

Complex selection on life-history traits and the maintenance of variation in exaggerated rostrum length in acorn weevils

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Abstract Trophic interactions can trigger the development of exaggerated specialized characters and promote morphological diversification. For example, acorn weevils (genus *Curculio*) present strikingly long rostrums, which are used by females to perforate oviposition holes through the seed coat. Species exhibiting longer rostrums are known to exploit larger acorns, and therefore rostrum length is thought to be subject to selection to match the preferred acorn type. However, rostrum length is strongly correlated with body size, and morphological divergence could result from either selection on rostrum length for optimal food exploitation or from other pressures acting on body size. We collected infested acorns at oak forests where the large *Curculio elephas* and the small-bodied *Curculio glandium* co-occur.

There were no interspecific differences in adult female body size to rostrum length allometric relationships, and rostrum length is equally correlated with body size in either species. MtDNA-based species identification showed that *C. glandium* larvae were present within acorns of all sizes, whereas *C. elephas* larvae were restricted to acorns above a minimum size, irrespective of oak species. Hence, exploitation of large acorns can hardly have triggered rostrum enlargement, as the small sized *C. glandium* adults (with short rostrums) could perforate and oviposit in both small and large acorns. Rather, increased rostrum length is probably a by-product of the larger body sizes of individuals emerging from bigger acorns, which allow increased larval size and enhance larval survival likelihood. Summarizing, when exaggerated feeding traits co-vary with other body features, interspecific morphological variability may result from contrasting selective pressures acting on these correlated characters.

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Introduction

Ecological interactions play an important role in generating and maintaining the biological diversity of living organisms, as they influence adaptive morphological traits and thus species diversification (Mitter and Futuyma 1979; Mitter et al. 1979; Farrell and Mitter 1998; Benkman et al. 2001). Where species interactions are mediated by overlapping feeding interactions, morphological divergence in foraging-related traits permits the coexistence of species with different modes of resource utilization (Ruber et al. 1999; Schluter 2000). Feeding specialization thus constitutes a major factor promoting species diversity, of which insects,

and Coleoptera in particular, are a paradigmatic example. In beetles, high species richness is frequently associated with phytophagy and diversity of host plants (Farrell and Mitter 1998; Kergoat et al. 2005; Hunt et al. 2007). Host plant–herbivore interactions may result in differences in allometric shape or in body size in response to specialized feeding, which may lead to species diversification (e.g., Farrell and Mitter 1998; Kergoat et al. 2005; Toju and Sota 2006a; see Strauss and Zangerl 2002 for a review).

Phytophagous beetles are an ideal example to study the role of trophic interactions in species diversification through morphological divergence. However, invertebrate food webs remain insufficiently known, which constitutes an important handicap for ecological studies on interspecific trophic segregation. Fine-grain data on niche partitioning between closely related species is frequently lacking. Moreover, species identification itself can be problematic, especially in the developmental life stages. In this context, recent advances in molecular techniques are shedding light on insect diets (Pinzon-Navarro et al. 2010), and allow herbivore identification at all stages of the life cycle (Ahrens et al. 2007). Moreover, molecular data permit hidden patterns of ecological segregation to be detected at the intraspecific level (undetectable in studies of morphological variation), which may eventually lead to sympatric genetic differentiation (see Orr and Smith 1998 for a review). In the present paper we apply these molecular tools to assess the interplay of ecomorphological diversification and resource segregation among co-existing species, studying the interaction between oaks (*Quercus*) and acorn-feeding weevils (*Curculio*).

The genus *Curculio* (Coleoptera: Curculionidae) is widespread in the Holarctic. Most species are seed parasites and their main host plants are oaks, chestnuts, hazelnut trees and camelias (Hughes and Vogler 2004b). They exhibit long legs and a vastly extended rostrum that measures up to three times their body length, particularly in the females (Hughes and Vogler 2004a; Toju and Sota 2006b). The mandibles at the terminus of the rostrum are used to drill an oviposition hole by slowly chewing a narrow opening into the thick coating of the growing seed (Desouhant et al. 2000; Bonal and Muñoz 2008, 2009). Both the exaggerated legs and the extended rostrum are thought to be adaptive for oviposition into seeds of various sizes and shapes, as they may determine the kind of seeds that ovipositing females can grip and penetrate (Hughes and Vogler 2004a). Studies on the camelia weevil (*C. cameliae*) have shown that differences in rostrum length among populations are correlated with seed coat thickness (Toju and Sota 2006b). The plant and the insect are involved in a coevolutionary race in which thick seed coats promote the development of longer rostrums and vice versa (Toju and Sota 2006b). The pressure on rostrum length is strong, as evident from the disproportionately

enlarged rostrum relative to body size in populations feeding on seeds with thick seed coats (Toju and Sota 2006a).

