

Decision Making for Food Choice by Grasshoppers (Orthoptera: Acrididae): Comparison Between a Specialist Species on a Shrubby Legume and Three Graminivorous Species

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ABSTRACT Dwarf gorse bush (*Ulex minor*) heathlands in Limousin, France, are ecological islands often separated by tens of kilometers of grasslands and hedges, where several species of grasshoppers belonging to the genus *Chorthippus* (Acrididae: Gomphocerinae) coexist. *Chorthippus binotatus* (Charpentier) feeds only on *Ulex minor*; nymphs feed exclusively on leaves whereas adults become florivorous at the end of the season. The other species studied (*C. biguttulus* (L.), *C. albomarginatus* (De Geer), and *C. parallelus* (Zetterstedt)) are all graminivorous. The importance of sugars, nitrogen content, sparteine (a quinolizidine alkaloid), and plant architecture in food selection was investigated. *Chorthippus binotatus* is sensitive to sucrose and fructose, consistent with the high sugar content of *Ulex minor* flowers. Experiments with grass coated with sparteine showed that this molecule associated with sucrose is a phagostimulant for this grasshopper. Different behavioral responses of graminivorous species are observed with sparteine alone, but never phagostimulation. We compared the response times corresponding to decision-making between the different species toward several components involved in food selection. The food choice toward host plant and sugars is as quick for *C. binotatus* as for the two graminivorous species (*C. parallelus* and *C. albomarginatus*), whereas *C. biguttulus* is slower and exhibits atypical reactions. *Chorthippus binotatus* can feed on Poaceae, but with more time spent, leading to an increasing predation risk. This situation is a limitation toward dispersal between different heathlands (patchy habitats).

KEY WORDS decision making, patchy habitats, alkaloids, *Chorthippus*, diet shift, graminivory

CHAPMAN (1982) NOTED that in the majority of phytophagous insect orders, >50% of the species are oligophagous (they feed on one plant family only) or monophagous. The Orthoptera, and particularly Acridoidea, stand apart from the other orders: 60% of grasshoppers have been classified as polyphagous, and a further 25% are graminivorous.

In North America, at least two Gomphocerinae species have evolved specialized diets from graminivory under conditions in which grasses were often very sparse (Otte and Joern 1977). *Ligurotettix coquilletti* McNeill feeds on *Larrea* (Zygophyllaceae), *Atriplex* (Chenopodiaceae), and *Lycium* (Solanaceae) (Chapman and Joern 1990). *Boottettix argentatus* Bruner lives and feeds on the creosote bush *Larrea tridentata* (Zygophyllaceae) (White and Chapman 1990). The basis for this preference depends partly on the presence of the lignan nordihydroguaiaretic acid (NDGA) (Chapman et al. 1988).

In France, the genus *Chorthippus* (Gomphocerinae) comprises 16 species (Bellmann and Lucquet 1993). The diet of all species was characterized by

Gangwere and Morales-Agacino (1973). All species are graminivorous with the exception of *Chorthippus binotatus* (Charpentier) (Picaud et al. 1999). This species is present in France, Spain, and Morocco and comprises six subspecies, five of which occur in France (Default 1999). *Chorthippus binotatus binotatus* feeds almost exclusively on various species belonging to the Genisteae tribe (Fabaceae) (Grassé 1929), with some regional specialization: *Ulex minor* Roth in Périgord and Limousin, *Ulex europaeus* L. and *Cytisus scoparius* (L.) Link in Charente-Maritime, *Genista scorpius* (L.) DC. in the French Pyrenees (Default 1999), and *Ulex parviflorus* Pourret in Vaucluse (Mossot and Petit 2000). In Limousin, the dietary preference of *C. binotatus binotatus* during the season is synchronized with the life history of *Ulex minor*: the grasshopper feeds shoots before blooming and flowers during the summer (Picaud et al. 1999, 2002). In the laboratory, the entire life cycle can be completed with *Ulex minor* as the only food supply.

It is certain that *C. binotatus* evolved from graminivorous species: the 15 other species in the genus *Chorthippus* feed on Poaceae, as well as the French species of the sister genera *Omocestus* and *Stenobothrus* (Bonnet 1996). It is well known that grass-feeding

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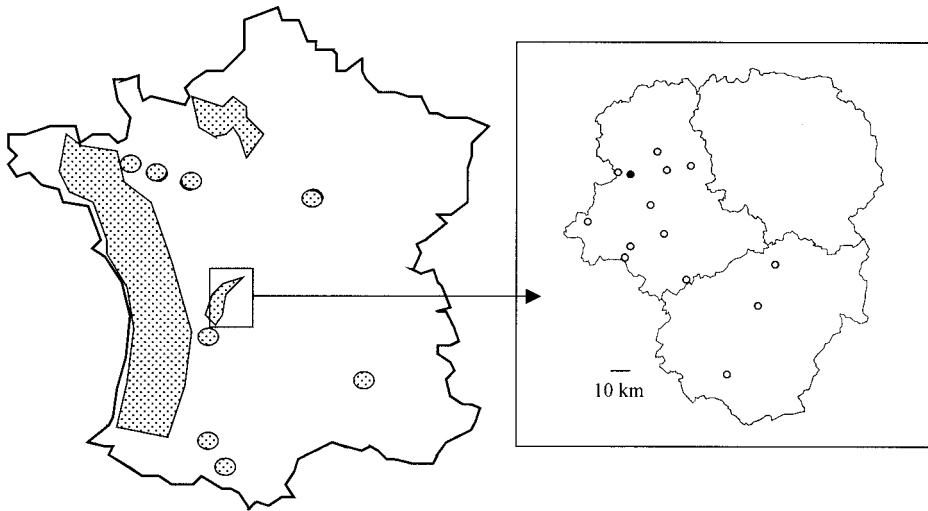


