# Predispersal seed predation in leguminous species: seed morphology and bruchid distribution

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We measured and calculated seed morphological parameters [axonometric linear measures, weight, volume and minimum-maximum seed size ratio (SMIMA)] of 110 leguminous plant species occurring in Hungary. We collected seed samples, reared the predispersal seed predators from the samples, took morphological data from the bruchids (elytra length, body width, thorax length) and calculated the body size ratio (BSR). We found that SMIMA and BSR were fairly good predictors of bruchid infestation patterns at plant tribe, species and sample (locality) levels. Bruchid body length was much less variable than the parameters (e.g., volume) of seeds from which they were reared.

We also found that the closer the shape of the seeds of a leguminous plant species to a sphere, or the larger the seed volume, the higher the probability of bruchid infestation. Seed morphological traits (weight, volume and SMIMA) clearly separated the hosts of the genera *Bruchus* and *Bruchidius*. For the three related bruchid genera studied, phylogenetic and ecological effects (regarding seed shape preference) were also separated.

We concluded that the evolution of the seed size of plant species studied is not likely driven by bruchids or by other seed predators.

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Components of seed morphology such as size and shape can be of decisive importance for endophagous predispersal seed predators (en-PSPs) that develop inside seeds. For example, bruchids, which are generally stockily built, are not able to colonise very flat seeds, the smallest dimension of which leaves no space for an adult (Bridwell 1918, cited by Center and Johnson 1974, Janzen 1969).

Janzen (1969), studying seed size (seed weight) and seed predator relationship, found with woody Central American leguminous species that the seeds of species attacked by bruchids were significantly smaller than the seeds of non-attacked species. He assumed that bruchid-attacked plant species evolved towards smaller seed size that provided an escape from seed predation also through better dispersal, masting and predator satiation. However,

Ernst et al. (1989) found a distribution of seed weights of attacked or not-attacked seeds by bruchids opposite to Janzen's results. The idea of masting generated by seed predators was also doubted (Waller 1993). Therefore, the central question remains whether en-PSPs may select for decreasing seed size. We argue that en-PSPs are not likely to affect evolution of seed size. However, we provide data indicating that bruchid colonisation is strongly affected by seed morphological parameters.

Seed size is but one seed trait among many (Janzen 1969, 1971, Center and Johnson 1974) that limit access to and actual use of seeds. We consider these other traits, most notably chemicals, when discussing the relationships between seed size and bruchid infestation. Any morphological or chemical changes effective against a PSP should take effect before attack. Moreover, a precon-

dition for seed size selection is the ability of seed predators to show seed size preference; the evidence for this is scanty (Willson 1983). The situation is even more complicated, because temperate zone bruchids oviposit on pods mostly when the seeds are embryos. Thus, the size/shape of the pod does not provide information about the quality and size of seeds.

Bruchid species considered in this paper and regarded as en-PSPs may be divided into two groups. The first group comprises species of the genus *Bruchus* plus *Bruchidius fasciatus* Ol., and may be called "real" en-PSPs (ren-PSPs). Their body size is limited by seed dimensions, since the larvae develop to adults within a single seed. The second group, "quasi" en-PSPs (qen-PSPs), comprises the rest of the *Bruchidius* species, the larvae of which enter a seed, consume its content (frequently leaving testa untouched), but may move into another neighbouring seed and complete development within the pod. Thus, in such species, seed size does not limit body size. The latter feeding style is markedly different from ecto-PSPs (curculionids and lepidopterous larvae) where both testa and endosperm are removed.

Restricting our considerations on bruchids, we are seeking answers to the following questions:

1. whether seed morphology (size and shape) at plant tribe and species levels correlated with bruchid infestation patterns or were independent of them;

2. whether seed morphological parameters (size and shape) subdivided resources among bruchid taxa;

3. whether bruchid morphological (size) features corresponded with plant morphology data; and

4. what sort of evolutionary relationship could be detected between en-PSPs and seed size and shape?

## Materials and methods

### Collection and handling of samples

Seed samples of woody and herbaceous leguminous plant species have been collected over 10 yr in Hungary. Ripening or completely mature pods were picked without stem or other plant parts in order to avoid 'contamination' by organisms not living inside the pods. (Herbarium specimens of the plants were preserved for identification). Samples were first stored in the laboratory until emergence of bruchids and were finally evaluated only after one yr of storage. Several samples were obtained from various arboreta (see Acknowledgements).

### Bruchid infestation records

Bruchids were identified by professional taxonomists. Besides our own collection and rearing records, information on host plants of bruchids were compiled from the literature or were provided by expert curators of national collections (Hoffmann 1945, Lukjanovitch and Ter-Minasjan 1957, Kaszab 1970, Wendt 1981, Strejček 1990, Merkl pers. comm., Podlussány pers. comm.).

### Morphological measurements and calculations

The seed size of 110 leguminous species [ca 50% of the species listed for Hungary (Soó and Kárpáti 1968)] was measured. Only data of native or naturalised species (such as *Gleditsia*, *Robinia*, *Amorpha*, *Cladrastis*, *Sophora*, *Cercis*, etc.) in Hungary were used for the analyses.