However, in the case of acorn feeders, which constitute the bulk of *Curculio* species, the adaptive role of rostrum enlargement is not clear. In interspecies comparisons, morphometric analysis has shown that, while rostrum length is correlated with the use of greater seeds (Hughes and Vogler 2004a), species with longer rostrums are also larger sized (Hughes and Vogler 2004a). More importantly, these studies have not investigated whether the geometric relationship between rostrum length and overall body size differs between species. The tight correlation between body size and rostrum length makes it difficult to disentangle the role of host (acorn) size in the morphological diversification of *Curculio* species, as either trait might mediate resource segregation in assemblages of co-occurring beetles (see Pyron and Burbrink 2009 for an example of body size-related ecological traits in a different taxon). Moreover, potential effects related to resource segregation among species may be overlain by the fitness advantages of a larger body size. It is well known that *Curculio* larvae finish their development inside a single seed, and that seed size can constrain their final body size (Bonal and Muñoz 2008, 2009), which is strongly correlated with an individual's fitness, specifically higher larval survival, larger adult size and enhanced fecundity, as demonstrated in *C. elephas* (Desouhant et al. 2000).

Investigating the details of host utilization in co-occurring species in natural populations may shed light on the roles of the different selective pressures acting on *Curculio* morphological diversification. We carried out a field study at two sites (a monospecific and a mixed oak forest) where the larger *Curculio elephas* and the smaller *Curculio glandium* co-occur. We investigated whether rostrum length and/or body size can constrain the size of the acorns exploited and whether other fitness-related pressures (e.g., larval survival likelihood) may have promoted morphological diversification within the group. Our specific objectives were: (1) to examine whether there is a disproportionate development of rostrum length with respect to body size in either species; (2) to measure the degree of interspecific niche segregation by using mtDNA for species identification of the larvae collected in different sized acorns; (3) to assess the relationship between larval body size and survival likelihood.

Materials and methods

Study area

The study was carried out in two localities. Collserola Natural Park (Barcelona, Spain; 41°24'N, 2°6'E) (locality

A) is a Mediterranean mixed oak forest dominated by two species: the evergreen *Quercus ilex* and the winter deciduous *Q. humilis* (present in 95 and 75% of the forested area, respectively). The acorns of both species grow and mature within a single year, but *Q. humilis* acorns are bigger than those of *Q. ilex* (mean dry masses $1.1 \text{ g} \pm 0.2 \text{ SD}$ and $0.5 \text{ g} \pm 0.1 \text{ SD}$, respectively) (Espelta et al. 2009b). Montseny Natural Park (Barcelona, Spain; $41^{\circ}46'N$, $2^{\circ}23'E$) (locality B) is a monospecific *Q. ilex* forest where acorns are larger than those of *Q. ilex* in locality A (mean dry mass $0.8 \text{ g} \pm 0.2 \text{ SD}$); the distance between the two localities is 52 km. In locality A, we monitored *Curculio* interspecific segregation across the acorn size range, but also recorded additional phenological variables, adult and larval sizes and larval survival likelihood. Locality B was used as a control for interspecific segregation, as *Q. ilex* partially covers the missing acorn size range in locality A and allows the effects of acorn size to be separated from those of the host species.

Sampling methods

In locality A, we selected three groups of ten trees (five *Q. ilex* and five *Q. humilis*) at a mean distance of 350 m, and installed four seed traps in each to collect dropped infested acorns (see Espelta et al. 2009a for methodological details). In locality B, each of the three groups consisted of five *Q. ilex*, and we used the same collecting protocol. We sampled acorns of both *Quercus spp.* throughout the acorn growing season because acorn size varies between oak species and with collection date. This permitted an assessment of the effect of acorn size on interspecific trophic segregation, controlling for potential effects of oak specificity or phenological disparities between beetle species.

