

“Caterpillar, Snail and Slug Bioassays with Rare and Common Plant Species”

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From left to right and top to bottom: Caterpillar of *Spodoptera littoralis*, leaf damages of *Spodoptera littoralis*, *Arion vulgaris*, flowering *Sideritis hyssopifolia*, leaf damages of *A. vulgaris*, *Cepaea nemoralis*

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Abstract

Conservation of rare plant species is an integral part of preserving biodiversity. However, the mechanisms which determine why some species are rare, and the others are common, are not yet fully understood. My study investigated this question about rarity with focus on the differences in plant-herbivore interactions between rare and common plant species. I used a multispecies experiment with thirty-five plant species, examining ten common and twenty-five rare species. I analysed whether herbivores perform better on leaves of rare or of common plants. Performance assays were conducted with three generalist herbivores, the caterpillar *Spodoptera littoralis*, the snail *Cepaea nemoralis* and the slug *Arion vulgaris*, and preference assays with *S. littoralis*. The results demonstrate differences between juvenile caterpillars and adult molluscs. They indicate that different herbivores as well as their life stages impact plants differently. The molluscs discriminate their food before they start to eat and then eat more of some species than of others, whereas *S. littoralis* generally attacks leaves from all plant species but performs better on rare species. Overall, the results indicate that rare species get proportionally more eaten and lead to better performance of herbivores *A. vulgaris* and *S. littoralis*. Thus, experiments about herbivores as drivers for rarity strengthen further investigations. Finally, the findings on the impact of different herbivores in different life-stages on rare plant species can help improve conservative measures for rare plant species.

Introduction

Biodiversity is defined as the diversity on the level of genetics over organisms up to diversity in ecosystems and interactions within and between the levels (Bundesamt für Umwelt (BAFU) 2017). It is “the basis for our shared wealth, health and well-being” (Ban Ki-Moon in Secretariat of the Convention on Biological Diversity 2014). However, biodiversity is threatened and under decline, putting our well-being at stake (Secretariat of the Convention on Biological Diversity 2010). Main risks for biodiversity are degradation and loss of natural habitats, increasing deposition of nutrients, climate change and invasive alien species (Secretariat of the Convention on Biological Diversity 2010). Since biodiversity, sustainable development and human well-being is tightly linked, the

Convention on Biological Diversity (CBD) targets the conservation of biological diversity on international level. As a member of the CBD, Switzerland has developed their own national Action Plan Biodiversity in 2017 (Bundesamt für Umwelt (BAFU) 2017). One part of the action plan is the special protection of endangered species, or species for which Switzerland carries international responsibility (Bundesamt für Umwelt (BAFU) 2017). Such species are registered in the National Priority Species List (BAFU 2011) consisting of 3606 species from 21 different groups of organisms, including around 700 vascular plant species. One criteria of the priority list is the worldwide, or in some cases European, distribution of species (BAFU 2011). Plant species on the priority list have low abundance or a small range size, and are considered as rare according to Gaston (1994). Rare plant species can become even more endangered when they exist in small populations only. In small populations, stochastic processes like inbreeding, loss of genetic variation, or genetic drift can further increase the probability of extinction (Conner and Hartl 2004).

In order to preserve rare and threatened species, it is imperative to know the causes of their rarity and endangerment. Yet, the mechanism why some species are rare, and others are common is not yet fully understood. One way to study causes of rarity is to analyse coexistence of species and community assembly. Coexistence theory (reviewed by Chesson 2000) explains maintenance of species diversity as an interplay of environmental factors, biotic interactions, feedbacks and inter- and intraspecific interactions. Furthermore, differences in niche breadth and fitness between species considerably shape communities. The assembly of a community combines, in addition to the processes within coexistence theory, the processes operating on regional scale like species pool, dispersal and stochasticity (reviewed by HilleRisLambers et al. 2012).

Another way to study causes of rarity is to look at factors influencing species abundance and comparing environmental factors and life-history traits or functional traits of rare and widespread plant species. Previous studies analysed differences in single traits within pairwise comparisons of a rare species and a widespread congener (see Bevill and Louda 1999). However, only more recent studies could find general causes of rarity because they observed and compared life-history traits over a broader range of species. They found that common species have better establishment traits as they are generally larger (Lavergne et al. 2003, 2004), have bigger and more flowers (Lavergne et al. 2004) and respond more positively to nutrients (Dawson et al. 2012). Wamelink and his colleagues (2014) found that rare species have more limited habitat preferences, i.e. narrower niche breadth, for abiotic soil conditions. Moreover, rare species suffer more from negative soil-feedback (Kempel et al. 2018). Furthermore, Hugo Vincent (2017) showed not only that seeds of rare species germinates with lower percentage than seeds of common ones, but also that rare species have narrower niche breadth concerning climate.