Three linear dimensions of the seeds were measured. Seeds were oriented uniformly for measurement with hilum up and the lens (strophiole) to the right. Then the vertical and horizontal measures were taken, followed by the third measure perpendicular to the first two directions. The linear size parameters were determined by a microscope ocular meter or a mechanical calliper with 0.01-mm accuracy. Only visibly mature and intact seeds were measured, randomly chosen from the sample. In most cases 35 seeds per plant species were measured. (The measurements of seeds infested with bruchids gave inaccurate readings because of the presence of exit holes. Therefore, these data were not used).

The mass of the same seeds was also measured on an electronic balance with 0.1-mg accuracy. We did not want to kill the seeds by drying them in an oven in the hope of using them for further purposes, therefore they were only air-dried. These contained ca 8% water.

To determine seed volume, the three respective axonometric projections of one randomly chosen seed from the 35 seeds measured above were enlarged and drawn on paper. The three figures were picked up by a video camera and fed into a computer program to calculate volume. The volumes of seeds in the rest of the sample (5–34 seeds) were calculated similarly by substituting the actual linear dimensions.

We calculated also the minimum-maximum seed size ratio (SMIMA) by dividing the smallest linear dimension by the largest one for each seed measured. As a value between 0 and 1, it described the shape of the seed: the closer SMIMA was to 1, the more spherical was the seed.

The resulting matrix ($6 \times 3625$ data) was further coded for presence/absence of bruchids per plant species, their taxonomic affiliation, etc. Similarly, seed size parameters were coded for plant tribes, genera and species affiliations according to the systematics of the Leguminosae (Polhill and Raven 1981).

We measured the following body size parameters with bruchids: length of one elytron, the largest width of elytra, and length of thorax. The sum of elytra and thorax lengths was considered as the total body length. The bruchids were measured as dry specimens under a binocular microscope with ocular micrometer. We measured all available specimens from all infested samples. Sample sizes varied accordingly: 9 to 376 at species level, and 1 to 140 at sample level.

The matrix ($5 \times 1726$ data) of body parameters was further coded for taxonomic affiliations, etc., and the body size ratio (BSR), i.e. elytra width/body length, was also calculated. Body size parameters were means of specimens originating from several samples.

Table 1. Mean (SE) seed size parameters of plant species by tribes they belong to without regard to bruchid infestation.

| Plant tribe[1] | SMIMA[2] | | Volume | | Weight | | N | NP[3] | NB[4] |
|---|---|---|---|---|---|---|---|---|---|
| Caesalpinieae | 0.4019 | (0.006)f | 48.56 | (3.79) | 72.03 | (5.84) | 70 | 2 | 0 |
| Sophoreae | 0.4048 | (0.008)f | 43.53 | (1.94) | 60.88 | (2.35) | 60 | 2 | 0 |
| Tephrosieae | 0.2297 | (0.003)h | 385.71 | (16.92) | 438.96 | (19.57) | 35 | 1 | 0 |
| Robinieae | 0.3413 | (0.006)g | 15.46 | (0.40) | 22.75 | (0.63) | 70 | 2 | 3 |
| Phaseoleae | 0.5130 | (0.012)d | 286.47 | (21.94) | 348.89 | (24.72) | 175 | 5 | 1 |
| Amorpheae | 0.2934 | (0.006)g | 3.11 | (0.09) | 4.03 | (0.14) | 35 | 1 | 1 |
| Galegeae | 0.4691 | (0.007)e | 6.45 | (0.40) | 8.78 | (0.52) | 261 | 8 | 4 |
| Hedysareae | 0.4970 | (0.009)e | 8.27 | (0.29) | 8.82 | (0.50) | 70 | 2 | 1 |
| Loteae | 0.6785 | (0.007)b | 1.22 | (0.05) | 2.23 | (0.07) | 280 | 8 | 6 |
| Coronilleae | 0.2940 | (0.003)g | 2.93 | (0.10) | 4.81 | (0.14) | 162 | 5 | 5 |
| Vicieae | 0.7522 | (0.005)a | 53.59 | (4.18) | 72.80 | (5.45) | 901 | 29 | 17 |
| Cicereae | 0.6429 | (0.004)b | 163.59 | (3.87) | 189.65 | (4.35) | 35 | 1 | 1 |
| Trifolieae | 0.5221 | (0.004)c | 1.85 | (0.07) | 2.54 | (0.10) | 1037 | 31 | 4 |
| Genisteae | 0.4904 | (0.005)e | 8.21 | (0.30) | 11.89 | (0.44) | 434 | 13 | 7 |

[1] After Polhill and Raven (1981); [2] SMIMA = minimum-maximum seed size ratio; [3] NP = Number of plant species considered in this study; [4] NB = Number of bruchid species present in the tribe by collection and literature data within the geographic area studied; Means followed by the same letters are not significantly different at the $p = 0.01$ level (LSD-test; data were not homogeneous by Kolmogorov-Smirnov tests, but ANOVAs were significant).

## Data analyses

The following statistical and spreadsheet program packages were used: BIOSTAT, SPSS, CSS and QPRO. For selecting the most important seed and body size parameters in reference to the absence/presence of bruchid infestation, as well as separating the two bruchid genera, discriminant function analyses were used. Grouping of plant species according to similarity in seed shape was done by hierarchical, agglomerative, complete linkage cluster analysis using squared Euclidean distances. Other procedures are mentioned in the Results and in the footnotes of the tables. Data were first analysed for homogeneity by Kolmogorov-Smirnov's or Bartlett's tests, then submitted to ANOVA. If homogeneity conditions were not met, transformations were applied. If the subsequent ANOVA did not provide a significant F-value, it was noted in the table.

