Adult *Helix aspersa* snails were maintained individually for one week in plastic cages with 9 living *Lupinus albus* plants as their only food. Among these 9 plants, 3 chemotypes bitter, intermediate and sweet which differed in their alkaloid content were equally represented. Each day, the leaf surface grazed and the number of leaves attacked by the snails were recorded for each chemotype and each snail. A consumption/attack (C/A) ratio was calculated by dividing the surface grazed (C) by the number of attacks (A). The number of attacks and the grazed area were positively correlated for each chemotype during the whole experiment, and the snails ate similar quantities of lupin each day. After 4 and 6 days of experiment, we noticed a rejection of the bitter chemotype in favor of the intermediate and sweet ones respectively. After the 6th day, the surface grazed per attack was significantly higher on the sweet chemotype than on the bitter plants. We hypothesize that rejection of the bitter chemotype might be related to (i) an alkaloids reaction threshold associated with an increase in the amount of alkaloids in the wounded plants and/or (ii) aversive ingestive conditioning.

**INTRODUCTION**

Since Ehrlich & Raven (1964) promoted research on highly coevolved systems, many studies have focused on the relationship between plants and monophagous herbivores. However, as pointed out by Whelan (1982), the study of feeding preferences in polyphagous herbivores increases our comprehension of many adaptive mechanisms. In polyphagous terrestrial gastropods, such as slugs and snails, feeding preferences are influenced by the biochemical composition of the plants and especially by secondary metabolites. This has been shown for example for glucosinolates (Glen, Jones & Fieldsden, 1990; Moens, Couvreur & Cors, 1992), terpenoids (Rice, Lincoln & Langenheim, 1978; Gouyon, Fort & Caraux, 1983; Linhart & Thompson, 1995) and cyanogenic glucosides (Jones, 1962; Crawford-Sidebotham, 1978; Dirzo & Harper, 1982; Burgess & Ennos, 1987). The action of these chemicals may be toxic and/or deterrent. This is also the case for the alkaloids which are well represented in plants and especially in Fabaceae (Levin, 1976; Wink, 1984; Bruneton, 1993; Zarucchi, 1994). Their defensive role against different phytophagous taxa has long been known (Robinson, 1974; Cantot & Papineau, 1983; Kinghorn & Balandrin, 1984; Zuniga, Salgado & Corcuera, 1985; Metcalf & Metcalf, 1992; Razaka, Pothiers & Moncoulon, 1992). However, it has only been studied in pulmonates for pyrrolizidine (Speiser & Rowell-Rahier, 1991; Speiser, Harmatha & Rowell-Rahier, 1992) and quinolizidine alkaloids (Wink, 1984).


The aim of this study was to investigate the
effect of quinolizidine alkaloid concentration on the feeding choices of *Helix aspersa* and their eventual variations in relation to time using living plants to recreate natural conditions.

**MATERIALS AND METHODS**

*Biological materials*

Adult *Helix aspersa* (Gastropoda: Pulmonata) used in this experiment were obtained from a snail-farm in Brittany, France, and had never experienced any fresh plant food. They were maintained in covered plastic containers under the following conditions: temperature: 20 ± 1°C; relative humidity: 80 ± 5%; 12h/12h light/dark cycle. The snails were moistened and fed with a cereal-based flour for snail breeding produced by Arrieve Enterprises (St-Fulgent, France). Some small pots filled with moistened soil were also placed in the containers into which the snails could lay their eggs before the onset of the experiment. The food was removed from the containers two nights before the onset of the experiment in order to stimulate locomotor activity and feeding motivation (Adamo & Chase, 1991).

The plants used belonged to three chemotypes of white lupin, *Lupinus albus* L. (Fabaceae), which were grown in our greenhouse. The three chosen chemotypes differed in their leaf quinolizidine alkaloid content which was influenced both by seed's content and by genotypic expression (Table 1) (Harrison & Williams, 1983; Saito, Koike, Suzuki & Murakoshi, 1993). To summarize, some alleles at independent loci can affect the alkaloid levels in *L. albus* (Harrison & Williams, 1983). Amongst them, the alleles *pauper* and *nutricius* (Table 1) can both reduce the alkaloid biosynthesis in different manners, *nutricius* being the less efficient of the two. Those alleles are then responsible for the different alkaloid concentration between the ‘Lublanc’ sweet chemotype and the ‘Nyirsegui’ intermediate one.

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The Draggendorf test (Hackbarth & Trollh, 1959), carried out on the leaves, was used to verify the purity of the chemotype expressed by the bitter plants. It reacted positively to the presence of alkaloids, but only in concentrations higher than 0.1%. Thus, it did not react for the sweet and intermediate chemotypes and could not be used to discriminate between them. Each lupin leaf contained five to seven leaflets. Each morning, we observed the snails’ attacks on the leaves, traced and measured the consumed foliar areas with a scanner (Epson GT-8500) and Canvas 3.5 software. We wanted to take into account the eventual displacements of the snails during feeding. Considering that a snail can easily extend its foot over two or more leaflets without getting out of place, we scored all the visible injuries on each leaf as one “attack” (A), so that two leaflets wounded on the same leaf were considered as a single attack. Thus, we obtained daily and for each snail, the grazed areas and the number of attacks corresponding to each chemotype.