Biotic interactions, particularly with herbivores, have been also suggested as an important mechanism driving species abundance (Kempel et al. 2015). In theory, herbivores can stabilise biodiversity in a community either when specialist herbivores regulate their hosts independently, or when generalist

herbivores switch their hosts according to their abundance. In effect, generalist herbivores, alone and synergistically, increase plant species richness and decrease productivity (Allan and Crawley 2011). They could even override bottom-up effects, i.e. effects through nutrients, at least in intermediate productive grasslands (Alberti et al. 2017). The effects were even stronger with diverse herbivores. Herbivores affect fitness of plants as it affects the plant's ability for reproduction and competition (Züst and Agrawal 2017). Since resource allocation in defence, reproduction and growth are costly, their relationships are in trade-off (Züst and Agrawal 2017). Such a growth-defence trade-off was shown by Kempel and her colleagues (2015). They investigated widespread and less abundant species in grassland systems and showed that less abundant species were more palatable and profited more from herbivore exclusion by increasing their abundance. Thus, this more palatable plant species invest less in defence but more in growth. However, their study is limited to montane hay meadows (Stein et al. 2010) and neither can it show the impact of herbivores on common and rare species in general. Alternatively, one could also argue based on the growth-defence hypothesis that common species are less defended because they invest their energy into growth and competition. In contrast, rare species would be more defended compared to common ones. In addition to the growth-defence hypothesis, the appearance hypothesis (Feeny 1976) should be considered as well to assess the general patterns of herbivore impacts on common and rare plant species. The appearance hypothesis predicts that widespread, common species are more defended because they are more apparent and thus under higher herbivore pressure.

This study starts to fill this gap of how herbivores are related to rarity of plant species in general. To that effect, I performed several herbivore performance and preference bioassays to unravel the influence of herbivores on plant rarity. I conducted a multispecies experiment with thirty-five plant species, ten common and twenty-five rare priority species in Switzerland (BAFU 2011). The experiments were mainly performance assays with three different generalist herbivores, *Spodoptera littoralis* caterpillars, adult snails of *Cepaea nemoralis* and adult slugs of *Arion vulgaris*. Additionally, I conducted preference assays with *S. littoralis* caterpillars. My main hypotheses were: (0) The null hypothesis states there is no difference between rare and common species in respect to herbivores. (1) Alternatively, common plant species invest more in competition and thus more in growth and less in defence, whereas rare species invest likely more in defence. So, common species are more preferred and more valuable to generalist herbivores than rare plant species. Therefore, herbivores perform better on common plants. (2) A second alternative would be that rare species are generally more attractive. They are less exposed to selection pressure by herbivores as they are less apparent. Therefore, they could worse adapt for defence against herbivores. Thus, herbivores perform better on rare species.

Material and Methods

Biological Material

Plants

Seeds of rare and common plant species were collected during summer and autumn in 2016 and 2017 by Adrian Möhl. For this study, all plant species on “The Swiss List of National Priority Species” (BAFU 2011) were considered as rare, and all the other ones as common. The seeds were collected from different parts of Switzerland (Canton of Bern, Fribourg, Jura Mountains, Ticino, Wallis and Vaud) according to their partly very restricted distribution range (Table 1).

In February 2018, the seeds were treated with a cold stratification at around 4° Celsius over 6-8 weeks in a dark cold chamber within the greenhouse (Ostermundigen, Switzerland). The seeds were sown in pots of 10 cm in diameter filled with seedling substrate (Klasman-Deilmann GmbH). At the end of March 2018, the plant pots were randomly distributed on three tables in the greenhouse (Ostermundigen, Switzerland), and raised for two weeks. Then, the plants were transplanted individually into 11 × 11 cm pots filled with 9:1 Kultursubstrat (potting soil with 25% Landerde [normal soil]: sand). Pots were watered when necessary. Because of strong infestation of black flies and mites in the greenhouse, all plants were treated with a short-lasting insecticide (Pyrethrum FS, Andermatt Biocontrol AG, Grossdietwil, Switzerland) before the start of the experiment. Finally, we had thirty-five plant species from fourteen different plant families (1 –11 species per family), from which ten species belong to the group “common” and twenty-five species to “rare” (Table 1).

As some plant species were already in senescence or had not enough leaves when the mollusc experiments started, the number of species decreased to twenty-three species from ten different plant families, with eight common species and fifteen rare species.

Table 1: Final species list with their three letter shortcut, area of origin, plant family and in which herbivore assay they are used.