Phylogenetic effects (relatedness of bruchid genera) confound ecological traits (e.g., adaptation to a plant trait) considered as adaptive in case of closely related taxa (Gittleman 1985, Pagel and Harvey 1988, Brooks and McLennan 1991). In our case, the separation of the

Table 2. Body size parameters of bruchid species.

| Species | PSP type | BSR[1] | (SE) | Body length (mm) | (SE) | N |
|---|---|---|---|---|---|---|
| *Bruchus*[2] | | | | | | |
| *luteicornis* Illig. | ren | 0.6341 | (0.001)b | 2.69 | (0.01)f | 376 |
| *venustus* Fahrs. | ren | 0.6378 | (0.001)a | 2.85 | (0.01)e | 225 |
| *affinis* Fröl. | ren | 0.6230 | (0.002)c | 3.46 | (0.01)b | 115 |
| *atomarius* L. | ren | 0.6207 | (0.001)c | 3.12 | (0.02)c | 161 |
| *pisorum* L. | ren | 0.6017 | (0.001)d | 4.12 | (0.01)a | 31 |
| *rufipes* Herbst | ren | 0.6373 | (0.003)b | 3.03 | (0.02)d | 30 |
| Mean | | 0.6281 | (0.001) | | | |
| *Bruchidius*[2] | | | | | | |
| *marginalis* L. | qen | 0.5700 | (0.001)b | 2.75 | (0.01)b | 144 |
| *fasciatus* Ol. | ren | 0.6442 | (0.001)a | 2.94 | (0.02)a | 145 |
| *pusillus* Germ. | qen | 0.6533 | (0.003)b | 2.02 | (0.02)d | 55 |
| *varius* Ol. | qen | 0.5540 | (0.003)c | 2.44 | (0.03)c | 53 |
| *pauper* Boh. | qen | 0.5500 | (0.007)cd | 2.03 | (0.05)d | 9 |
| Mean | | 0.5931 | (0.002) | | | |
| *Acanthoscelides*[3] | | | | | | |
| *pallidipennis* Mots. | ren | 0.6005 | (0.001)a | 1.83 | (0.01)b | 129 |
| *obtectus* (Say) | ren | 0.6044 | (0.002)a | 2.73 | (0.02)a | 120 |
| Mean | | 0.6025 | (0.001) | | | |

[1] BSR = body size ratio (elytra width/body length); [2] LSD-tests preceded by ANOVAs that were significant; [3] t-test ($t = 39.43$, $df = 180.6$, 2-tailed $p < 0.001$). Means followed by the same letters within the same genus and in the same column are not significantly different at the $p = 0.01$ level. "ren" and "qen" = real and quasi endophagous predispersal seed predator, respectively.

Table 3. Body length variability of *Bruchus* species between host species and between localities (in samples).

| Bruchid and host plant species | Body length (mm) | (SE) | N |
|---|---|---|---|
| *Bruchus affinis* | | | |
| By plant species | | | |
| *Vicia grandiflora* | 3.62a | (0.01) | 5 |
| *Lathyrus latifolius* | 3.54ac | (0.01) | 70 |
| *L. silvestris* | 3.40bc | (0.06) | 4 |
| *L. tuberosus* | 3.31b | (0.03) | 36 |
| *Bruchus atomarius* | | | |
| By plant species | | | |
| *Lathyrus venetus* | 3.23a | (0.04) | 30 |
| *L. vernus* | 3.09b | (0.02) | 109 |
| *Bruchus venustus* | | | |
| By samples | Body length (SE) ranges | | |
| *Vicia tenuifolia* | 2.95 (0.03)a–2.36 (0.15)b | | 9–3 |

Means followed by the same letter in the same column within a *Bruchus* species are not significantly different at the p=0.01 level (ANOVAs followed by LSD-tests).

genera *Bruchus, Bruchidius* and *Acanthoscelides* according to size and shape of the host plant seeds was necessary because data from these related taxa were not independent. For this purpose first, a cluster analysis (see above) was done on SMIMA and seed width, resp., of only those plant species from which bruchids were reared (34 spp.). Seed shape groups, numbered 1–6, were obtained (see a similar presentation in Fig. 1). Bruchid body size parameters (BSR or body width) corresponding to seed shapes were then arranged for ANOVA to determine whether relatedness of bruchid genera or seed shape influence distribution among plant species. Because of missing values in some cells (Table 7), 2-way ANOVAs could not be performed. Therefore, only a series of one-way analyses was done. Finally, we also tested a null-hypothesis assuming that bruchids belonged to seed shapes by chance. One-way ANOVA analyses were repeated with three separate sets of randomised seed shape data.

## Results

### Seed size dimensions
Seed size parameters of leguminous tribes are shown in Table 1. There were significant differences between tribes concerning SMIMA: Vicieae, Loteae and Cicereae had values greater than 0.6 indicating a shape approaching a sphere. (Data for the 110 species can be provided on request).

### Bruchid size relations
Fourteen bruchid species were reared from the seed samples. Body size ratios (BSR) and body lengths differed significantly among bruchid species (Table 2). The dif-

ferences between the largest and smallest means were 7–15% for BSR, and 31–35% for body length within the genera. The BSR data show that *Bruchidius fasciatus* is more similar to *Bruchus* spp. than to the other *Bruchidius* spp.