Fig. 1. Distribution map of *Chorthippus binotatus* subspecies *binotatus* in France (hatched parts) and in Limousin Region (open circles), divided in three departments (after Kruseman, 1982; Defaut, 1999). Plant samples for analyses were collected in Ceinturat heathland (black circle).

grasshoppers feed mainly on the most abundant Poaceae species (Bernays and Chapman 1994). Because the chemical composition of secondary metabolites of Graminae are rather uniform (Bruneton 1999), one can assume that grass-feeding grasshoppers are oligophagous and therefore relatively specialist species.

In Limousin, the heathlands correspond to ecological islands (Brugel et al. 2001). Thus, for species using food resources that are limited and patchy, the distance between these bush formations can be a constraint for dispersal. A less obvious constraint that may be associated with oligophagy is the problem of food quality, including nitrogen and/or carbohydrates levels, and the balance between deterrents and phagostimulants.

Bernays and Bright (2001) showed that food choice of *Schistocerca americana* (Drury) between different combinations of two plant species causes interrupted feeding as a result of inefficient decision making. So, feeding on only one plant species saves time and potentially benefits grasshoppers. One can ask several questions about the properties linked to feeding upon a given plant: (1) in the case of binary choice (*Ulex minor* versus Poaceae), do the insects feed exclusively on their host plants; (2) what are the characteristics of *Ulex minor* involved in the diet preference of *C. binotatus*; and (3) is the specialist species on Genistee bushes as efficient as each graminivorous species in their food selection?

In this work, we examined the feeding behavior and the food selection of all the *Chorthippus* species coexisting with *Chorthippus binotatus* in Limousin heathlands: *C. biguttulus* (L.) (subgenus *Glyptobothrus*, as *C. binotatus*), *C. parallelus* (Zetterstedt), and *C. albomarginatus* (De Geer) (subgenus *Chorthippus*). It is known that the three latter species are graminivorous (Bonnet 1996). We investigated food quality as the

basis of food selection in grass feeding and *Ulex minor* specialist grasshoppers. We analyzed sugars and nitrogen content in the plant species with a special focus on *Ulex minor* and Poaceae. As for secondary compounds, it is well known that numerous quinolizidine alkaloids characterize the tribe of Genisteeae (Bruneton 1999). Our investigation was limited to sparteine, the major alkaloid in the genus *Ulex* (Picaud et al., unpublished data). The influence of plant architecture was also tested: ramified in the case of *Ulex*, straight in the case of Poaceae.

We discuss the findings in relation to the benefit of quick decision times (Bernays and Bright 2001, Abrams and Schmitz 1999) and the disadvantage of being a specialist on *Ulex minor*, as the *Ulex minor* heathlands form islands between grasslands and pastures.

Materials and Methods

Experimental Animals. *Chorthippus* adults (*C. binotatus*, *C. biguttulus*, *C. albomarginatus*, and *C. parallelus*) were collected in September and October 1993–2001 in heathlands in the Limousin region (45–46 degrees N. latitude, 1–2 degrees E. longitude), France (Fig. 1). If we take into account the mean of absolute plant cover in seven heathlands where *C. binotatus* was present, the most abundant plant species are: *Ulex minor* (48%), *Calluna vulgaris* (34%), *Molinia caerulea* (23%), *Erica cinerea* (17%), *Pteris aquilinum* (7%), and *Erica tetralix* (4%). Insects were placed individually in glass jars (6 cm diameter × 10 cm height) and kept at room temperature (20 ± 2°C), under a L:D 12:12 h photoregime and 50% humidity for the duration of the experiments. They were fed daily with fresh plants collected at the Limoges University: *Ulex minor* for *C. binotatus* and a mix of *Agrostis tenuis*, *Holcus lanatus*, and *Lolium perenne* for the graminivorous species.

Every week after the experiments, all living insects were returned to the wild and replaced by newly collected specimens.

Tested Plants and Food Selection Experiments. We chose *Ulex minor* (flowering time in July–October) and the most abundant grass in heathlands (*Molinia caerulea*) for testing. Another grass species, *Lolium perenne*, was also tested because of its abundance at the Limoges University. Before each experiment, food was withheld from insects for 4–6 h (Bernays and Chapman 1970).

Food choice tests with 16–32 individual adults of both sexes of each species were performed in a Petri dish (14.2 cm diameter \times 2 cm height) isolated in a green enclosure. A 40 W-lamp above the center of the dish provided constant light and minimized temperature fluctuations. Each experiment was limited to 15 min to avoid postingestive feed-backs. Thus, decisions by grasshoppers depended on receptor sensitivity and the physiological state of individuals. The grasshopper was transferred to the center of a dish in a small glass jar. It was given a choice between two separate sources, each consisting of fresh plants (\approx 1 g), cut with scissors just before the start of the bioassay. Three different behavioral responses were recorded (Williams 1954, Bernays and Chapman 1970, Uvarov 1977): orientation (the grasshopper walked to the plant (item 1), palpated the plant (item 2), and ate the plant (item 3). To help understand food choice, we examined sugars, architecture, and the secondary metabolite sparteine.