Shortcut	Species	Origin	Rarity	Family	Herbivores
ACHMIL	<i>Achillea millefolium</i>	Visp, VS	common	Asteraceae	none
AIRELE	<i>Aira elegantissima</i>	Geimen Naters, VS	rare	Poaceae	none
ARATUR	<i>Arabis turrata</i>	Felsch Biel, BE	common	Brassicaceae	<i>A. vulgaris</i>
AREGRA	<i>Arenaria grandiflora</i>	Mont Tendre, VD	rare	Caryophyllaceae	<i>S. littoralis</i>
ARTBOR	<i>Artemisia borealis</i>	Blauherd, VS	rare	Asteraceae	<i>A. vulgaris</i>
ARTGLA	<i>Artemisia glacialis</i>	Blauherd, VS	rare	Asteraceae	<i>S. littoralis</i> , <i>A. vulgaris</i>
BROSQU	<i>Bromus squarrosus</i>	Visperterminen, VS	rare	Poaceae	<i>S. littoralis</i>
CARCER	<i>Carpesium cernuum</i>	Pregassona, TI	rare	Asteraceae	<i>S. littoralis</i> , <i>A. vulgaris</i>
CAUPLA	<i>Caucalis platycarpos</i>	Visp, VS	rare	Apiaceae	<i>S. littoralis</i>
CENJAC	<i>Centaurea jacea</i>	Bern Muristrasse, BE	common	Asteraceae	<i>S. littoralis</i> , <i>A. vulgaris</i>
CENPUL	<i>Centaureum pulchellum</i>	Turtig, VS	rare	Gentianaceae	<i>S. littoralis</i>
CENVAL	<i>Centaurea valesiaca</i>	Central Valais, VS	rare	Asteraceae	<i>S. littoralis</i> , <i>A. vulgaris</i>
COCPYR	<i>Cochlearia pyrenaica</i>	Gantrisch BE	rare	Brassicaceae	<i>A. vulgaris</i>
CREFRO	<i>Crepis froelichiana</i>	Alpe Boglia, TI	rare	Asteraceae	<i>S. littoralis</i> , <i>A. vulgaris</i>
ERYCHE	<i>Erysimum cheiranthoides</i>	Holligen, BE	common	Brassicaceae	<i>S. littoralis</i>
HYPPER	<i>Hypericum perforatum</i>	Bern Güterbahnhof, BE	common	Hypericaceae	<i>S. littoralis</i> , <i>A. vulgaris</i>
HYPRAD	<i>Hypochaeris radicata</i>	Holligen, BE	common	Asteraceae	<i>S. littoralis</i> , <i>A. vulgaris</i>
HYPRIC	<i>Hypericum richeri</i>	Mont Tendre, VD	rare	Hypericaceae	none
KNAARV	<i>Knautia arvensis</i>	Holligen, BE	common	Caprifoliaceae	<i>A. vulgaris</i>
LEOINC	<i>Leontodon incanus</i> ssp <i>tenuiflorus</i>	Alpe Boglia; TI	rare	Asteraceae	<i>S. littoralis</i> , <i>A. vulgaris</i>
LINVUL	<i>Linaria vulgaris</i>	Visp, VS	common	Plantaginaceae	<i>S. littoralis</i> , <i>A. vulgaris</i>
MINCAP	<i>Minuartia capillacea</i>	Mont Tendre, VD	rare	Caryophyllaceae	<i>S. littoralis</i>
ORIVUL	<i>Origanum vulgare</i>	Egelsee Bern, BE	common	Lamiaceae	<i>A. vulgaris</i>
POAREM	<i>Poa remota</i>	Bern, BE	rare	Poaceae	<i>A. vulgaris</i>
POLMAJ	<i>Polycnemum majus</i>	Leuk, VS	rare	Amaranthaceae	<i>S. littoralis</i>
POTMUL	<i>Potentilla multifida</i>	Gornergrat, VS	rare	Rosaceae	<i>A. vulgaris</i>
		Zermatt, VS	rare	Rosaceae	<i>S. littoralis</i>
RORISL	<i>Rorippa islandica</i>	Schwarzsee; FR	rare	Brassicaceae	<i>S. littoralis</i>
RUMHYD	<i>Rumex hydrolapathum</i>	Wohlensee, BE	rare	Polygonaceae	<i>A. vulgaris</i>
SCRNOD	<i>Scrophularia nodosa</i>	Magnedens, FR	common	Scrophulariaceae	<i>A. vulgaris</i>
SCUALP	<i>Scutellaria alpina</i>	Mauvoisin, VS	rare	Lamiaceae	<i>A. vulgaris</i>
SIDHYS	<i>Sideritis hyssopifolia</i>	Mont Tendre, VD	rare	Lamiaceae	<i>A. vulgaris</i>
TARDIS	<i>Taraxacum dissectum</i>	Combe des Ambourneux, VD	rare	Asteraceae	<i>S. littoralis</i> , <i>A. vulgaris</i>
THLROT	<i>Thlaspi rotundifolium</i> ssp <i>corymbosum</i>	Rotenboden, VS	rare	Brassicaceae	<i>S. littoralis</i>
THLSYL	<i>Thlaspi sylvium</i>	Gagenhaupt, VS	rare	Brassicaceae	<i>A. vulgaris</i>
		Findelalp, VS	rare	Brassicaceae	<i>S. littoralis</i>
XERINA	<i>Xeranthemum inapertum</i>	Visp, VS	rare	Asteraceae	<i>S. littoralis</i> , <i>A. vulgaris</i>

Herbivores

For the first part of herbivore assays, I used caterpillars of the species *Spodoptera littoralis* (Boisdval) (Lepidoptera: Noctuidae). They were chosen as they are generalists known to feed on a broad range of plant species from different families including families of our experimental plant species, e.g. Asteraceae, Caryophyllaceae, Lamiaceae, Poaceae, Rosaceae; Brown and Dewhurst 1975). *S. littoralis* is also known to be a model organism to study plant – herbivore interactions (e.g. Erb et al. 2011; Kempel et al. 2011, 2015; Züst and Agrawal 2017).

Around thousand caterpillars were hatched from eggs (Syngenta, Stein, Switzerland) and grown in plastic boxes (16 × 13 × 6 cm) at room temperature (24 ± 4° C) until the begin of the experiment. They were reared with a maize-based artificial diet kindly provided by Robert CAM.

For the second herbivore assay, I collected *Cepaea nemoralis* (Linnaeus, 1758) (Pulmonata: Helicidae) four days before the experiment in the botanical garden Bern (Bern, Switzerland). They were chosen because they are easy to collect, handle and label, and they accept cellulose in form of tissue paper as food source (Grime et al. 1968; Boch et al. 2014). Tissue paper is a neutral, starvation diet, which is used to preclude pre-adaptation of the snails to specific plant species they had eaten before the collection. Whether the snails have eaten the tissue paper can be easily checked by the white colour of their faeces (Grime et al. 1968).