A comparison of BSR by host plant species showed small, although in some cases statistically significant differences. For example, there were significant differences among BSR values with *Bruchus affinis* Fröl. reared from *Lathyrus latifolius* L.: 0.6274 (0.002) and from *L. tuberosus* L.: 0.6158 (0.003) (means, SE; ANOVA followed by LSD-tests; N=70 and 36, resp., p=0.01), although the difference is only 1.85% of the larger mean.

A similar comparison of body parameters by samples (i.e., among samples of a particular plant species collected at different localities and at different dates) revealed that from 17 samples the largest and smallest mean (SE) BSR values for *Bruchus affinis* were: 0.6375 (0.003) vs 0.6045 (0.003) (ANOVA was not significant, LSD-test was, p=0.01). Among *Bruchidius* spp. only *Bruchidius pusillus* Germ. had significant differences among BSR from 16 samples: 0.5980 (0.02), the largest, vs 0.5467 (0.05), the smallest (ANOVA was not significant, but LSD-test was, p=0.01). The same slight plasticity was noted with body length at host-plant species level and with samples with the two bruchid genera (Tables 3 and 4).

BSR produced the largest (0.6498) correlation coefficient between the discriminating variables (body length, body width, elytra length, thorax length and BSR) (discriminant function analysis using a pooled within-group correlation matrix) and gave the best separation between the genera *Bruchus* and *Bruchidius*. However, this variable is not independent of the others listed, as it was calculated from these, nor were the data behind the variables normally distributed even after transformation. The *Bruchus* and *Bruchidius* genera, as a whole, significantly differed by the mean BSR (SE) values: 0.6281 (0.001) and 0.5931 (0.002), respectively (t-test, t=15.85, df= 477.25, 2-tailed p<0.001), although the difference is only 5.6% of the larger mean.

Table 4. Body length variability of *Bruchidius* species between host plant species and between localities (in samples).

| Bruchid and host plant species | Body length (mm) | (SE) | N |
|---|---|---|---|
| *Bruchus fasciatus* | | | |
| By plant species | | | |
| *Laburnum agagyroides* | 3.02a[1] | (0.02) | 126 |
| *Cytisus scoparius* | 2.39b | (0.05) | 17 |
| By samples | Body length (SE) ranges | | |
| *B. fasciatus* in | | | |
| *Cytisus scoparius* | 2.22 (0.07)–2.53 (0.07) | | 6–6 |
| *B. varius* in | | | |
| *Trifolium alpestre* | 2.33 (0.11)–2.47 (0.04) | | 5–23 |

[1] t-test (t=13.07, df=141, 2-tailed p<0.001).

Table 5. Characters of seeds of plant species infested or noninfested with bruchid species. ("Infested" includes seeds inhabited by *Bruchus*, *Bruchidius* and *Acanthoscelides*).

| Seed parameters | In-fested | (N = 59) | Non-infested | (N = 51) |
|---|---|---|---|---|
| Mean (SE) SMIMA | 0.60 | (0.04)a | 0.52 | (0.04)b |
| Mean (SE) volume of seeds (mm³) | 49.95 | (3.25)a | 21.34 | (1.55)b |
| Mean (SE) weight of seeds (mg) | 64.13 | (3.95)a | 27.34 | (1.82)b |

Small letters denote significant differences in the same row (t-tests, $p < 0.001$). Means were calculated with 1666 (noninfested) and 1959 (infested) data, resp. (Variances were not equal and homogeneous for volume and weight).
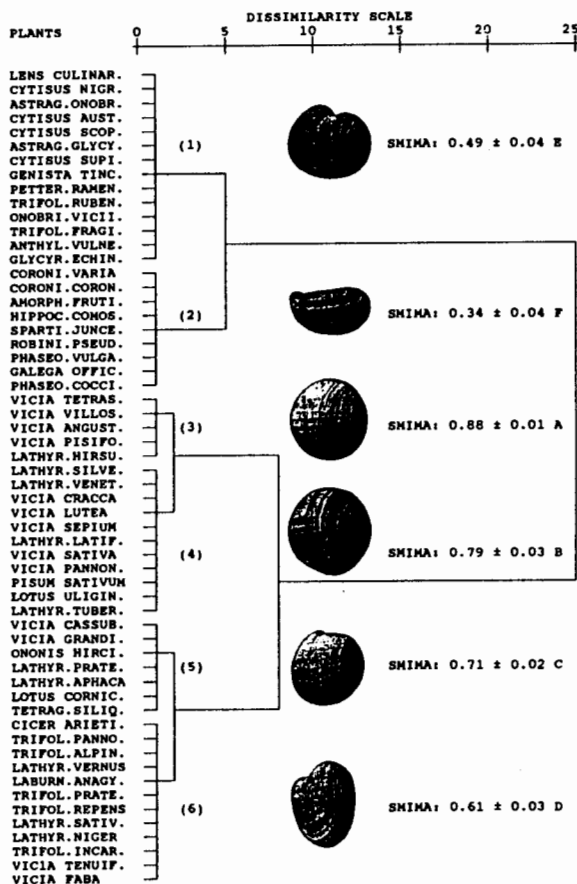


Fig. 1. Classification of leguminous plant species infested with bruchid predispersal seed predators, by hierarchical, agglomerative, complete linkage cluster analysis using squared Euclidean distances. Means (SD) of SMIMA signed with the same letters are not significantly different at the $p = 0.01$ level. Comparisons were done by LSD-test after homogeneity testing and ANOVA. Seed shapes: 1 = flattened; 2 = elongated and to some extent cylindrical; 3 = close to perfect sphere; 4 = spherical; 5 = spherical, but also somewhat elongated; 6 = with some irregularities in shape.