In locality A, sampling started on 30th July 2006 and continued until the end of the acorn falling season in late October (see Espelta et al. 2009a). Traps were emptied every 15 days and the fallen acorns were categorized as being either aborted (not included in this study), infested by *Curculio* sp. (recognizable from a small dark spot marking the drill hole produced during oviposition), or sound (fully developed). Weevils oviposit throughout the acorn growing period, so the acorns attacked later are larger when they are abscised (Bonal and Muñoz 2007, 2008; Bonal et al. 2010). To check the temporal changes in acorn size, we weighed ten sound acorns from each tree to the nearest 0.001 g every 15 days. The mass of the infested acorns was estimated from their linear dimensions (to the nearest 0.01 mm) using the formula $M_{ac} = 5.6 \times 10^{-4} L W^2 - 7.13 \times 10^{-8} L^2 W$, where M_{ac} is the acorn mass (in grams) and L and W the length and the width (in mm) of the acorns, respectively. This method is very accurate and explains 97% of the total variance (see Bonal et al. 2007

for a detailed description), and avoids errors from variations in the mass of infested acorns due to the consumption of cotyledon by larvae. At locality B, we sampled infested acorns all at once in early October. Our main interest in this place was to assess acorn use by the different *Curculio* species according to acorn size and to compare it with locality A. Infested acorn size was estimated using the same method based on linear measures above.

To monitor larval emergence, infested acorns were placed individually in open plastic vials and checked every day for emerging larvae. We collected larvae from both localities and preserved them in 100% ethanol for molecular analysis. At locality A, in addition, all larvae were carefully measured to assess the effect of body size on survival. We used head capsule width at the end of the last instar as an estimate of body size, which was measured to the nearest 0.01 mm under a microscope. The head capsule is the only strongly chitinized part of the larva. Its width can be measured easily and is strongly correlated with body size (see Barthell and Baird 2004; Cressa et al. 2008; Etile and Despland 2008). We chose this measure because, unlike body mass, it is not susceptible to temporal change after larval death. Two months after the larvae stopped emerging, we opened all acorns to assess the degree to which larvae had depleted the cotyledons, and to check for any dead larvae that had failed to emerge from the acorn. Each time we revisited the seed traps we also collected adult weevils by gently shaking tree branches to confirm the presence of the different species and to assess their phenology. All adults were sexed and measured (elytra and rostrum) to the nearest 0.001 mm; elytra length was used as a measure of body size. Once measured, all individuals were preserved in 100% ethanol for molecular analysis.

Laboratory methods and genetic analyses

We extracted larval DNA and sequenced a fragment (826 bp) of the mitochondrial cytochrome oxidase subunit 1 (*cox1*) using the primers Pat and Jerry following methods described previously (Hughes and Vogler 2004b). Sequencing allowed the identification of both live and dead larvae and was performed using Big-Dye (Perkin-Elmer) technology and an ABI3700 sequencer. Forward and reverse strands were edited using Sequencher 4.1 (Gene Codes, Ann Harbour, MI, USA).

Species were identified against a set of reference sequences of Holarctic *Curculio* (Hughes and Vogler 2004b). This study contained phylogenetic trees based on four genes coding for nuclear elongation factor 1-alpha and phosphoglycerate mutase and the mitochondrial *cox1* and *cob*, which were used as backbone constraints in tree searches (Fig. 1). All trees were obtained under the parsimony criterion using PAUP 4.0 (Swofford 2001),

performing heuristic searches with 1,000 random addition replicates and tree bisection reconnection (TBR). Support values were obtained from bootstrap analysis with 100 replicates. Phylograms were used to represent the number of character changes.