Thirty-six snails were kept for three days within a plastic box (38 × 28 × 20 cm, GLES box, IKEA) covered with a perforated plastic wrap (polyethylene, ALDI SUISSE AG, Ostermundigen, Switzerland) and filled with mosses, water and salad leaves, in an 11° C fridge. One day before the experiment, I brought them to the greenhouse (Ostermundigen, Switzerland) and separated the snails into black rectangular trays (17 × 12.5 × 5.5 cm, polystyrene) closed with perforated plastic wrap and fed them with wet tissue paper (solo, Plantos Prd. und Vertriebsges. mbH & Co. KG, Buchholz, Germany). The snails were labelled on the shell.

The third chosen herbivore was *Arion vulgaris* (Moquin-Tandon) (Syn.: *A. lusitanicus* Mabille; Pulmonata: Arionidae). They are abundant and widespread in Europe and known as a general pest (Pfenninger et al. 2014; Rowson 2017). I collected more than fifty individuals one day before the first experiment in the botanical garden Bern (Bern, Switzerland) and brought them to the greenhouse (Ostermundigen, Switzerland). Like in the *C. nemoralis* bioassay, I separated them into rectangular trays closed with perforated plastic wrap and fed them with wet tissue paper to empty their stomach. Forty-six of the pots with the individual slugs were labelled. The other slugs were kept as a reserve.

After the first round of slug assays, all pots were cleaned and filled with a piece of salad leaf and wet tissue paper to prepare the snails for the second round. Four days later, the slugs had eaten the salad and started eating the tissue paper.

Herbivore Assays

Performance of *Spodoptera littoralis*

The performance assays with *S. littoralis* were conducted with three-day old caterpillars. For the experiments, I randomly chose one individual per plant species, and punched four circles out of a few leaves with a whole puncher of 1 cm in diameter. When the leaves didn't allow punching out entire plant material (e.g. due to a pinnate leaf shape), I cut similar amounts of leaf area with scissors. The leaf discs were placed in petri dishes (94 × 16 mm, Greiner BioOne International GmbH) filled with 25 ml Agar-Agar (1.5% w/v) (granuliert, BioScience, Carl Roth GmbH & Co. KG, Karlsruhe, Deutschland). Agar-Agar ensures constant humidity, preventing a rapid wilting of the leaf discs. The leaf discs within the petri dishes were photographed from a standardised distance before and after the experiment (Nikon D750 [Nikon Corporation, Japan], Lightning desk, RSX Copy Stand with RTX camera arm [Kaiser Fototechnik GmbH & Co.KG, Buchen, Switzerland]).

Always three larvae were weighted (Sartorius Cubis, Sartorius Lab Instruments GmbH & Co. KG, Göttingen, Germany) together and placed into the prepared petri dishes. I closed the petri dishes with parafilm with some air holes.

During the experiment of two-day length, the larvae were kept in a climate chamber (16 h 22 °C / 8 h 18 °C day / night and 70% hum) (Percival Scientific Inc., Perry, Iowa, USA). In-between, I checked the larvae and recorded their mortality.

After two days, mortality and the weight of the surviving larvae were recorded.

Preference of *Spodoptera littoralis*

In addition to the performance assays, I conducted preference assays with 6-day old caterpillars of *S. littoralis*.

The Agar-Agar petri dishes were divided into four squares. Within the middle of each square I placed one leaf disc, with 1 cm distance from the edge. Leaf discs from the opposite side belong to the same plant species. Plant species were randomly paired resulting in overall forty pairs of twenty-one common-rare, fourteen rare-rare and five common-common-pairs.

Petri dishes were photographed before and after the experiment. The experiment ran for twenty-four hours.

As the preference assays with *S. littoralis* did not generate sufficient data due to high mortality rate, an analysis of the experiments was not feasible.

Performance of *Cepaea nemoralis*

I randomly chose one individual per plant species from the reduced species range and cut a part of a large leaf or several small leaves from one plant, respectively, and took a photo of them. *C. nemoralis* were weighted and put into new rectangular pots with the photographed pieces of plant leaves. After

one day, I made ventilation slots in the bottom part of the pots in addition to the holes in the plastic wrap.

Performance assays with snails was broken off after two days because the snails refused to eat the leaves and were retracted during most of the experimental time. Even tissue paper was more attractive (personal observation). Thus, no data analysis was performed.

Performance of *Arion vulgaris*

The experimental set up for *A. vulgaris* was identical to *C. nemoralis*, except for that the slug experiment ran over twenty-four hours.

The morning after the experiment leaf leftovers were collected and photographed. Because the weight of the slugs strongly depends on their water content (which I could not control for), I decided to focus on the leaf area eaten by the slugs instead of their weight gain.

The second round of experiments was conducted four days after the end of the first round. Tissue paper was removed and replaced with pieces of leaves of randomly chosen plant species. The only condition was that no slug received twice the same plant species. The leaves were photographed before and after the experiment.

Analysis of leaf area

Photographs of all herbivore assays were analysed using Fiji by calculating the leaf areas from particles (Schindelin et al. 2012). Thereby, scale was set on a known distance and colours were converted to binary.

Statistical Analysis

Statistical analyses were carried out in R Studio (R Developmental Core Team 2017). Figures are made with ggplot() out of the package “ggplot2” (Wickham 2016).