## Colonisation pattern of bruchids

The correlation between SMIMA and BSR was low: 0.3771 (all data combined). A relatively high correlation was found between the smallest seed measure (usually "width") and bruchid body width (0.6854). (This is why this parameter was also used in subsequent calculations besides BSR).

We found that it was the smallest linear seed parameter that gave the largest correlation coefficient (0.7936, discriminant function analysis) along which infested and bruchid-free plant species could be separated. However, in the following we used SMIMA, because it directly referred to seed shape.

All seed parameters were significantly larger for bruchid-infested species than for noninfested species (Table 5).

Based on seed shape (SMIMA), six clusters were formed (Fig. 1) in bruchid-infested species that corresponded to six shapes: (1) flattened, (2) elongated and to some extent cylindrical, but still flattened, (3) close to a perfect sphere, (4) spherical, (5) spherical, but also somewhat elongated, and (6) with some irregularities in shape. There were significant differences among clusters in mean (SD) SMIMA, both with infested and noninfested species (LSD-test, $p = 0.01$, Fig. 1); not, however, in volume or weight. The cluster analysis of noninfested species gave an identical pattern of shapes.

The total number of plant species used by the two genera *Bruchus* and *Bruchidius* is almost identical with relatively little overlap (Table 6). *Bruchus* spp. are present in 9 plant genera, while *Bruchidius* spp. are present in 20 genera. This significant heterogeneity in host use at genus-level is also demonstrated by Shannon's H' (Table 6). On the average, *Bruchidius* spp. use 12–13 times smaller seeds by volume or weight reflecting the fact that 4 of the 5 species studied are qen-PSPs. Also, SMIMA values substantially differ, while legume species not utilised by bruchids are in between the two.

For the same reason, the host-use of the two genera by seed shape also differs. *Bruchus* spp. use, on average, more spherical seeds; while *Bruchidius* spp. can colonise less spherical ones. This is indicated by the *Bruchus/Bruchidius* ratios in the six seed shape clusters of Fig. 1: (1) 1:6, (2) 1:5, (3) 5:1, (4) 3:1, (5) 2:1, and (6) 1:1. Three plant species infested with *Acanthoscelides* spp. occur in cluster (2).

A comparison of mean seed volume and smallest linear seed measure (Fig. 2) indicated that the range of *Bruchus*-infested plant species is considerably wider than that of the *Bruchidius*-infested ones, and they overlap with noninfested plants.

Within the genus *Bruchus*, mean body width was significantly distributed among seed shapes (Table 7). Thus, ecological factors seemed to be more important in explaining the distribution patterns of this bruchid genus among plant species. The null-hypothesis that bruchid species (by body widths or BSR) can randomly belong to any seed shape, was rejected in 5 from 6 runs, and only

Table 6. Seed characteristics and host-plant spectrum differences among *Bruchus* and *Bruchidius* genera.

| Traits | *Bruchus* | *Bruchidius* |
|---|---|---|
| No. bruchid species[1] | 17 | 18 |
| No. of plant tribes used | 7 | 7 |
| No. of genera used | 9 | 20 |
| No. of plant species used | 31 | 34 |
| Shannon's H' for genera[2] | 1.5680a | 2.7299b |
| Overlap in species (genera) | 7 (6) | |
| Mean (SE) SMIMA | 0.7283 (0.005)a | 0.4945 (0.005)b |
| and (N) | (811) | (864) |
| SMIMA of plants not infested by bruchids[3] (N) | 0.5158 | (0.004) c (1600) |
| Mean (SE) volume of seeds | 61.01(4.67)a | 4.16 (0.14)b |
| used (mg) and (N)[4] | (805) | (839) |
| Mean (SE) weight of seeds | 81.79(6.02)a | 6.03 (0.21)b |
| used (mg) and (N)[5] | (805) | (839) |

[1] Based on collection and literature data; [2] t-test: $t = 6.4822$, df = 32.9, $p < 0.001$; [3] t-tests, a–b: $t = 33.04$, df = 1600, $p < 0.001$; a–c: $t = 31.75$, df = 1808, $p < 0.001$; b–c: $t = 3.45$, df = 2133, $p = 0.001$; [4] t-test: $t = 12.85$, df = 1642, $p < 0.001$; [5] t-test: $t = 12.84$, df = 1642, $p < 0.001$.

one randomisation produced a close enough series resembling the distribution observed in nature (ANOVA was not significant on BSR: $F = 1.378$, df = 4, $p = 0.2856$; LSD test was significant between seed shapes 3 and 4, $p = 0.05$).

## Discussion

### Seed morphology and bruchid colonisation

As expected, the intertribal variability of seed size (volume, weight) is very large in leguminous plants. For instance, mean (SE) seed volume varies between $1.22 \pm 0.05$ and $286.5 \pm 21.94$ mm³ among tribes attacked by bruchids (Table 1), i.e. the ratio is 1:235, while the size of bruchid species is much less variable, e.g. body length varies between $1.83 \pm 0.01$ and $4.12 \pm 0.01$ mm corresponding to a ratio of 1:2.25 (Table 2).