Statistical analyses

We performed ANOVAs to compare the body size (elytra length) and rostrum length between the adult females of *C. elephas* and *C. glandium*. The same analysis was used to compare head capsule width between the larvae of both species. To detect any interspecific difference in the scaling relationship between adult rostrum length and body size, we compared females of both species, adapting the methodology used by Toju and Sota (2006a) for intraspecific population analyses. We carried out these analyses only on females, as their rostrums are longer, used for drilling oviposition holes, and thus susceptible to a stronger selection pressure. Allometry was assessed using the function $y = ax^b$, where x and y correspond to body and rostrum length, respectively. This was log-transformed to give $y = \log a + b \log x$ and the slope of b was calculated to assess the allometric relationship between body size and rostrum length. We then performed an ANCOVA in which log-transformed rostrum length was the dependent variable, the species was the independent factor, and log-transformed body size was the covariate. We assessed whether the allometric relationship between rostrum length and body size (the slope of the function) differed between species and whether the species membership had any independent effect on rostrum length.

To analyze the interspecific niche segregation between *C. elephas* and *C. glandium* (over acorn size, *Quercus* spp.,

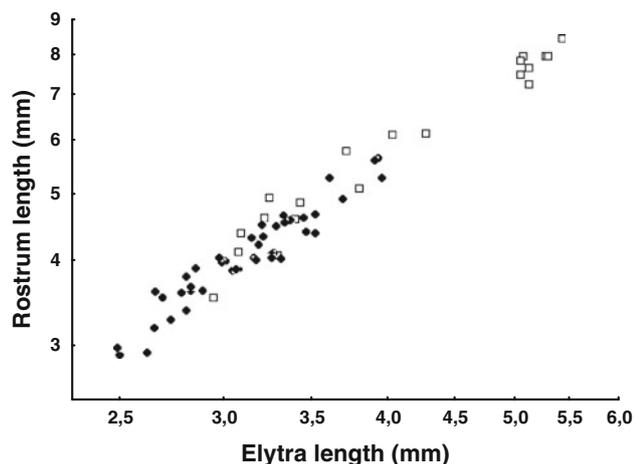


Fig. 1 Relationship between elytra length and rostrum length in females of *C. elephas* (empty squares) and *C. glandium* (filled circles). The scaling on both axes is logarithmic

phenology) we used generalized linear models (GLZ) for a dependent variable with a binomial distribution (i.e., the presence of one species or the other) and a logit link function. The independent variable was acorn size. The same type of GLZ was performed to test the effects of larval size and species membership (independent variables) on larval survival (binomial dependent variable).

We performed analyses of the molecular variance (AMOVAs) using ARLEQUIN software (Excoffier et al. 2005) to assess intraspecific genetic differentiation among the two localities or among individuals exploiting acorns from different *Quercus* species. These analyses were performed for both *C. elephas* and *C. glandium*, and partitioned the total genetic variance among the different levels of the factors locality (A and B) or *Quercus* spp. (*Q. ilex* and *Q. humilis*).

Results

The test for the allometric relationship between female body size and rostrum length revealed that the slope (b) of the common function for both species was larger than unity ($b = 1.30$; 95% CI: 1.23–1.37; $df = 61$; $t = 38.17$; $P < 0.001$). This slope did not differ between *C. elephas* and *C. glandium*. The ANCOVA confirmed the strong positive effect of body size on rostrum length ($F_{1,59} = 833.45$; $P < 0.001$; Fig. 1). Conversely, neither the factor species nor its interaction with body size were significant (respectively, $F_{1,59} = 1.15$; $P = 0.28$ and $F_{1,59} = 0.54$; $P = 0.56$; Fig. 1), indicating that rostrum length is determined by body size irrespective of species membership, and that the allometric relationship of body size/rostrum length does not differ between these two beetle species. Hence, the greater average rostrum length of *C. elephas* females compared to *C. glandium* ($6.04 \text{ mm} \pm 0.23\text{SE}$ vs. $4.10 \text{ mm} \pm 0.23 \text{ SE}$; $F_{1,61} = 45.75$; $P < 0.001$) is basically a consequence of the larger body size of the former ($4.14 \text{ mm} \pm 0.13\text{SE}$ vs. $3.14 \text{ mm} \pm 0.09\text{SE}$ in *C. glandium*; $F_{1,61} = 13.78$; $P < 0.001$) rather than a disproportionate development.