During plant choice experiments, the sum of orientation and palpation times from initiation of movement until the decision (feeding upon or rejecting it) was documented. Concerning the experiments with sugars and the alkaloid, non-volatile chemical compounds, only palpation time before decision was taken into account.

Chemical Analysis of Plants (soluble carbohydrates and nitrogen content). The samples of *Ulex minor* flowers, shoots, and stems were collected from Ceinturat heathland (Fig. 1), five in September 1997 and four in July 1999 at the beginning of blooming for a series of analyses. Four samples of two species of Graminae were collected in grasslands of the Limoges University in early July 1999. The separation and identification of soluble sugars (glucose, fructose and sucrose) was adapted from Harisson et al. (1997) and Cataldi et al. (1999) and performed by high performance liquid chromatography (HPAEC-PAD). Elution of the samples was carried out in isocratic conditions by using 0.16 M aqueous NaOH at a flow rate of 1 ml \times min⁻¹. Calculations of mono- and disaccharide concentrations were made from dry weight extraction residues, and then expressed relative to initial sample dry weight. The measurement precision was tested by duplicating analyses of the samples.

The nitrogen content was evaluated by Kjeldahl analysis (AOAC 1984).

Sugar Sensitivity. The experimental apparatus was the same as that described above, with the exception that the length of the test was reduced to 5 min to

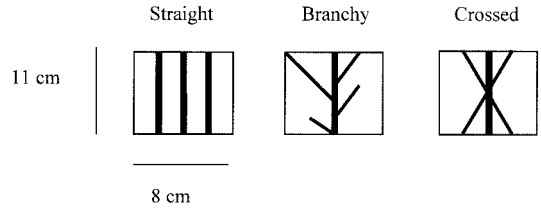


Fig. 2. Drawings presented to insects in plant architecture experiments.

avoid consumption of the piece of filter paper. The choice consisted of two pieces of filter paper (each one measuring 6 \times 6 cm, and four folds), placed at random on opposite edges of the dish, one with one M sugar solution (1 ml), and one with distilled water (20–21 individuals for each species).

Decomposition Analysis of Shape of the Plant. Individual grasshoppers were placed for 10 min in a Y-maze with opaque walls (30 \times 8 \times 11 cm) covered with Plexiglas. The grasshoppers had to choose between two drawings (8 \times 11 cm) placed vertically and at random ends of the two branches (18 \times 8 \times 11 cm). Choice between the drawings was recorded when the insect reached the end of the Y-maze and climbed on the drawing. The time from initiation of movement until climbing onto the drawing was recorded. Two pairs of green patterns on a yellow background, to mimic *Ulex* branches in the environment, were presented: each individual (19–21 by species) had to choose between straight versus branching aspects and straight versus crossed aspects in separate bioassays (Fig. 2).

Alkaloid Sensitivity. Preliminary experiments demonstrated (data not shown) that *C. binotatus* and *C. biguttulus* have no preference or rejection behavior for 1% acetic acid versus water, on filter paper. Alkaloid content in Leguminosae vary generally between 0.1 and 3% of plant dry weight (Bruneton 1999). Our analyses of different organs of *Ulex minor* (Picaud et al., unpublished data) have shown that sparteine represents between 30–48% of nonlinked alkaloids. Different concentrations (in 1% acetic acid) of sparteine 99% (Sigma Aldrich, Saint-Quentin, France) were tested under the same conditions as described in “sugar sensitivity” trials, with a 1% acetic acid treatment as a control. Experiments were repeated three times with different sets of 16–24 individuals of *C. binotatus*, tested separately with 0.1%, 0.5%, 1% and 2% of sparteine. The principal behavioral response recorded was the arrestment on the filter paper.

Interspecific comparisons of behavioral responses toward sparteine were undertaken with a solution of 1% sparteine in 1% acetic acid on filter paper in the center of a glass container (5 cm diameter at base, 7 cm high), with a 1% acetic acid treatment as a control. For each species, we observed 20–24 separated individuals. For each individual, we recorded 10 items during a 5-min continuous observation. The behaviors were: palpation in the air (Pair); palpation of filter paper (Palp); arrestment on the filter paper (Arrest); put-