A C/A ratio (= meal size per attack) was calculated by dividing the cumulative consumption (C) by the cumulative number of attacks (A) for each chemotype.

**Experimental conditions**

Each plant was grown individually in a compost-filled pot (8 × 8 × 7 cm). The pots were placed nine by nine in 20 containers (25 × 25 × 8 cm) as soon as the plants were three-week-old and possessed from five to seven well-developed leaves. The space between the pots was filled with moistened compost. The three chemotypes were distributed in latin-square combination in the container (so that plants belonging to the same chemotype were not placed side by side). To prevent snails from eating cotyledons, which do not have the same alkaloid content as the leaves, each cotyledon was covered with a plastic film held in place with a clip. At this stage, the cotyledons were not essential for the plants’ survival as photosynthesis was active in the leaves. The containers were maintained under the thermohygrometric and photoperiodic conditions previously described. Each container was covered with a cage of plastic net (mesh: 1 × 1 cm). One snail was placed in each container two hours before the onset of the dark phase. The experiment lasted one week. At the end of the experiment, only 14 replicates were analysed because 6 snails did not show any feeding activity.

**Table 1.** Chemotypes of *Lupinus albus* used in the experiment and alkaloid contents of seeds (data from Cantot & Papineau, 1983)

<table>
<thead>
<tr>
<th>Designation</th>
<th>Chemotype</th>
<th>Seeds’ alkaloids content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bitter</td>
<td>Spanish ecotype, E20</td>
<td>1.5</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Hungarian variety, «Nyirsegui»</td>
<td>0.1</td>
</tr>
<tr>
<td>Sweet</td>
<td>French variety, «Lublanc»</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>
Temporal changes of the ingestion of each chemotype

There was no significant difference in the overall amount of lupin ingested (i.e. daily global consumption of the three chemotypes together) over time (ANOVA, $F = 1.12$, $P > 0.05$) (Figure 1). The daily consumption rate was around $1600 \text{ mm}^2$. Inter-individual variation was high throughout the experiment. The cumulative daily ingestion of the intermediate and sweet chemotypes showed similar linear increases (Figure 2), and no significant difference could be found between them throughout the experiment (ANOVAs, $P > 0.05$). During the first three days, the daily ingestion of the bitter chemotype seemed to evolve in the same way as the two others and no significant difference could be found between the three chemotypes (ANOVAs, $P > 0.05$). It then decreased each day and remained at a relatively low level, indicating a regular decrease of the daily ingestion of the bitter chemotype. The consumption difference became significant after the fourth day of experiment between the bitter

![Figure 1](image-url)
and the intermediate chemotypes (Fisher’s PLSD = 1261.4, P < 0.05) and after the sixth day between the bitter and the sweet chemotypes (Fisher’s PLSD = 1811.8, P < 0.05). This indicates that the bitter chemotype was progressively rejected in favour of the sweet and intermediate ones.

Temporal changes in the number of snail attacks on the three chemotypes

The bitter chemotype was significantly less attacked than the intermediate one after the third day (Fisher’s PLSD = 1.62, P < 0.05), and then the sweet chemotype after the sixth day (Fisher’s PLSD = 2.61, P < 0.05) (Figure 3). Moreover, there was a regular decrease in the number of attacks on the bitter chemotype and, to a lesser extent, on the sweet one. The trends in the number of leaves attacked (Figure 3) were similar to those of the consumed areas (Figure 2). The inter-individual variation of the number of attacks was, again, quite high.

Relation between ingestion and number of attacks

Each day, the cumulative ingestion and the cumulative number of attacks were highly correlated for each chemotype (P < 0.0001, R^2 bitter = 0.92; R^2 inter = 0.67; R^2 sweet = 0.79).

The C/A curves of the bitter and intermediate chemotypes followed similar patterns with no significant difference between them (ANCOVA, P > 0.05). They exhibited a stabilisation after the two or three first days and remained low throughout the experiment (Figure 4). The C/A ratio of the sweet chemotype rose considerably after the third day of experiment (ANCOVA, P < 0.05), it seemed to stabilise after day 6.

There was no significant difference in the area grazed per attack (C/A) between the three chemotypes during the five first days (ANOVA, P > 0.05), but the sweet and bitter chemotypes differed significantly after the sixth day (Fisher’s PLSD = 415.8, P = 0.03).