The intraspecific variability of body size in bruchids emerging from different host plant species and/or samples turned out to be very small. Body length was most variable with *Bruchidius fasciatus* between adults from *Laburnum anagyroides* Medic. and *Cytisus scoparius* (L.) Wimm.: $3.02 \pm 0.02$ and $2.39 \pm 0.05$ mm, respectively, i.e. a ratio of only 1:1.26. In all other instances body length was even less variable (Tables 3 and 4).

Interspecific variation in body width (variance expressed as percentage of mean) showed a tight interval for the bruchids (*Bruchus* spp. 4.05%, *Bruchidius* spp. 5.97%), whereas seed width of infested plant species varied much more (*Bruchus*-inhabited spp. 62.93%, *Bruchidius*-inhabited spp. 17.42%). These data indicate that bruchid body size is quite a constant trait. Thus, even if opportunity is provided to develop in a larger seed, a bruchid would not grow larger, although, in theory *Bruchus* spp. could prefer plants with larger seeds because of

possible advantages of larger body size within genetically determined limits. Nelson and Johnson (1983, cited in Johnson 1990) found that larger seeds of an *Astragalus* species were more successful in germination, but at the same time were also more infested by bruchids than smaller ones.

It is self-evident that especially ren-PSP bruchids can colonise leguminous species only whose seeds measure above a certain volume limit. Indeed, as shown in Table 5, mean volume and mean weight of seeds were significantly smaller in uninfested leguminous species than in bruchid-infested ones. From this it could be speculated that small seed size evolved as an escape from bruchid selection pressure.(But see discussion in "Evolutionary considerations".) The crucial body size parameters of ren-PSP bruchid species is body width as indicated by the correlation found with the smallest linear seed parameter ($r = 0.5513$, $t = 3.304$, $n = 27$, $p = 0.003$). However, in the case of bruchids belonging to the quasi-endophagous group, such a limitation does not exist (Center and Johnson 1974) or another type of body size limitation occurs as in *Acanthoscelides alboscutellatus* (Ott and Lampo 1991).

On the other hand, oviposition behaviour of temperate region bruchids excludes any direct seed size preference of the females, because egg-laying takes place on the green pods containing developing embryos only. Furthermore, young pods were frequently found overburdened with bruchid eggs regardless of the number and size of embryos (Labeyrie 1991, Szentesi unpubl.).

Seed shape may also be a limiting factor for endophagous bruchid colonisation (Bridwell 1918, cited in Center and Johnson 1974). As could be expected by the life style differences the SMIMA ratio segregated the host plants of the genera *Bruchus* and *Bruchidius*, too. In general, the host plant species of bruchids had significantly more spherical seeds than the non-host species
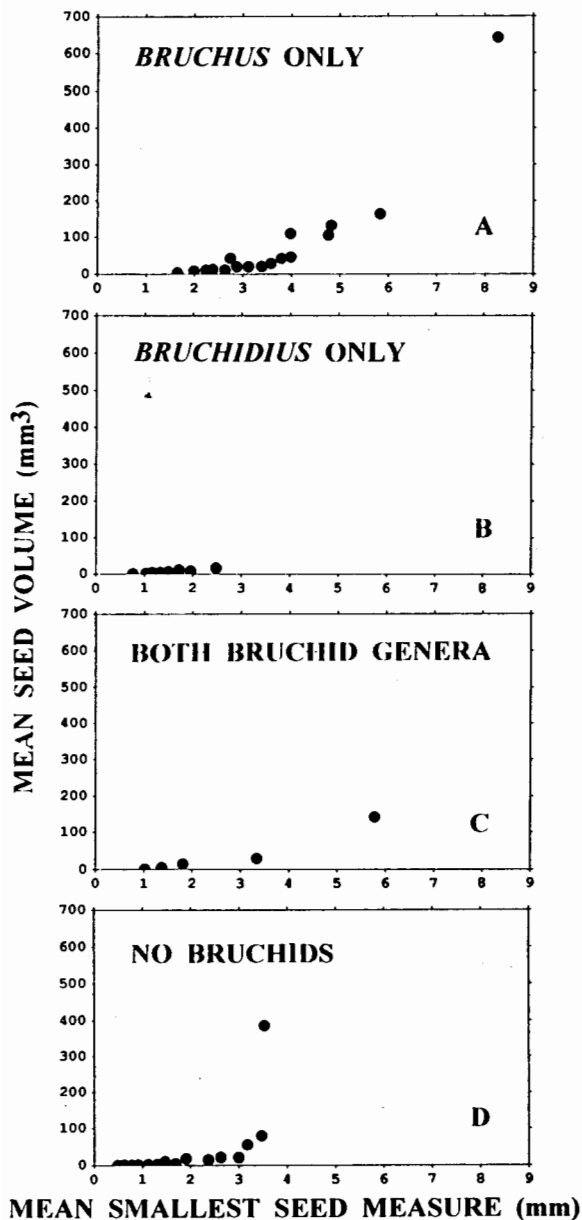
Fig. 2. Distribution of plant species infested with *Bruchus* (A), *Bruchidius* (B), with both genera (C), or with no bruchids at all (D). Mean seed volume (mm³) and smallest seed measure (mm) values.