Because of a temporary contamination in the breeding facility of Syngenta, most caterpillars of the experiments were infected with a nuclear polyhedrosis virus (NPV) causing a high mortality rate (Jones et al. 1994, Robert CAM personal communication). Hence, the preference assays gave not enough observations for their analysis. Evaluable observations for the performance assays were likewise strongly reduced because I included only observations where all three larvae survived the two-day experiment. At the end, the data frame was reduced from two-hundred and nine to thirty observations. Thereby, plant species range was reduced to twenty-two species, with seventeen rare and five common species, from ten families (see Table 1). This reduction also resulted in a very unbalanced dataset. A Jackknife resampling technique was attempted to handle the imbalances, but balanced models were too small to analyse the multi-species experiment. Hence, I decided to analyse the data with mixed effects models as they are considered to be robust with unbalanced data (Kuznetsova et al. 2017).-I calculated the performance of caterpillars as their response in weight gain using the difference between final and initial weight (final weight - initial weight). I could not analyse

the binary response whether caterpillars have started to eat leaves or not as almost all of the caterpillars started to eat. Leaf damage during the feeding trials was analysed using the difference between leaf area before and after (leaf area before – leaf area after). Some leaf damages resulted in negative values due to measurement errors. I redefined those values to zero because it is a conservative method (it underestimates the amount of leaf damage) in order not to lose more observations. I checked the relationship between weight gain and leaf damage using simple linear regression. Furthermore, I used linear mixed effects models (lmer, package "lme4", Bates et al. 2015) to compare the relationship of the response variables (weight gain, leaf damage) in dependence of plant rarity. Plant species were taken as random factors. I visually checked the residuals of all models for normality and homoscedasticity. To improve normality of errors and homogeneity of variances, leaf damage was square root transformed. P-values resulted from a likelihood ratio test of the full model with the effect plant rarity against a model without plant rarity as effect. Plant species were tested with a likelihood ratio test for random effects (ranova, package "lmerTest", Kuznetsova et al. 2017a).

Performance of *A. vulgaris* was analysed in two steps: (1) whether they have started to eat leaves or not (binary response variable), and (2) for those which fed on leaves, how much of a leaf area was eaten (leaf damage). I excluded the observation with slug individual number 31 from the analysis because it died during the experiment. For (1), the observations were divided into the two groups “eaten” and “not eaten”. I set a threshold for not eaten leaves at ninety-five percent of their initial leaf area instead of hundred percent to account for measurement errors. The other observations were treated as eaten. I checked the reasonableness of these two groups while re-examining the photos by eye. I used general mixed effects models (glmer, package “lme4”, Bates et al. 2015) to analyse the binomial variables due to plant rarity. The hypotheses were tested with a likelihood ratio test against a model without plant species as effect. Model estimates and standard errors were obtained using the package “effects” (Fox 2003; Fox and Hong 2009) and displayed by inversed logit values (inv.logit, package "boot", Davison and Hinkley 1997; Cauty and Ripley 2017). For (2), I calculated the response of the slugs in leaf damage like the leaf damage of *S. littoralis* (final weight - initial weight) and redefined as well negative leaf damages as zero leaf damage. Means of leaf damages explained by plant rarity were analysed with linear mixed effects models (lmer, package “lme4”, Bates et al. 2015). For all slug models, plant species and the slug identity were taken as random factors. I visually checked the residuals of all models that normality and homoscedasticity was given. ranova() from the “lmerTest” package (Kuznetsova et al. 2017) was used to get an ANOVA-like table for random effects for all linear mixed effects models.

Results

There was no correlation of leaf damage and weight gain of the caterpillars in the experiment (adjusted $R^2 = 0.044$, p -value = 0.137, Figure 1 (1)), suggesting independency of plant palatability and nutritious value. Plant rarity affected caterpillar's weight gain (p -value = 0.036; Table 2, Figure 1 (2)). Whereas for leaf damage, rarity did only marginally influence the amount of eaten leaves by the caterpillars (p -value = 0.092; Table 2, Figure 1 (3)). Plant identity (species) did not significantly affect the weight gain nor leaf damage of the caterpillars (p -value = 0.272, p -value = 0.517; Table 2). Hence, caterpillars did not seem to particularly prefer certain plant species.

A. vulgaris showed no difference in its performance between rare and common plant species, neither in the binary response eaten or not eaten (p -value = 0.195; Table 3) nor in amount of eaten leaves (p -value = 0.583; Table 3). However, performance of slugs depends on individual plant species. There are some species not at all eaten by the slugs (p -value < 0.001; Table 3) and some species noticeably more eaten (p -value < 0.001; Table 3).

Table 2: Results of linear mixed effects models testing the fixed effect plant rarity and the random effect plant species on weight gain of and leaf damage by *S. littoralis* (square root transformed) as dependent variables.

<i>Spodoptera littoralis</i>	weight gain			$\sqrt{\text{leaf damage}}$		
fixed effects	χ^2	Df	p -value	χ^2	Df	p -value
plant rarity	4.4179	1	0.03556	2.8354	1	0.09221
random effects	χ^2	Df	p -value	χ^2	Df	p -value
plant species	1.2091	1	0.2715	0.42054	1	0.5167

Table 3: Results of generalized linear mixed effects model and linear mixed effects model testing the fixed effect plant rarity and the random effects plant species and slug individuals on eaten vs. not eaten leaves and leaf damage, respectively, with *A. vulgaris* as dependent variable.