DNA sequences were obtained for 134 larvae from locality A and 160 larvae from locality B. The *cox1* sequences obtained from larvae grouped into two separate clusters that also included sequences of previously identified adults of *C. glandium* and *C. elephas* collected in northern France (Hughes and Vogler 2004b) (Fig. 2). Bootstrap analysis showed that both clusters had high support (BS = 100), confirming the unequivocal grouping of these sequences with the previously identified species. Mapping the branch length of *cox1* sequences on the backbone constraint tree of Hughes and Vogler (2004a) from four genes confirmed that both groups (*C. elephas* and *C. glandium* clusters) are distantly related to each other and

other species on the tree (Fig. 2). The number of distinct haplotypes was similar in both species (18 vs. 17 haplotypes), but genetic diversity was higher in *C. glandium*, with a mean number of pairwise differences of 6.51 and 4.34 among any two haplotypes at localities A and B, and intragroup nucleotide diversity $\pi = 0.005$ and 0.008, respectively. In *C. elephas*, these values were lower in both localities (mean number of pairwise differences in localities A and B were 1.26 and 0.91, respectively; intragroup nucleotide diversity $\pi = 0.0016$ and 0.0010).

In locality A, all emerging larvae were identified to the species level by means of mtDNA and then measured. Head capsule width was significantly smaller in *C. glandium* (mean \pm SE; 1.37 mm \pm 0.4 vs. 1.65 mm \pm 0.45 in *C. elephas*; ANOVA $F_{1,132} = 90.17$; $P < 0.0001$), although there was a certain degree of overlap. The interspecific size difference was not merely a consequence of limited food availability, as these differences held up when comparing only those individuals that had grown ad libitum (i.e., they left cotyledon uneaten after finishing their development). As expected, head capsules were larger in larvae that had grown unconstrained, but interspecific differences remained significant (1.47 mm \pm 0.65 SE in *C. glandium* vs. 1.67 mm \pm 0.6 SE in *C. elephas*; ANOVA $F_{1,34} = 17.65$; $P < 0.0001$). The patterns of acorn size choice did not differ between localities. In both places, *C. glandium* (the smaller species) occupied a wider range of acorns (from the smallest to the largest ones), whereas *C. elephas* was restricted to those acorns with >0.4 g dry mass in either locality (GLZ, $df = 1$; Wald statistic = 3.60; $P = 0.03$; Fig. 3).

Larval survival was higher in *C. elephas* (82 vs. 63% in *C. glandium*). However, this difference was due to the larger size of *C. elephas* rather than to species membership. In fact, larval size had a very strong positive effect on larval survival likelihood (GLZ, $df = 1$; Wald statistic = 14.62; $P < 0.001$; Fig. 4), whereas there was no pure effect of species membership (GLZ, $df = 1$; Wald statistic = 0.31; $P = 0.57$). Indeed, survival increased similarly for larger larval size in both species (interaction larval size \times species membership not significant; GLZ, $df = 1$; Wald statistic = 0.10; $P = 0.74$; Fig. 4).

There was no apparent host preference for either *Q. ilex* or *Q. humilis*, and interspecific segregation over acorn sizes did not differ between locality A (mixed *Q. ilex* and *Q. humilis* forest) and locality B (monospecific *Q. ilex* forest) (GLZ; $df = 1$; Wald statistic = 0.62; $P = 0.42$; Fig. 4). At locality A, *C. elephas* larvae were found in *Q. ilex* acorns less frequently than in *Q. humilis* (29 vs. 48%), but this correlates with acorn size, not preference of host species. In fact, at locality B, where only *Q. ilex* is present, there was a trend for *C. elephas* to be more abundant than at locality A (52 vs. 35% of all specimens; $df = 1$; Wald

statistic = 3.65; $P = 0.057$). Moreover, at locality A (both host species present), the probability of host use by one species or the other depended on the acorn size (GLZ, $df = 1$; Wald statistic = 4.55; $P = 0.03$), irrespective of the oak species ($df = 1$; Wald statistic = 0.29; $P = 0.58$). This confirms that the low frequency of *C. elephas* on *Q. ilex* at locality A was due to the small acorn size of this species at that site.

The molecular analyses showed no genetic differentiation among the localities, neither in *C. elephas* nor in *C. glandium*. Although some haplotypes were unique to each locality, the most common ones were shared and present in similar proportions in both. The AMOVA showed that genetic variation among localities accounted for $<1\%$ of the total variance in either *C. elephas* or *C. glandium* (see Table 1; $P > 0.25$ in both cases). With respect to a potential genetic differentiation within either species between those individuals exploiting acorns from different *Quercus* species at locality A, the AMOVA was not significant in all cases (Table 1; $P > 0.65$ in both cases). This makes it very improbable that there is a potential hidden intraspecific subdivision according to host (oak) species.