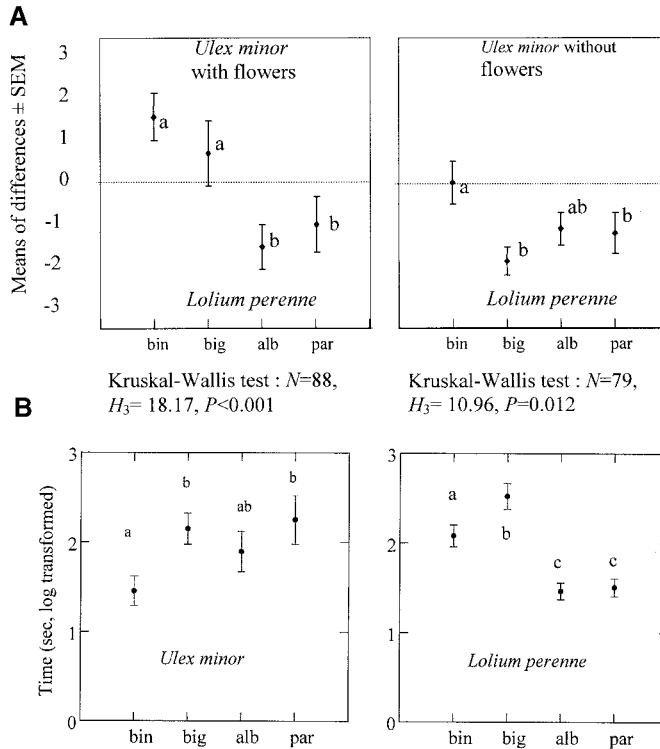


Fig. 3. Interspecific comparison of three behaviors of *Chorthippus binotatus* (bin), *C. biguttulus* (big), *C. albomarginatus* (alb), and *C. parallelus* (par) in binary food plant experiments. (a) Results (mean \pm SEM of differences) from Wilcoxon test on behaviors (orientation: 1, palpation: 2, consumption: 3) for plant choice. Kruskal-Wallis tests of all combined data are noted on each figure. Different letters correspond to significant differences between species (Mann-Whitney *U* tests, $P < 0.05$). (b) Mean reaction times \pm SEM (seconds, Log_{10} -transformed data) to move and palpate the plant. ANOVA of the combined data of all species concerning reaction time for flowered *Ulex minor*: $F_{3,27} = 3.62$, $P = 0.026$. ANOVA for *Lolium perenne*: $F_{3,30} = 17.16$, $P < 0.001$. Different letters correspond to significant differences between species (Tukey's test, $P < 0.001$).

ting the fore legs on the filter paper and touching the mouth parts (Leg); putting the labrum on the filter paper (Lab); biting and masticating (Bmas); leg withdrawing above the filter paper (Wleg); regurgitation (Reg); leaving filter paper (Leave); jumping resulting from filter paper contact (Jump).

We tried to enhance grass consumption by *C. binotatus* in adding some characteristic molecules present in *Ulex minor*: we studied its behavior with *Lolium perenne* covered, with a brush, with different combinations of solution diluted in 1% acetic acid: sucrose one M, sparteine 1%. *Lolium perenne* covered with 1% acetic acid were used as control. We recorded the time spent palpitating before decision and the frequency of individuals (16–22 individuals for each experiment) consuming *Lolium perenne*.

Statistical Tests. The individual behaviors for each choice were analyzed with Wilcoxon tests. Differences given by these tests were used to perform Kruskal-Wallis tests for interspecific comparisons using SYSTAT ver. 7.0 (SPSS 1997).

The reaction times were compared using a multi-way analysis of variance (ANOVA) because all of the data were normally distributed. Log-transformed palpation times were compared using multi-way ANOVA

for interspecific comparisons. In each case, Tukey's tests were performed for pair-wise comparisons. Frequencies of different items with sparteine trials and coated grass experiments were compared with a Mann-Whitney *U* test.

Results

Behavioral Comparison among Species. *Chorthippus binotatus* and *C. biguttulus* preferred *Ulex minor* flowers (Fig. 3a) to *Lolium perenne* (Kruskal-Wallis test: $H_3 = 18.166$, $N = 88$, $P < 0.001$) and *Molinia caerulea* (Kruskal-Wallis test: $H_3 = 16.30$, $N = 79$, $P = 0.001$). When the *Ulex minor* branches have not flowered yet, the graminivorous species (*C. biguttulus*, *C. albomarginatus*, and *C. parallelus*) preferred Graminae (Kruskal-Wallis test: $H_3 < 10.96$, $74 < N < 79$, $P < 0.015$ with both binary plants choice).

The shortest time (Fig. 3b) between the beginning of the experiment and the palpation of *Ulex minor* in the case of binary choices was observed for *C. binotatus* (ANOVA: $F_{3,27} = 3.62$, $P = 0.026$). *C. albomarginatus* and *C. parallelus* show the quickest reaction time to *Lolium perenne* (ANOVA: $F_{3,30} = 17.16$, $P < 0.001$).