<i>Arion vulgaris</i>	eaten - not eaten			leaf damage		
fixed effects	χ^2	Df	p -value	χ^2	Df	p -value
plant rarity	1.6769	1	0.1953	0.3008	1	0.5834
random effects	χ^2	Df	p -value	χ^2	Df	p -value
plant species	12.747	1	< 0.001	38.884	1	< 0.001
slug individuals	0	1	0.9999	0	1	1

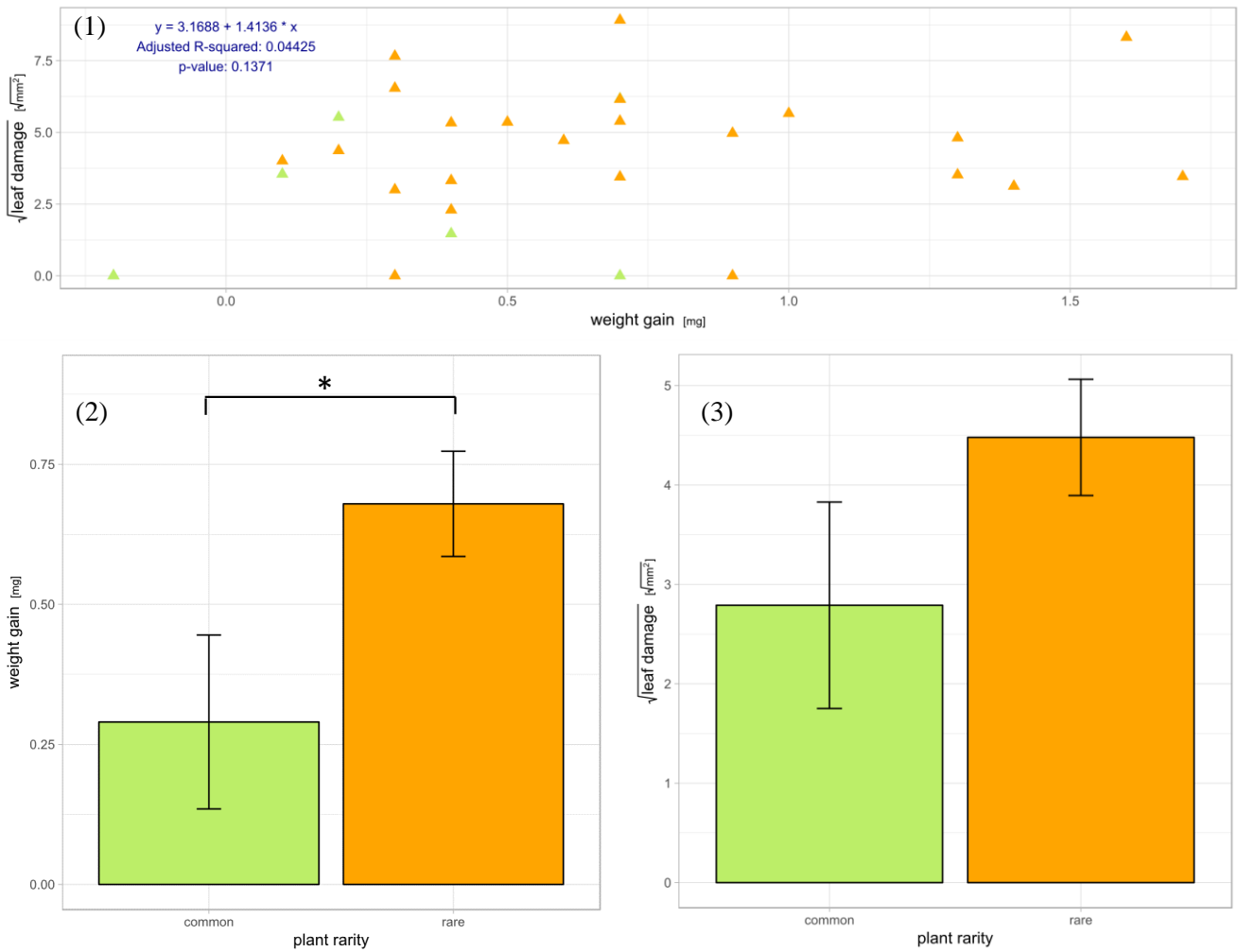


Figure 1: Relationship between performance of *S. littoralis* as (1) relation between leaf damage (square root transformed) and weight gain, and as relation between plant rarity and (2) weight gain (difference between final and initial weight), or (3) leaf damage (square root transformed difference between initial and final leaf area). (2,3) Depicted are the means of the species means for rare and common plants, with standard error bars. Statistically significant values are indicated with an asterisk. *, p -value < 0.05.

