In insects, whilst variations in life cycles are common, the basic patterns typical for particular groups remain generally conserved. One of the more extreme modifications is found in some subterranean beetles of the tribe Leptodirini, in which the number of larval instars is reduced from the ancestral three to two and ultimately one, which is not active and does not feed. We analysed all available data on the duration and size of the different developmental stages and compared them in a phylogenetic context. The total duration of development was found to be strongly conserved, irrespective of geographical location, habitat type, number of instars and feeding behaviour of the larvae, with a single alteration of the developmental pattern in a clade of cave species in southeast France. We also found a strong correlation of the size of the first instar larva with adult size, again regardless of geographical location, ecology and type of life cycle. Both results suggest the presence of deeply conserved constraints in the timing and energy requirements of larval development. Past focus on more apparent changes, such as the number of larval instars, may mask more deeply conserved ontogenetic patterns in developmental timing.

1. Introduction

Insects are extremely diverse in their morphological and physiological traits [1], and many of these modifications affect the life cycle, allowing fine-tuned adaptation to most environments [2]. Despite this diversity, there are developmental constraints and ontogenetic patterns that characterize general types of life cycles common to all members of a lineage. In holometabolous insects, the basic pattern is characterized by embryonic development, followed by several larval stages alternating growth and moulting. After the last larval moult, the metamorphic transition is completed in the pupal stage, resulting in the formation of an adult with a morphology substantially different from the previous stages (see [3] for an overview). Variations on this basic pattern are rare, occurring for instance in cases of heterochrony, in which either reproductive maturity is reached before transforming into an adult or the insect becomes an adult that retains some larval characters [4,5]. In addition, many groups show extreme stability in larval development, with a fixed number of larval instars before pupation [6].

Detailed knowledge of the life cycle and its modifications is restricted to relatively few species, and it is far from clear how often and how easily life cycles diverge from the general pattern. A spectacular case of life cycle modification is the reduction in the number of larval instars observed in different groups of subterranean insects, leading in its most extreme forms to a non-feeding larva that does not moult until pupation [7,8]. In the subterranean species of Leptodirini beetles, these modifications of larval development have been documented for several species in different geographical areas and have been traditionally assumed to have evolved multiple times independently, even in the case of closely related species [8,9]. However, with the use of a comprehensive molecular phylogeny, it has recently been shown that the number of independent origins of life cycle modification was low, with a single one in the Pyrenees, the
geographical area for which most data are available [10]. In this lineage, there was a single transition during the Late Oligocene—Early Miocene from an ancestral 3-instar cycle [11] in species living in forest litter or under stones to a 2-instar cycle in species with fully subterranean habits (either in deep or shallow subterranean environments), in which the active larva still feeds. From within this lineage with a 2-instar cycle, there was then a single transition to a 1-instar, non-feeding larva in the Lower Miocene, in a lineage found today only in the deep subterranean environment of caves [10].

In this work, we analyse in detail the available data on the duration and size of the different developmental stages of subterranean leptodirine beetles in the phylogenetic context provided by Cieslak et al. [10]. We find that the total duration of development is strongly conserved in these insects, despite variation in the number of instars and feeding behaviour of larvae; this developmental duration differs only in a clade of cave species from southeast France. We also find a strong correlation between first instar larval and adult body sizes, again regardless of number of larval moults. Both results suggest the presence of deeply conserved constraints in the timing and energy requirements of larval development, and at the same time higher variability in traits traditionally given greater biological relevance, such as the number of larval instars [9,10].

2. Material and methods

We obtained detailed data on the life cycle of 15 species from the literature, including average duration and size of individual developmental phases obtained from breeding experiments usually conducted through multiple generations under controlled conditions (electronic supplementary material). To place these species in a phylogenetic context, we used the phylogeny in [10], which includes 14 of these 15 species (the missing species is presumably related to another that is included) plus a supplementary material). Most species showed the ancestral, plesiomorphic condition, with a relative duration of the larval stages—irrespective of the number of instars—of ca 60% of the total development (figure 1a). This was significantly longer than the embryonic and pupal stages, which took up ca 20% each. In the second group, however, embryonic, larval and pupal development were each of similar duration, thus taking a 33% relative duration of total development (figure 1b). This group comprised a clade of five species from southeast France with a 1-larval instar cycle only (b). Bars, absolute range of the values (in days).

To compare the duration of the different stages, we grouped species according to different criteria: (i) phylogenetic: a derived clade of five species with 1-larval instar from southeast France versus the rest (showing the plesiomorphic condition [10]); (ii) geographical: species from the Pyrenees versus those from southeast France; and (iii) developmental: the species with 1-instar larvae versus those with 2- or 3-instar larvae. We compared the duration (in days) of the egg, larval and pupal stages and the total developmental time among species using a two-tailed ANOVA with the uncorrected data and using phylogenetically independent contrasts as implemented in the PDAP package run in MESQUITE v. 2.7 (http://mesquiteproject.org). We also used discriminant analysis with membership of one of the three groups as the independent variable and the duration of egg, larvae and pupae as predictors to identify misclassified species (electronic supplementary material, table S3).