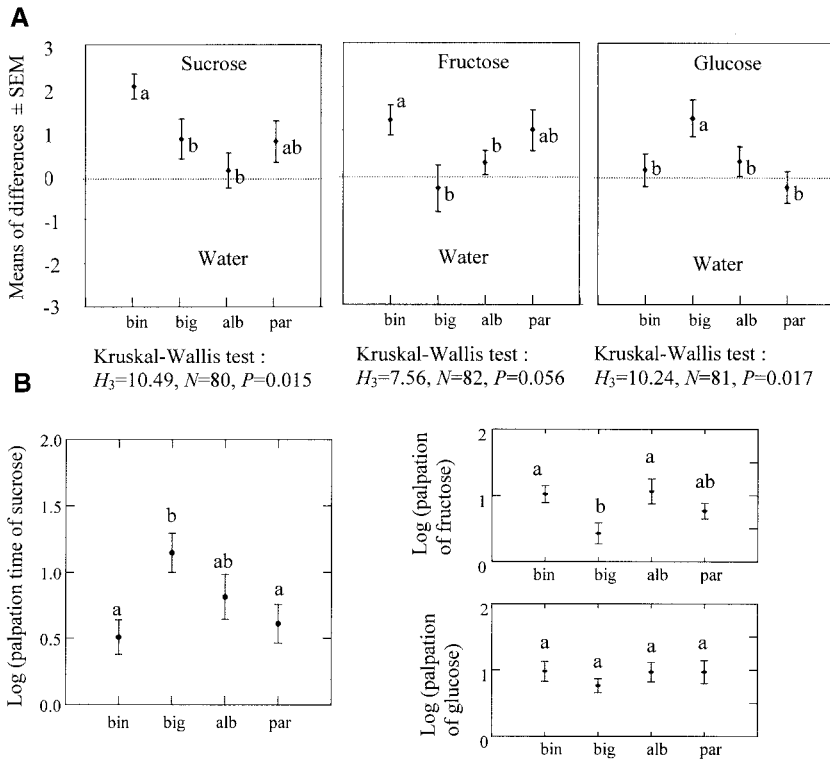


Fig. 4. Interspecific comparison of three behavioral items of *Chorthippus binotatus* (bin), *C. biguttulus* (big), *C. albomarginatus* (alb), and *C. parallelus* (par) in an experiment testing the response to different sugars versus water. (a) Mean \pm SEM of differences from Wilcoxon test on behaviors (orientation: 1, palpation: 2, consumption: 3). Kruskal-Wallis test of all combined data are noted on each figures. Different letters correspond to significant differences between species (Mann-Whitney *U* tests, $P < 0.05$). (b) Mean palpation time \pm SEM (seconds, log-transformed data) of soluble carbohydrate solution (1M). ANOVA of the combined data of all species for each sugar experiment: see text. Different letters correspond to significant differences between species (Tukey's test: $P < 0.05$).

The most marked preference for sucrose (Fig. 4a) was observed for *C. binotatus* (Kruskal-Wallis test: $H_3 = 10.49, N = 80, P = 0.015$). This result is corroborated by the fact that *C. binotatus* showed the quickest palpation time (Fig. 4b) before making a decision in the presence of sucrose (ANOVA: $F_{3,28} = 3.91, P = 0.019$). Curiously, *C. biguttulus* responded slowly to sucrose. As for fructose, the most complete series of behaviors was performed by *C. binotatus* and *C. parallelus* (Kruskal-Wallis test: $H_3 = 7.56, N = 82, P < 0.056$). Unexpectedly, *C. biguttulus* had the quickest reaction time (Fig. 4b, ANOVA: $F_{3,31} = 3.53, P = 0.026$) in spite of its low motivation for fructose (Fig. 4a). *C. biguttulus* is the only species sensitive to glucose (Kruskal-Wallis test: $H_3 = 10.23, N = 81, P = 0.017$), but no difference between decision time was recorded (ANOVA: $F_{3,29} = 0.77, P = 0.52$).

Finally, *C. binotatus* was the only species attracted (Fig. 5) by branching aspects (Kruskal-Wallis test: $H_3 = 13.41, N = 82, P = 0.004$).

Soluble Carbohydrates and Nitrogen Contents. Flowers of *Ulex minor* had high levels of sucrose (mean \pm SEM = $18.9 \pm 0.7 \text{ mg} \times \text{g}^{-1}$ of extract residue) in the autumn but not summer (Table 1). Glucose and fructose were both present at very high

levels in *Ulex minor* ($>58 \text{ mg} \cdot \text{g}^{-1}$ of extract residue). Shoots of *Ulex minor* also contained fructose (mean \pm SEM = $3 \pm 1.4 \text{ mg} \cdot \text{g}^{-1}$) and sucrose (mean \pm SEM =

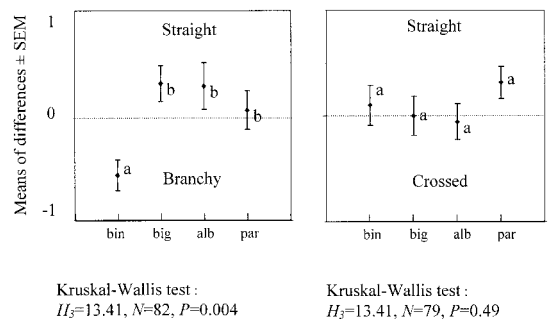


Fig. 5. Interspecific comparison of orientation behavior of *Chorthippus binotatus* (bin), *C. biguttulus* (big), *C. albomarginatus* (alb), and *C. parallelus* (par) in a Y-maze. Results (mean \pm SEM of the differences) of Wilcoxon test on orientation behaviors an experiment comparing different plant architecture. Kruskal-Wallis test of all combined data are noted on each figures. Different letters correspond to significant differences between species (Mann-Whitney *U* tests, $P < 0.05$).