Regarding any potential confounding effect of reproductive phenology and acorn temporal growth, we were able to confirm that the larger average acorn size exploited by *C. elephas* compared to *C. glandium* is independent of the collection date per se; i.e., temporal effects are only through the growth of acorns over the season (GLZ, Wald statistic = 3.01; $df = 1$; $P = 0.10$ and Wald statistic = 4.57; $df = 1$; $P = 0.03$ for the effects of the independent variables “date” and “weevil species membership,” respectively).

Discussion

Our results show no divergence in adult female allometry between these species of acorn feeding beetles. Rostrum length was found to be correlated with body size in both species, and *C. elephas* females were on average larger and their rostrums were longer than in *C. glandium* because of the correlation with body size. However, rostrum length per se was not critical for exploiting large acorns, as the small sized *C. glandium* females (with short rostrums) could perforate and oviposit into small and large acorns. Thus, the small size of *C. glandium* resulted in a wider trophic niche compared to *C. elephas*. However, there were also advantages associated with a large body size, which was strongly correlated with survival in both species, and therefore the larger average body size of *C. elephas* larvae resulted in lower death rates during development compared to *C. glandium*.

The slope of the allometric function relating body size and rostrum length was significantly larger than unity,



Fig. 2 Parsimony tree based on *cox1* showing the positions of haplotypes obtained from the larvae at the study area and published reference sequences. Sequences correspond to the distinct haplotypes, and terminals are labeled with the host (*Q. ilex*/*Q. humilis*) and collection locality (A or B). Bootstrap values are shown only for

nodes defining the relationships of *C. elephas* and *C. glandium* haplotypes; all other nodes were backbone-constrained in the tree search. The scale bar at the bottom indicates the number of changes on the branches

showing that rostrum length does not increase isometrically with body size. The slope value did not differ between *C. elephas* and *C. glandium* and was very similar to that calculated in different populations of *Curculio cameliae* (Toju and Sota 2006a). This shows that larger specimens have proportionally longer rostrums. The fact that the same slope is found in different species, independent of the type of host exploited, suggests a strongly conserved allometric rule within the taxa. This might be due to past phylogenetic constraints, as—at least in the case of acorn curculios—the longer rostrum would not provide any advantage to larger sized specimens. This finding differs from that of the study

performed on *C. cameliae*, which showed that specimens with the same body size in different populations exhibit rostrums of different lengths, whereas the rostrum lengths of *C. elephas* and *C. glandium* females of the same body size did not differ significantly. The type of host seed may explain such a pattern. Camelia seeds show a great regional variability in seed coat thickness, which triggers a disproportionate increase in rostrum length with respect to body size at localities with thick-coated seeds (Toju and Sota 2006a, b). In contrast, the outer coating of an acorn is usually not very thick and does not change greatly with acorn size, locality or oak species (J.M. Espelta and