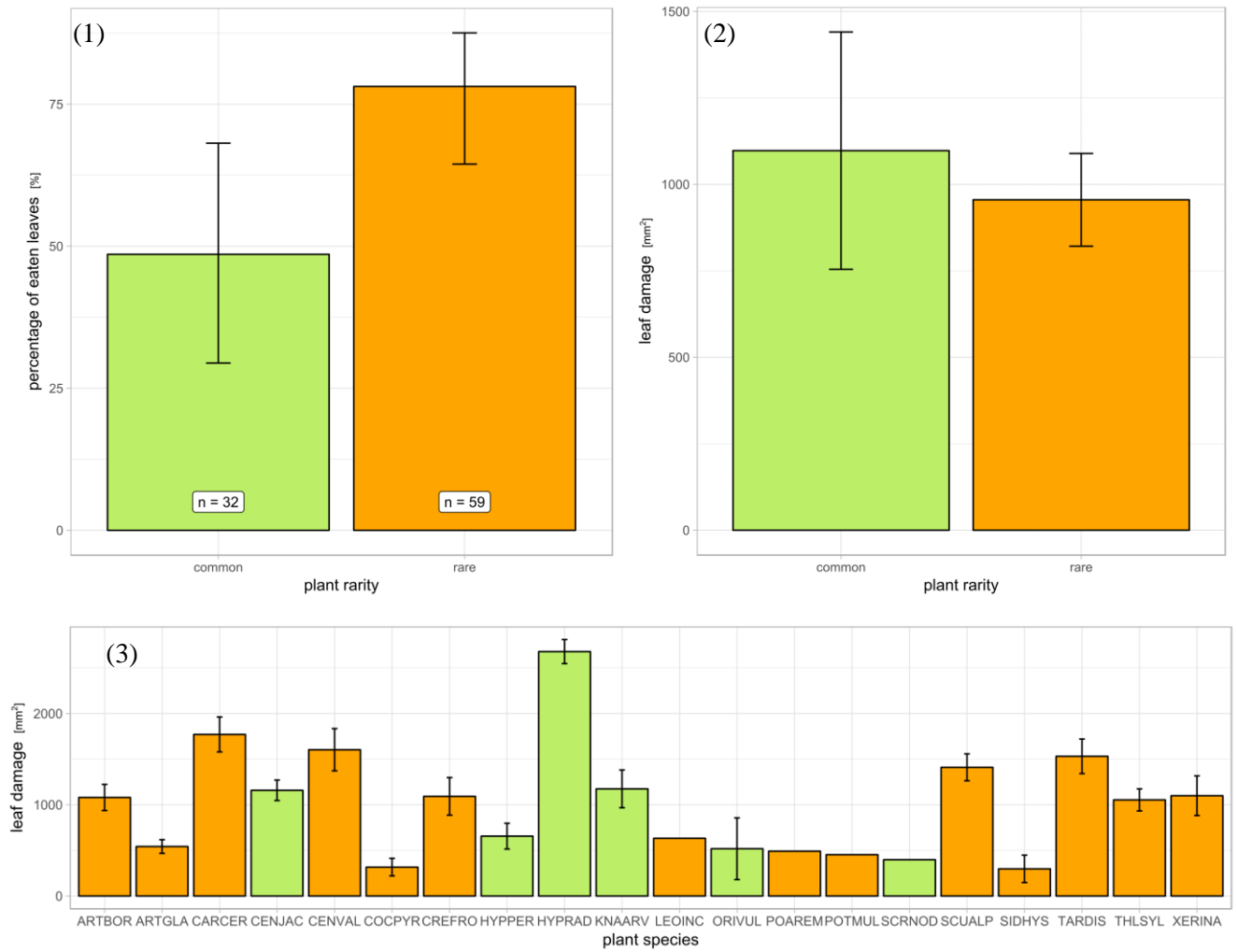


Figure 2: (1) Percentage of the number of eaten leaves, vs not eaten leaves, grouped by plant rarity of the leaves. (2) Relationship between performance of *A. vulgaris* as leaf damage (difference between initial and final leaf area) in dependency of plant rarity or (3) in dependency of plant species (shortcuts see Table 1). Depicted are (1) mean percentages of the number of eaten leaves with standard error bars and number of observations, and (2) means of the species mean, or (3) species mean, with standard error bars (where more than two observations exist).

Discussion

This study investigated the broad question whether herbivores could contribute as a driving factor for plant rarity by applying herbivore bio-assays with three generalist herbivores on more than twenty plant species. Based on current literature, two different assumptions can be made. (1) Common species can be assumed to be stronger competitors and invest more in growth compared to rare species. Due to the growth-defence hypothesis (reviewed in Züst and Agrawal 2017), common species would therefore invest less in defence. Thus, generalist herbivores would overall perform better on common plants. (2) Alternatively, herbivores perform better on leaves of rare plants because they are worse adapted to herbivores. This might be a result of the small distribution area of rare species. Such plants are less apparent and less likely found by herbivores. Hence, low density of herbivores reduces the selection pressure for plants to defend against herbivores. Thus, rare plants are worse adapted to herbivores and are more palatable to newly exposed herbivores.

I found indications (significant effect of rarity on caterpillar weight gain, marginally significant effect of rarity on caterpillar leaf damage; Table 2, Figure 1) that rare plants are more eaten by the generalist caterpillar *S. littoralis*. However, I found no correlation between weight gain and leaf damage (Figure 1 (1)). Yet, this might be a consequence of the restricted data set. Due to a high mortality of the caterpillars, I only considered the small number of resistant caterpillars, but this reduced the statistical power. Furthermore, for those resistant caterpillars, it cannot be totally excluded that the virus affected them in their feeding behaviour. Nevertheless, or particularly for this reason, it is remarkable that rare plant species lead to better weight gain of *S. littoralis*.