Table 1. Determination of soluble carbohydrates by HPAEC-PAD analyses and nitrogen content by Kjeldahl analysis. Sugars and nitrogen (mean ± SEM) are expressed in mg·g⁻¹ for dry weight. Analyses were performed on 500-mg plant tissues of 5 samples of *Ulex minor* and 4 samples of each grass

| | Glucose | Fructose | Sucrose | Nitrogen |
|--|------------|------------|------------|------------|
| Flowers of <i>Ulex minor</i> (end of July) | 58.3 ± 0.9 | 67.7 ± 6.7 | 3.5 ± 0.2 | 5.16 ± 0.5 |
| Flowers of <i>Ulex minor</i> (September) | 69.1 ± 0.5 | 77.4 ± 0.9 | 18.9 ± 0.7 | No data |
| Stems of <i>Ulex minor</i> | 5.6 ± 2.1 | 2.6 ± 0.4 | 8 ± 0.8 | 9.6 ± 1.2 |
| Shoots of <i>Ulex minor</i> | 3.95 ± 0.8 | 3 ± 1.4 | 6.6 ± 0.8 | 9.8 ± 0.6 |
| Leaves of <i>Agrostis tenuis</i> | 7 ± 2.5 | 8.4 ± 4.1 | 9.6 ± 0.9 | No data |
| Leaves of <i>Holcus lanatus</i> | 4.2 ± 1.7 | 3.6 ± 2 | 8.7 ± 1 | No data |

6.6 ± 0.8 mg · g⁻¹), representing <10% of the quantity present in flowers. The two species of grasses contained sugars in comparable amounts to stems of *Ulex minor* (Table 1). *Ulex minor* flowers were low in nitrogen content (0.5–0.6% of dry weight). Nitrogen made up ≈1% of the dry weight of shoots and stems of *Ulex minor*.

Sparteine Sensitivity. At concentrations between 0.5 and 2% sparteine, 25% of *C. binotatus* individuals were arrested by the piece of filter paper. For weaker concentrations, the percentages of responses clearly fall (12% of individuals for 0.1% sparteine). Consequently, the sparteine concentration used in interspecific experiments was 1%.

Among the 10 behaviors considered, four were of special interest because of statistical differences between species (Fig. 6): air palpation, arrestment on the piece of filter paper, leaving filter paper and regurgi-

tation. It should be noticed that except one individual of *C. albomarginatus*, arrestment and leaving filter paper were never associated. *C. binotatus* differed from remaining species by three behaviors when sparteine is dropped on filter paper. *C. binotatus* palpated the air more than the other species (Kruskal-Wallis test: $H_3 = 18.94, N = 57, P < 0.001$). Arrestment on the piece of filter paper characterized *C. binotatus* (Kruskal-Wallis test: $H_3 = 16.27, N = 57, P = 0.001$). In the same way, *C. binotatus* was the only species never leaving filter paper (Kruskal-Wallis test: $H_3 = 19.03, N = 57, P < 0.001$).

Otherwise, regurgitation characterized *C. albomarginatus* compared with other species (Kruskal-Wallis test: $H_3 = 21.12, N = 57, P < 0.001$).

Within each species, we tested to determine whether there was a significant difference between the frequencies of each behavior in response to acetic

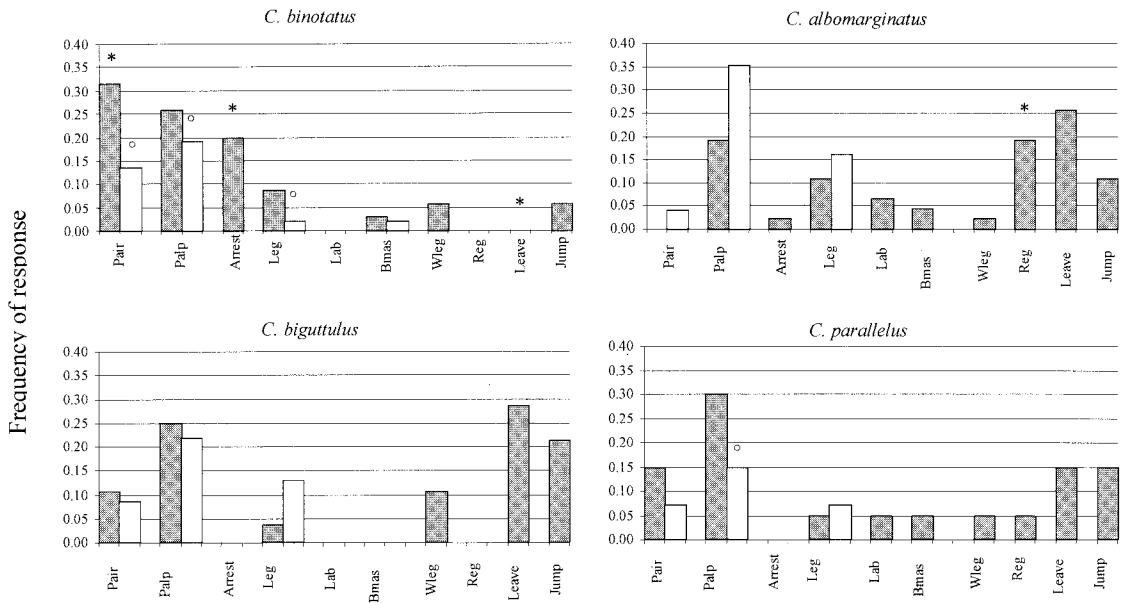


Fig. 6. Frequency of different behaviors in response to 1% sparteine diluted in 1% acetic acid on filter paper (shaded boxes) or to 1% acetic acid alone (open boxes). Asterisks indicate significantly different values from all other species tested (Kruskal-Wallis test, $P \leq 0.001$ in each case). The circles indicate significant differences between frequencies of response to sparteine and the acetic acid control (Mann-Whitney U test, $P < 5\%$ in each case), when both frequencies are not null. In the case of acetic acid alone, the behavior “no-response” is not represented. Pair: palpation of air; Palp: palpation of filter paper; Arrest: arrestment on filter paper; Leg: placing fore legs on filter paper and touching with mouth parts; Lab: touching filter paper with labrum; Bmas: biting and masticating; Wleg: withdrawing leg above filter paper; Reg: regurgitation; Leave: leaving filter paper; Jump: jumping in response to contact with filter paper.