To compare larval and adult size, we used phylogenetically independent contrasts in the PDAP package, also using the phylogeny in [10].

3. Results

The phylogenetic criterion was the best for comparing the duration of the different developmental stages, with no misclassified species in the discriminant analysis and the highest significances in pairwise PDAP comparisons (electronic supplementary material). Most species showed the ancestral, plesiomorphic condition, with a relative duration of the larval stages—irrespective of the number of instars—of ca 60% of the total development (figure 1a). This was significantly longer than the embryonic and pupal stages, which took up ca 20% each. In the second group, however, embryonic, larval and pupal development were each of similar duration, thus taking a 33% relative duration of total development (figure 1b). This group comprised a clade of five species from southeast France with a 1-larval instar life cycle. The total duration of development ($N = 8$, average $= 305$ days, s.d. $= 27$) was not significantly different between these two groups (electronic supplementary material, table S3).

The application of alternative criteria—either geographical (Pyrenees versus southeast France) or type of development (species with 1-larval versus 2- and 3-larval instars)—resulted in heterogeneous groups with respect to the duration of different developmental stages, with lower or no significant differences between them (electronic supplementary material).

There was a strong correlation between the total length of the freshly hatched larva and the adult, irrespective of developmental type and geographical origin of species (regression using phylogenetically independent contrasts, $N = 12$, $r^2 = 0.92$, $p < 0.000001$; figure 2). The exclusion of an outlier species with large body size (Leptodirus hochenwartii) only slightly
increased the correlation (N = 11, r² = 0.71, p < 0.001). There was a negative allometric relationship (slope of the uncorrected regression line a = 0.73, s.e.m. = 0.06), with the first instar larvae being slightly larger than the adult for most of the species except for the largest. There was no significant correlation between the size of the adult and the size of the second instar larvae (N = 6; r² = 0.16, p > 0.3).

4. Discussion

We found a strong constraint in the total duration of developmental stages in subterranean leptodirines, irrespective of the number of larval instars, type of habitat (forest litter or subterranean) and geographical region. Even though we obtained all raw data from published sources, these data had never been used in comparative studies (neither in a phylogenetic context nor otherwise) and none of the developmental patterns we uncover have been proposed before.

According to our results, the ancestral, plesiomorphic condition of the relative duration of the developmental stages remained unchanged through life cycle evolution in the Pyrenees in both epigean and subterranean species, and also through at least one transition to a life cycle with 2-larval instars in southeast France. It was, however, modified at the origin of one clade with 1-larval instar in southeast France, but even in this clade the total duration of development, from egg to adult, did not change. The origin of this constraint, and whether it is linked to the colonization of the subterranean environment, remains speculative, but may be related to the existence of programmed timers for the completion of metamorphosis [12] or the specific cell division rate and determination [13,14]. Our results show an increased evolutionary variability of the number of instars with respect to other developmental constraints, in agreement with the view that growth and development are decoupled from traditionally recognized strict boundaries in insect ontogeny, such as moulting [6,11].

In all species of Leptodirini, the reported size of the freshly hatched larvae was similar to that of the adult, which is unusual both in other groups of the same family (Leiodidae) and in Coleoptera as a whole, in which first instar larvae are generally much smaller than the adult. The large size of the first instar larva suggests a general trend in Leptodirini to accumulate resources in the egg, reducing the contribution of larvae to the global input necessary to complete development. It has been suggested that such a non-feeding larva may allow a species to survive in more extreme environments, as the more mobile adult can forage over a larger area to provide enough resources for the whole life cycle [10]. In Leptodirini, the non-feeding, inactive larva is protected inside a cocoon (‘logette’) [7,8], and thus less vulnerable to predators, which may be an additional advantage [10].

The preservation of the size of first instar larvae with respect to adult size, irrespective of whether the larva feeds or not, is surprising and counterintuitive, but could be explained by differences in dry weight. The only available data report a dry weight of 41.3% in the recently hatched larvae of Troglodromus bucheti, with a 1-instar cycle and a non-feeding larva, compared to 18% for Speophyes lucidulus, with 2-instars and a feeding larva [15]. Through the ontogeny, the relative dry weight of the non-feeding larvae decreases due to water absorption through the integument in the saturated atmosphere of the cave, and just before pupation, the ratio of dry to total weight was shown to be similar in both species (18% versus 22%, respectively) [15].

In summary, our results reveal a number of surprising developmental constraints in this lineage of beetles acting through multiple evolutionary transitions both in habitat type and number of larval instars. These constraints are best explained by the existence of intrinsic factors related to cell-cycle regulation determining developmental rates [13].

Data accessibility. All primary data are provided in the electronic supplementary material.

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