In contrast, *A. vulgaris* does not show an overall better performance on rare species (Table 3, Figure 3). However, slugs discriminate their food stronger compared to caterpillars. Almost all caterpillars started to eat their leaves whereas more than a third of the slugs refused to eat. Therewith, a slight trend is visible towards rare species being preferred nourishment for slugs (Figure 2 (1)). As soon as slugs have started to eat their leaves, they do not distinguish between rare and common species anymore (Figure 2 (2)). However, the few observations lead to reduced power here as well. It cannot be falsified that rare plants get generally more eaten. Even with low number of observations in the experiments, I found a clear signal for species-specific effects. *A. vulgaris* eat some species more than other ones, despite its reputation of being a generalist (but see Biner and Frank 1998). Surprisingly is likewise that six of the seven most eaten plant species belong to Asteraceae (see Figure 2 (3); *A. borealis*, *A. glacialis*, *C. valesiaca*, *C. froelichiana*, *H. radicata*, *X. inapertum*), of which only one (*H. radicata*) is a common species. It is most likely that *A. vulgaris* prefers those species in choice-experiments or in field conditions. Therefore, multispecies preference assays and observational studies in the field should be performed in addition to multispecies performance assay. The question whether slugs perform better on and prefer rare species can then be re-evaluated in a more detailed analysis for the family Asteraceae.

Although, I could not evaluate the bioassays with *C. nemoralis*, the snails give hints to be choosy. Such a behaviour was described already earlier by Grime et al. (1968). However, when environmental conditions meet the requirements of *C. nemoralis*, i.e. being natural, their feeding behaviour might be considerably different. Accordingly, experiments under natural or field conditions holds promise for more conclusive results.

The overall view of all herbivores shows differences between molluscs and caterpillars. Caterpillars do not really distinguish what they eat, though they eat slightly less and perform worse on certain plants and might have preferences for other ones. One reason for this might be that caterpillars are in their growing, juvenile phase. The most important thing for them is to eat and grow. In contrast, slugs and snails are adult individuals and do not grow anymore. They even might have resource reserves and can themselves allow to restrict food for some days (indicated by *C. nemoralis* assays). Yet, to my knowledge, there are no studies about the degree of specialization in herbivores among different life stages. Hence, further research is needed in order to generalise that juvenile herbivores are even more generalists than adults. Assuming that rare plant species are generally more threaten by generalist herbivores than common plant species, it might be worthwhile for conservationists to distinguish between adult and juvenile herbivores.

It is especially remarkable that rare species tend to be more likely to get eaten by slugs as well as caterpillars and to show higher performance for caterpillars. Hence, the second hypothesis finds (somewhat) more support in the implemented experiments. This might be due to different herbivore density the plant species are acclimatised to at their site of origin. Herbivores and their density varies locally, whereas herbivore density leads to different adaptation in defence (Züst et al. 2012). Especially, plants in higher elevation might fight against less and surely different herbivores. *A. vulgaris*, for example, is limited by an upper elevation of 1700 m (Rowson 2017). That means for this study, that some used plant species never experienced pressure of *A. vulgaris* before. As plant rarity increases with higher elevation (Levin et al. 2007), rare plants could have experienced lower herbivorous pressure, have less adapted to herbivores and therefore get more eaten by them. One way to investigate these speculations is to analyse the activated genes and their products upon herbivore attack. Accordingly, rare plants would have less defence genes activated and fewer defence metabolites.

It may seem that rare plant species are more threaten when they are generally more and more likely eaten. It can thus be useful for conservation biologists to know better herbivores' performances on and preferences for certain plant species. It allows them to give special attention to presence of herbivores. It allows also to account for species specific differences among herbivores and for their food preferences. However, damage by herbivores might not equally affect plant's fitness. Likewise, higher herbivore density is not automatically considered to cause a loss of plant reproduction fitness (see Machado et al. 2016). Experiments to analyse different types and densities of herbivores in field conditions and their impact on plants and their offspring are needed. Similarly, it is yet to evaluate

which life stage of a plant is mostly affected, following the example of Kurkjian et al. (2017). They found for the rare species *Lupinus constancei*, that seeds are the most vulnerable life stage within a population. Even more, seed predation in particular leads to extinction. Therefore, the next important steps are the evaluation of performance and preferences of herbivores, for different life stages, from different taxa and from different feeding guild, in the field. It is then important to evaluate the next plant generation to assess the reproduction fitness. That is to analyse whether propagules grow better or worse when parents suffered under herbivores. Moreover, the impact of herbivores on different life stages of the plant, like flowering, germination, establishment of seedlings or contribution to seed banks, may be relevant. In addition, analysis of defence genes and metabolites is needed to link plant rarity with adaptation to herbivores by defence.

Based on the trend of my results, rare plant species seem to be more valuable to herbivores. They should be generally protected against juvenile herbivores like caterpillars. Additional protection should be provided for rare species of the family Asteraceae against slugs. Furthermore, this study leads the way for future studies on herbivore performance and preference in the field, and evaluations of herbivore effects on plants reproduction fitness. Lastly, knowing the vulnerabilities of rare species is essential for the application in conservation biology and maintenance of biodiversity.

Conclusion

Overall, this study investigated herbivores from different taxa, different life stages and different feeding guilds, and showed various outcomes for the different herbivores. Molluscs like *A. vulgaris* and *C. nemoralis* distinguish their food source more than caterpillars. Despite their reputation as generalists, *A. vulgaris* shows clearly species-specific responses. Slugs refuse their food or eat all of some preferred plants, whereas caterpillars eat what they find but leave different amount of damage. In conclusion, herbivores visually tend to eat more and to perform better on rare species what may indicate that rare species, with some species dependent differences, are more threaten by herbivores. This study rises hope for further research to gain insight into the mechanisms driving plant rarity and to contribute to conservation of rare species.

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