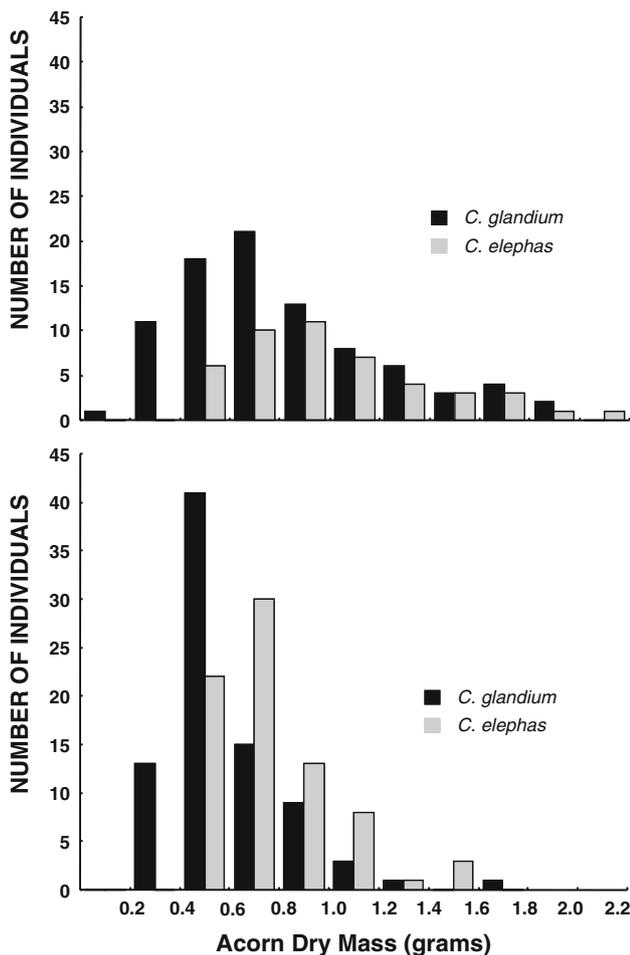


Fig. 3 Distributions of *C. elephas* (gray bars) and *C. glandium* (black bars) larvae over the range of acorn sizes in locality A (upper panel) and locality B (lower panel). The y-axis shows the number of individuals and the x-axis gives the dry acorn mass (g)

R. Bonal, unpublished results). As Toju and Sota (2009) have suggested, selective pressures acting on *Curculio* morphology may differ between groups exploiting different host plants.

A longer rostrum (and correlated body size) is thus not critical for attacking large acorns. In fact, rostrum length in *C. glandium* was significantly shorter than in *C. elephas*, but it was still sufficient to perforate and oviposit successfully into the largest acorns available. However, acorn size was critical for host selection by *C. elephas*, which rarely oviposited into small acorns. It was not possible to establish how this selection was made, but the size range required may be determined by mechanical limitations. Females need to hold firmly to the seed surface with the extended legs when drilling in a downward direction (the usual onset of the perforating routine; personal observation), and this could be hampered when the seed surface area is too small with respect to female size. On the other hand, it has been demonstrated that *C. elephas* larval size

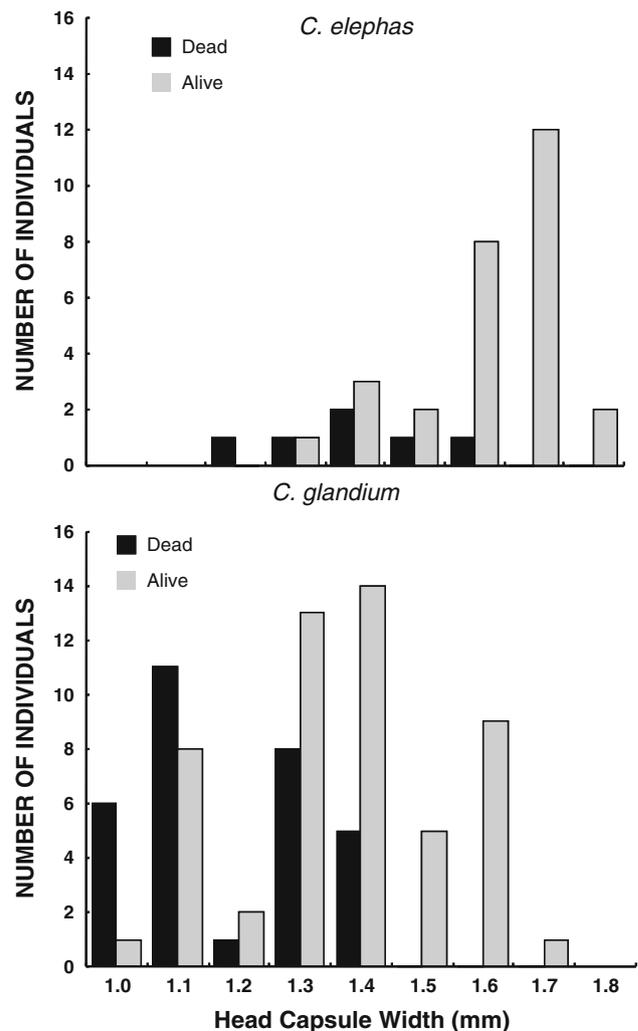


Fig. 4 Relationship between larval size and survival likelihood in locality A. The y-axis shows the number of larvae that finished developing (gray bars) and those that failed to do so (black bars) in *C. elephas* (upper panel) and *C. glandium* (lower panel). The x-axis indicates the size of the larvae (head capsule width in mm)

can be constrained by food availability when they consume the cotyledons completely (Bonal and Muñoz 2009), which may prevent *C. elephas* adult females from ovipositing into small acorns.