Figure 2. Temporal changes in the cumulative daily leaf ingestion of three chemotypes of *Lupinus albus* by adult land snails *Helix aspersa* (sample size is 14 for each data point) (mean ± standard error).
FEEDING CHOICE OF *HELIX ASPERSA* IN RELATION TO ALKALOIDS

DISCUSSION

The low level of consumption on the first day can be explained by introduction into a new environment, as a consequence of food deprivation (Adamo & Chase, 1991), or by the fact that the snails were just careful before eating a novel food. The stability of the daily global ingestion throughout the experiment shows that when a snail rejects one chemotype, it compensates by a higher ingestion of the other chemotypes. The ingestion of the bitter/repellent chemotype does not lead to any decrease of the total ingestion.

After the fourth day, the rejection of the bitter chemotype was significant. However, *Helix aspersa* was never able to discriminate between the intermediate and the sweet chemotypes and seemed to exhibit a reaction threshold to alkaloids, only reacting to concentrations between those of the bitter and intermediate chemotypes, as was also observed in *Sitona lineatus* (Curculionidae) which is an oligophagous species feeding only on Fabaceae (Cantot & Papineau, 1983). The rejection of the bitter chemotype seemed to increase over time. Two non-mutually exclusive hypotheses can be proposed. (i) The concentration threshold may only be reached by the bitter chemotype when the plants are wounded. A quick increase in lupins’ foliar alkaloid production may occur within 2–4 hours after an injury caused by a herbivore (Wink, 1983). Thus, a leaf wounded by a snail would become more repellent within a short time. (ii) Aversive conditioning may be responsible for this temporal modification of feeding choice as has been shown in different mollusc species (Delaney & Gelperin, 1986; Sahley et al., 1990). Suboski (1992) also indicates that an aversive conditioning may occur more rapidly than a non-aversive one. Here, the relatively quick response of snails towards alkaloids seems to be consistent with this hypothesis.

After non-aversive ingestive conditioning, Croll & Chase (1977; 1980) observed some modifications in the rate of *Achatina fulica* individuals that orientated themselves towards 2 plants, and Desbuquois & Daguzan (1995) showed that 10 to 15 days of conditioning could...
induce a modification in the amount of food ingested by *Helix aspersa*. The temporal change of the number of attacks for the 3 chemotypes might reflect snail differential orientation that probably includes distance chemoreception and post-ingestive mechanisms.

Alkaloids are stored in foliar vacuoles and are only released after crushing (Wink, 1986). Thus, alkaloid concentration could probably not be detected at distance, at least at the beginning of the experiment when plants were intact.

Chemoreception in snails is well developed (Rogers, 1971; Farkas & Shorey, 1976; Croll, 1983) but the respective roles of olfaction, taste and post-ingestive effects in feeding choice are not very clear, especially under natural conditions. At the end of our experiment, the C/A ratio of the sweet chemotype differed significantly from the bitter one, i.e. a single attack on a sweet plant led to the ingestion of a higher amount of tissue than an attack on the bitter chemotype. The gustatory abilities of the snails were attested by the positive correlation between the number of attacks and the quantity of lupin ingested.

It is advantageous for terrestrial molluscs to be able to quickly recognize the chemical characteristics of a plant in order to assess its nutritional value and toxicity, because locomotion carries a high energetic cost in these organisms (Denny, 1980; Bailey, 1989). Chemoreception and conditioning mechanisms should be linked with the foraging strategy of this species, and plasticity in feeding choice therefore emerges as adaptive.

The results described in this paper indicate that *Helix aspersa* has an ability to detect quinolizidine alkaloids, frequent in nature, even if *H. aspersa* and *Lupinus albus* are not commonly associated in the wild. Snail feeding-choice appears to be largely based on taste but the toxicity of the quinolizidine alkaloids do not seem to be an absolute defence against snails' grazing. However, quinolizidine alkaloids could still be a protection for alkaloid-bearing plants, as the high-alkaloid containing lupins were decreasingly attacked over time. Moreover, the fact that snails may prefer the intermediate-content lupins during the first days of the experiment suggests that (i) quinolizidine alkaloids

Figure 4. Temporal changes in the C/A ratio (cumulative daily ingestion / cumulative daily number of attacks) by adult land snails *Helix aspersa* for three chemotypes of *Lupinus albus* (sample size is 14 for each data point) (mean ± standard error).
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alone cannot entirely explain snail feeding-choice, or that (ii) selection for food material is a feedback mechanism controlled by post-ingestive effects.

Another interesting point that emerges from this study is that snails might compensate for a toxic ingestion by increasing consumption of a less-toxic plant. H. aspersa could thus exert a selective pressure upon the plants of its habitat.

Feeding-choice experiments under natural conditions should allow us to get further information about the foraging strategy of this opportunistic herbivore, trying to identify the prevailing parameters (both chemical and physical) in ingestion and avoidance of plants.

ACKNOWLEDGMENTS

We wanted to thank the I.N.R.A. of Lusignan for supplying the seeds. We are very grateful to Dr S.E.R. Bailey for his helpful comments and advice on this manuscript. We also wish to thank J.S. Pierre for his kind suggestions and V. Briand for her precious technical help.

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