenile development in high-altitude populations of *O. 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 an 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 analysed *ad hoc* linearly estimated individual growth rates from populations 2 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 ($F_{1,52} = 1{\cdot}64$, $P = 0{\cdot}21$). 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 tracked carefully, 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. 3). In further support, Telfer & 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 lowland population 2 and obtained an interesting result: females undergoing five stages were significantly smaller at hatching (5% on average) than four-stage individuals (t-test: $t_{15} = 4{\cdot}453$, $P < 0{\cdot}001$, 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 moulting 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 probably 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* moths 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 suggests selection against large male size, an issue that has received relatively little attention so far (but see Blanckenhorn 2000). A possible 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, personal observation). Higher mate search and courting effort by males thus probably 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. Because 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 estimated reliably 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.

With regard to 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 have presented a detailed analysis of grasshopper ontogenies at the level of multiple altitudinal populations, the sexes and individuals. Three important issues have emerged. First, the evolutionary response of growth rate to different types of time constraints can differ greatly. 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. Secondly, 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. Thirdly, 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 due probably to shifts in critical size thresholds. Clearly, our understanding of life history evolution will benefit greatly from similar studies in diverse taxa integrating time, size and intrinsic growth at different levels.

Acknowledgements

This work is a part of the doctoral thesis of Daniel Berner that would not have been possible without part-time employment at Agroscope FAL Reckenholz provided by T. Walter, and the supervision of Ch. Körner. G. Köhler, J. Samietz and E.-F. Kiel provided valuable advice at an early stage. M. Waldburger, S. Bosshart and P. Streckeisen made materials, greenhouse space and climate chambers available. All these people are gratefully acknowledged.

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Received 7 April 2005; accepted 9 August 2005