acid versus sparteine diluted in acetic acid. The responses of *C. binotatus* are generally stronger when sparteine is present (Mann-Whitney *U* test, $P < 0.01$ in each case), except for biting behavior. The behaviors related to avoidance (leaving filter paper, jumping, leg withdrawing, regurgitation) are generally expressed in graminivorous species when sparteine is present. Curiously, *C. parallelus* is the only graminivorous species palpitating filter paper with sparteine at a higher frequency than acetic acid (Mann-Whitney *U* test, $P < 0.05$).

Responses of *C. binotatus* toward *Lolium perenne* Coated with Different Combinations of Solutions. The time spent on palpation of *L. perenne* before decision (consumption or rejection) is shown in Fig. 7a. The shortest time, i.e., the quickest decision corresponded to the combination of sucrose and sparteine. This time differs significantly from the one recorded with *L. perenne* coated with acetic acid only (ANOVA: $F_{1,26} = 5.97$, $P = 0.02$).

Consumption frequency of *Lolium perenne* by *C. binotatus* (Fig. 7b) was enhanced by the same combination of sparteine + sucrose (Kruskal-Wallis test: $H_3 = 8.24$, $N = 69$, $P = 0.04$). The effect of addition of sucrose or sparteine alone did not significantly differ from the test.

Discussion

Analysis of Food Choice. *Chorthippus binotatus* prefers *Ulex minor*, especially flowers. Both *C. albomarginatus* and *C. parallelus* prefer Graminae to *Ulex*, whether blooming or not. Our experiments lead us to define three feeding groups. The first one is represented by *C. binotatus*, which specializes on *Ulex minor*, and the second one comprises *C. albomarginatus* and *C. parallelus*, which are "purely graminivorous." The third group consists of *C. biguttulus*, which behaves like the purely graminivorous species when *Ulex* is not flowering, but like the specialist species on Genisteae when *Ulex* is flowering. We can call this species "atypically graminivorous." Thus, both *C. binotatus* and *C. biguttulus* respond to *Ulex minor* flowers, which probably contain one or more attractants.

These observations are confirmed by measuring time to decision making. The two purely graminivorous species have the quickest reaction time for Graminae and the longest one for *Ulex minor*, whereas the specialist species on Genisteae shows the opposite behavior. Curiously, the "atypical graminivorous species" (*C. biguttulus*) is slow to make a decision, regardless of its choice (*Ulex minor* or Graminae) and has the broadest diet, accepting *Ulex* as well as grasses. This agrees with the findings of Bernays (1998, 2001) that the broader the food types accepted, the slower the response (assuming grasses to be rather uniform in secondary chemical compounds). This disadvantage is somewhat balanced by the fact that *C. biguttulus* has a larger habitat niche than either of the other species (Bellmann and Luquet 1993).

Chorthippus binotatus is the species most responsive to sucrose, and it is as responsive to fructose as

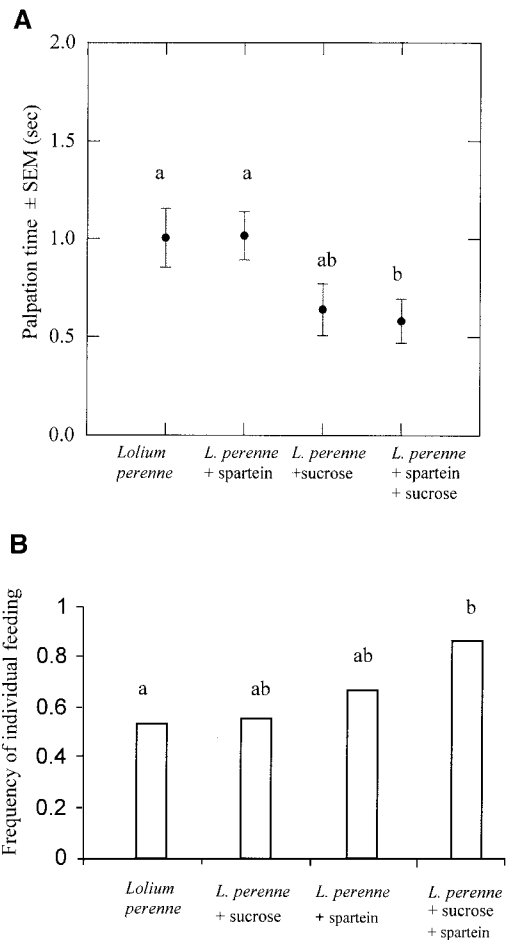


Fig. 7. Behavior of *Chorthippus binotatus* in response to *Lolium perenne* covered with different combinations of solutions diluted in acetic acid 1% (sucrose: 1 M, sparteine: 1%). (a) Mean time \pm SEM spent (seconds, log-transformed data) in palpation (global ANOVA: $F_{3,52} = 3.41$, $P < 0.05$). Different letters correspond to significant differences between treatments (Tukey's test: $P < 0.05$). (b) Consumption frequency. Combined data of each combination are compared with Kruskal-Wallis test: $H_3 = 8.24$, $N = 69$, $P < 0.05$). Different letters correspond to significant different frequencies between treatments (Mann-Whitney *U* tests, $P < 0.05$).