(Tables 5 and 6). Seed shape, however, appeared to be a plant species character independent of bruchid infestation. *Bruchus* spp. live in fewer plant genera, but use a wider range of host species, whereas *Bruchidius* spp. are more specialized (Table 6).

Naturally, not all plant species with seeds of suitable sizes and shapes are infested with bruchids. Fig. 2 clearly shows that infested and noninfested plant species are intermingled within the seed size spectrum. Surprisingly,

many plant species with large enough seeds are not infested. It is remarkable that considerable seed size intervals, mostly used by *Bruchus* spp., are not at all utilised by *Bruchidius* spp. The reason for this might be that the two genera differ in the way of host plant specialisation (see below). It also seems that the temperate zone bruchids that were studied here, colonise plant species whose seed size belongs to a relatively narrow range. Only species of tropical and subtropical origin such as *A. obtectus* (Say) (Szentesi 1972) and *Callosobruchus* spp. (Nakamura 1969) tend to occupy seeds in which several larvae can develop.

Clearly, considering seed morphological traits, there are many 'vacant niches' (Strong et al. 1984) which have not been colonised by bruchids for reasons other than seed morphology.

## Factors other than seed morphology affecting colonisation by bruchids

*Plant chemistry.* Most authors dealing with the host plants of bruchids looked for the chemistry of ripe seeds as plant characters determining bruchid specialisation (Janzen 1969, 1978, Rosenthal and Janzen 1979). However, temperate zone bruchids oviposit on unripe pod walls, the larvae bore through the pod wall into the unripe seeds and finish development before the seeds become fully ripe. Thus, the ovipositing female and the larva are exposed to a plant chemical profile that can be quite different from the chemical composition of the ripe seed (Szentesi and Wink 1991). Consequently, the key chemosensory stimuli that govern host plant selection by the bruchid females have to be sought on the surface of unripe pods. No such investigations have been carried out so far, although primarily the chemistry of the pod may account for the fact that from the 110 leguminous species studied here ca 50 (~45%) are not used by bruchids, whereas seed morphology of most species would enable colonisation. Plant chemistry and insect colonisation, however, may not correlate necessarily (e.g., Jones and Lawton 1991). In contrast to chemistry, Hare (1980) pressed the importance of morphological traits of plant tissue in defence against seed predators.

*Pubescence on pod wall* may prevent bruchid infestation in *Astragalus cicer* L. and *Vicia hirsuta* (L.) Gray (Szentesi and Jermy, unpubl.) or in *Astragalus newberryi* pods (Center and Johnson 1974). However, other species with hairy pods are successfully colonised (*Vicia pannonica* Cr., *Lathyrus hirsutus* L.; Szentesi and Jermy, unpubl., or *Astragalus mollissimus*, Center and Johnson 1974).

*'Hypersensitive reaction' of the pod wall.* Tissue proliferation induced by deposited bruchid eggs on the surface of pods (e.g., of *Lathyrus* spp.) affect egg-hatch, larval boring and survival (Annis and O'Keeffe 1984, Szentesi and Jermy, unpubl.).

Table 7. Distribution of body widths by both ecology (seed shape) and phylogeny (bruchid genera). The mean body width is given for each cell, sample sizes are in parentheses. Differences within a bruchid genus (row) or a seed shape (column) were tested with one-way ANOVA.

| Bruchid genus | Seed shapes corresponding to those on Fig. 1 (N) | | | | | |
|---|---|---|---|---|---|---|
| | 1–2 | 3 | 4 | 5 | 6 | F (df) |
| *Bruchus* | 1.8725 (1) | 2.0171 (3) | 1.8711 (15) | 2.2151 (1) | 2.4805 (1) | 4.924 (4)** |
| *Bruchidius* | 1.4346 (10) | – | 1.9505 (1) | – | – | 3.719 (1) |
| *Acanthoscelides* | 1.0986 (1) | – | – | 1.6520 (1) | – | |
| F (df) | 2.335 (2) | | 0.229 (1) | | | |

1 = flattened; 2 = elongated and to some extent cylindrical; 3 = close to perfect sphere; 4 = spherical; 5 = spherical, but also somewhat elongated; 6 = with some irregularities in shape. ** P < 0.01; (df) = degree of freedom.

## Evolutionary considerations

Seed size evolved under contrasting selection pressures. Shape and size strongly influence seed dispersal and subsequent events, such as speed of seed burial, germination ability, size of seedling, etc. (Fenner 1985, Murray 1986, Thompson et al. 1993). The observable high inter- and intrapopulational variations in seed morphology can be the trade-offs between life-history components, such as resource allocation (Lokesha et al. 1992), variable genetic quality among offspring and heterogeneous environment (Obeso 1993), habitat quality (Salisbury 1942), etc. It seems evident that plants most importantly optimised seed size for sufficient nutritional content for the seedling in a particular habitat type versus dispersive ability or preservative ability. Nevertheless, we have to consider whether or not selection may occur for avoiding consumption by ren-PSPs.