The dependence on seed size demonstrates that previous observations of *C. elephas* scarcity on *Q. ilex* (Espelta et al. 2009a) are explained by the small acorn size at some localities, rather than by a specificity for a certain oak species. At locality B, where only *Q. ilex* is present but larger-sized acorns are produced, *C. elephas* was common. Conversely, *C. glandium* uses acorns of any size at both sites, but with a preference for small acorns. Hence, the segregation between the two weevil species according to acorn size was similar whether in a monospecific *Q. ilex* or mixed *Q. ilex*/*Q. humilis* forest. This is consistent with the observed broad host ranges of *C. elephas* and *C. glandium*

Table 1 Results of the intraspecific AMOVA analyses showing the partition of the total genetic variance between localities and among *Quercus* species for both *C. elephas* and *C. glandium*

Source of variation	df	Sum of squares	Percentage of variation
<i>C. elephas</i>			
Among localities	1	0.56	0.23
Within localities	107	55.64	99.77
Among <i>Quercus</i> sp.			
Among <i>Quercus</i> sp.	1	0.34	0
Within <i>Quercus</i> sp.	107	54.86	100
<i>C. glandium</i>			
Among localities	1	2.94	0.10
Within localities	166	451.42	99.90
Among <i>Quercus</i> sp.			
Among <i>Quercus</i> sp.	1	0.36	0
Within <i>Quercus</i> sp.	166	454.00	100

development on various *Quercus* species elsewhere (Crawley and Long 1995, Branco et al. 2002). It is also unlikely that local host races have arisen, as no subdivision of genetic variation was evident between localities or with respect to host species in the mtDNA of both *Curculio*. The capacity to exploit small acorns also allows seasonally earlier host use by *C. glandium*, which provides preferential access to the resource. We have confirmed that the later use of acorns reported for *C. elephas* (Espelta et al. 2009a) occurred for no phenological reason other than the small size of acorns early in the season.

At this point, we may be left wondering what sort of selective pressures may have favored large-sized *Curculio*. In this context, it must be noted that the wider trophic niche and phenological advantages of small-sized species are not cost-free. Body size is tightly correlated with fecundity and reproductive success in many insect species (e.g., Engelmann 1984; Fox et al. 1996), which has also been confirmed in *Curculio* at the intraspecific level (Desouhant et al. 2000). Our results show that larval survival correlates with body size, irrespective of species membership. Consequently, the larger size of *C. elephas* larvae make them more successful at completing development compared to *C. glandium*.

The present work at the population level supports macroevolutionary studies proposing that fitness advantages can trigger body size increases together with the choice of a larger seed size (Hughes and Vogler 2004a). Larger body size increases the likelihood of larval survival, but larger larvae can only develop in acorns above a minimum size threshold. The longer rostrum of *C. elephas* would be a by-product of the selective pressures acting on body size, as rostrum length was proportional to body size irrespective of species membership. Moreover, a longer rostrum was not critical for the use of larger acorns, as the smaller *C. glandium* was able to exploit them as well as small acorns. Morphological variability can be maintained by a wider trophic niche and phenological advantages (access to earlier and smaller acorns) for small-sized individuals, in

spite of their lower survival likelihood. We must be cautious before inferring a role of these interactions in the speciation process, mainly because *C. elephas* and *C. glandium* are not sister species (see Fig. 2). Hence, morphological diversity could have an ancient origin due to ancestors with different life-histories. Conservation of life-history traits is not uncommon (e.g., the timing of life cycles in adephagan beetles: Sota 1994; Pearson and Vogler 2001), and determines niche width, geographic ranges and, ultimately, species interactions. In conclusion, opposing selection regimes for large and smaller body sizes could maintain morphological variability in *Curculio* and facilitate the co-existence of differently sized weevil species in local communities.

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