C. parallelus. *Chorthippus biguttulus* is most sensitive to glucose. As a result, sucrose and fructose are probably the major feeding stimulants, among sugars, on *Ulex minor*. The sugar analyses show that *Ulex minor* flowers in September had the highest content of sugars. However, the attraction of *C. binotatus* to its host plant in July is not explained by sugar content.

Presence of receptors that can detect sugars has been demonstrated in numerous insects (Mitchell 1981) and especially in grasshoppers (Bernays and Chapman 1994): the pyranose site is maximally sensitive to sucrose and glucose and the furanose site to fructose. Feeding on *Ulex minor* flowers is partly explained by sensitivity of *C. binotatus* to sucrose and of

C. biguttulus to glucose. Fructose does not play any role as the "strictly graminivorous" species are not attracted by *Ulex minor* flowers.

Sparteine alone leads to different behavioral responses according to the species. Leaving the substrate containing sparteine is a response characteristic for graminivorous species. *C. albomarginatus* regurgitates in response to sparteine. Contrary to the other species, *C. binotatus* palpates the air and remains on the substrate. Thus, sparteine is at least involved in the food selection of the insect species enabling it to feed on Genisteae. This situation can be compared with the phagostimulant power of pyrrolizidine alkaloids to *Zonocerus elegans* Thunberg (Boppré et al. 1984), although it is a polyphagous and opportunistic species.

Our study on *C. binotatus* with *Lolium perenne* coated with different solutions showed that the combination of sparteine and sucrose is probably involved in food choice of *C. binotatus*. This is in agreement with our results that the highest consumption level was recorded in presence of the same combination, demonstrating its role as a phagostimulant.

With respect to the plant architecture, we found that *C. binotatus* was attracted to the branched drawing over the straight one. This can partly explain its preference to bushes versus grasses. However, plant architecture cannot be the only explanation for the feeding behavior of this species, because it is never attracted to other flowering bushes in heathlands, e.g., *Calluna vulgaris*, *Erica tetralix*, and *Ulex europaeus*. Contrary to the results of Williams (1954) and Bernays and Chapman (1970), graminivorous species were not significantly attracted to a given architecture in our experiments. These authors used only three-dimensional lures and never drawings.

Benefits and Disadvantages of Being a Specialist on Fabaceae Bushes. *Chorthippus binotatus* has evolved from graminivorous species. We hypothesize that the shift could have been the consequence of an evolved sensibility to sparteine (and/or other quinolizidine alkaloids) in the shoots and not to Genisteae flowers because (1) young instars of the subspecies *C. b. binotatus* are never exposed to flowers, whereas (2) the adults of subspecies *C. b. moralesii* feed on both grasses and nonflowering Genisteae (Picaud et al. 1999). However, the diet shift within the genus *Chorthippus* has ecological consequences on nutrient balance, dispersal, and possibly predation risk.

Grasses growing on rich soils in spring contain as much nitrogen as Genisteae shoots and stems. However, nitrogen concentration of grasses decreases with plant maturity (Chapuis and Lefevre 1980). Finally, Genisteae in the autumn are richer in nitrogen than grasses growing on the poor soils of heathlands (Roze 1980, Glyphis and Puttick 1989). Consequently, feeding on Genisteae represents a gain in terms of nitrogen supply, at least when *Ulex minor* shoots and stems are consumed. However, when the specialist species shifts its diet toward *U. minor* flowers, we assume that the nitrogen supply falls. If compensatory feeding occurs, *C. binotatus* should eat twice as much on flowers as shoots. Nevertheless, the major advantage of this shift

should be an increase of fitness because of the presence of carbohydrates. We hypothesize that the sugars present in flowers favor the reproductive investment that occurs from the end of August until October, i.e., the main flowering season.

Graminivorous species have no constraint for dispersal as grasslands and hedges are connected. The occurrence of *C. binotatus*, however, is limited by the patchy distribution of *Ulex minor* heathlands. However, we showed that this specialist species can feed on Poaceae when there is no other available resource. Guéguen et al. (1975) found Poaceae phytoliths in *C. binotatus* faeces in Brittany. It should be pointed out that when the species specializing on Genisteae feeds on Poaceae, its vigilance level probably decreases, leading to a predation risk (Dukas 1998). Thus, population migrations between relatively close heathlands are possible but certainly infrequent. It would be interesting to know the percentage of individuals able to complete their life cycle with Poaceae as the only food source. Our preliminary experiments (Picaud et al. 2002) suggest that this percentage is very low at best. If a few generations could develop on grass, it would allow the species to colonize distant heathlands islands. Genetic distances between populations should be measured to estimate gene flow to address whether these populations are part of one or several metapopulations, and thus the extinction risk of each population.

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