Seed morphological and chemical traits powerfully influencing bruchid infestation patterns led to the interpretation of seed predator/host interactions as one of the textbook examples of coevolution (Janzen 1969 and later, Center and Johnson 1974, Rosenthal and Janzen 1979, Labeyrie 1991, and others). Janzen (1983: 645) stated explicitly: "… seed predators and dispersal agents undoubtedly serve as part of the selective forces that determine seed size". Unfortunately, no data were presented by him to support this argument. Janzen (1969) also supposed that leguminous plants were selected by seed predators for specific secondary plant substances such as non-protein amino acids, alkaloids, etc. as defence chemicals accumulating in the seeds. Recently, Johnson (1990) criticised many aspects of bruchid/host plant evolutionary relationships, because they have been based on assumptions, or on correlative data only, whereas experimental testing was totally neglected.

Janzen's (1975), and our own observations have shown that from some seed (pod) samples literally no PSPs emerged due to the very high parasitisation rate by chalcids or braconids or to the absence of PSPs at that locality. Also, recruitment of new plant individuals is considered to be episodic (Sallabanks and Courtney 1992). Thus, the selection pressure exerted by predispersal seed predation on plants acts very irregularly in space and time. Therefore, its effect must be negligible.

The negative impact of PSPs on plant fitness is further questionable if their beneficial roles are considered. Apart from the very specific case of fig wasps (Bronstein 1992), there are other instances when they may decrease density-dependent effects among host plant progenies (Harper 1977), increase outcrossing (Molau et al. 1989), maintain a higher plant diversity locally, pollinate the host-plant (Labeyrie and Hossaert 1985; Szentesi unpubl.), or promote germination of seeds by allowing moisture to enter through the bruchid exit holes (Sallabanks and Courtney 1992). Even if one finds a very severe effect on seed production imposed by seed predators, no real selective significance can be attributed to it, unless the impact on plant recruitment was also demonstrated, as convincingly done in a series of works by Louda (1982a, b, 1983). In plant species where vegetative reproduction prevails, seed predation may ab ovo hardly influence plant abundance (Louda 1994).

Thus, regarding the leguminous species reported in this study we can assume that severe and continuous bruchid infestation may affect only the demography of annual (fugitive) species, e.g. *Vicia angustifolia*, while their impact on species with vegetative propagation, such as *Vicia tenuifolia*, and on woody species would most likely be negligible or lacking.

There are several PSPs that cannot be supposed to select for reduced seed size in legumes. For example, gall-formers attacking pods, qen- and ecto-PSP species as well as plant bugs sucking the seeds through the pod wall are very unlikely to select for seed size. That seed size is subject to constraints other than defence, is supported by the wide spectrum of seed sizes in the Leguminosae (Harper et al. 1970 and Table 1).

Assuming a body size of ren-PSP species that is stabilised allometrically within certain limits, selection for a larger seed size would also be possible, if a ren-PSP preferentially kills small seeds, such as the acorn weevil (Forrester 1989, cited by Crawley 1989), or if larvae developing in a large enough seed do not destroy seed parts vital for germination. In such cases bruchid infestation may not affect germination or occasionally may

Table 8. Possible outcomes (hypothetical advantages and disadvantages) of seed size selection by en-PSPs.

| Selection for seed size | |
|---|---|
| smaller | larger |
| More seeds produced/plant | Less seeds |
| Less cost/seed | More cost/seed |
| Better dispersal | Less dispersive seeds |
| Less nutrients for germination | More nutrients/seed |
| More demanding for microsite | Less demanding |
| en-PSPs are less probable | More than one en-PSP is likely |

even promote it (Sallabanks and Courtney 1992). However, in the long run, the probability of using a large seed by two or more smaller-sized seed predators would act against this process. These contrasting selection scenarios are depicted in Table 8. From this it seems that the weights of the opposing trends are roughly balanced and the adoption of one or the other strategy may depend on the circumstances.

The seed predator guild could be supposed to participate in diffuse coevolution where traits are evolving under quite different and often contrasting needs, although, for reasons described above, the probability of even such a situation must be fairly low. We propose instead that en-PSPs adapt to the constraints set by leguminous plants without affecting the evolution of the same constraints (Jermy 1984, 1993).

As for the host plant specialisation of bruchids, data of this study do not support the resource partitioning (niche differentiation) hypothesis either, because there are many "vacant niches" (Fig. 2), while in several cases two or more bruchid species occurred in the same plant sample. For example, we reared *Bruchus brachialis*, *B. rufipes* Herbst. and *B. venustus* Fahrs. from *Vicia tenuifolia*. The partial segregation of *Bruchus*- and *Bruchidius*-host plants (Fig. 2) is also hardly explainable by assuming competition (niche differentiation). Instead, it most likely resulted from the tendency of host specialisation that may have genus-specific characteristics.

In conclusion, the data on bruchid colonisation presented here clearly indicate that bruchid host plant specialisation could not have resulted from a coevolutionary "arms race" that left only one or a few plant species as host(s) for each bruchid species. Instead, the explanation must be sought in the bruchids' host plant specialisation itself. The proximate cause of host plant preference is the recognition of plants by the egglaying female that in turn is based on the generally determined function of the nervous system (chemoreception and central processing of incoming information). Changes of the genome (mutations) may change plant recognition through changes in the function of the female's nervous system and thus may result in recognition of a new plant species as a host. Naturally, the latter would become a host only if it allows larval development. In this way plants exert a selection pressure on bruchids and thus shape host plant range secondarily, but the leading role in the evolution of host plant specialisation is played by the evolution of the insect's nervous system (Jermy 1984, 1993, Bernays and Chapman 1994).

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