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The nature of plant adaptations to salinity stress has trophic consequences

Wimke Fokkema, Wendy de Boer, Henk P. van der Jeugd, Adriaan Dokter, Bart A. Nolet, Luit J. De Kok, J. Theo M. Elzenga and Han Olff

W. Fokkema (wimke.fokkema@gmail.com), W. de Boer, L. J. De Kok, J. T. M. Elzenga and H. Olff, Groningen Inst. for Evolutionary Life Sciences, Univ. of Groningen, PO Box 11103, NL-9700 CC Groningen, the Netherlands. – H. P. van der Jeugd, A. Dokter and B. A. Nolet, Dept of Animal Ecology, Netherlands Inst. of Ecology (NIOO-KNAW), PO Box 50, NL-6700 AB Wageningen, the Netherlands. HPvdJ also at: Vogeltrekstation, Dutch Centre for Avian Migration and Demography (NIOO-KNAW), PO Box 50, NL-6700 AB Wageningen, the Netherlands.

In different ecosystems herbivores highly prefer particular plant species. This is often explained in a stoichiometric framework of nutrient-based plant adaptations to herbivory. We hypothesize that such super-palatability can also arise as an evolutionary by-product of osmoregulatory adaptations of plants to stressful environmental conditions, as salinity, drought and cold. Here, we investigate in a coastal salt marsh why some plant species are highly preferred by migratory brent geese *Branta bernicla bernicla* in spring while others are avoided. This salt marsh is an important spring staging site for the geese. Sufficient energy storage in a short period is critical to enable their northward migration to Siberia and subsequent reproduction. We test if geese prefer plants that balance their internal osmotic potential with the saline environment through energy-rich soluble sugars over plant species that use (compartmentalized) salts for this. We find that plant nitrogen and acid detergent fiber content, classic predictors of herbivore preferences, poorly explain which plants the geese prefer. Instead, plant species that are highly preferred by the geese adapt to salinity by high soluble sugar concentrations while avoided species do this by high plant salt concentrations. Thus, the type of osmoregulatory adaptation to stress displayed by different plant species is a good predictor for the food preference of geese on this salt marsh. We suggest that variation in other types of osmoregulation-based stress adaptations, as plant cold adaptations in tundras and plant drought adaptations in savannas, have similar important consequences for trophic interactions.

Specific adaptations of prey species to deal with environmental conditions can explain why some species or individuals within species are more or less attractive to predators than others. This has recently been shown for fish-eating pelicans, which prefer feeding on marine fish species that are able to exclude salt from their bodies over species which take up salts (Troup and Dutka 2014). Furthermore, diving ducks have been suggested to prefer feeding on smaller mussels with a lower salt load under conditions with higher salinities, thereby matching the preference for individuals within a prey species with the abiotic conditions (Nyström and Pehrsson 1988, Nyström et al. 1991). Similar effects of adaptations to harsh conditions may be expected in plant–herbivore interactions, since plants, like fish and mussels, cannot escape from generic stressful conditions and instead have to adapt in order to be able to persist. While some plants are completely avoided by herbivores, others are super-palatable and thereby lose large amounts of biomass to consumers (Crawley 1986, Vandermeijden et al. 1988). This may reduce their competitive ability relative to less-preferred species (Herms and Mattson 1992). However, super-palatable plant species generally persist in healthy populations under extremely high herbivore pressures. Examples include the halophytic dryland grasses *Sporobolus ioclados* and *Andropogon greenwayi* grazed by wildebeest *Connochaetes taurinus* on the Serengeti Plains (McNaughton 1983), the halophytic salt marsh plants *Puccinellia maritima*, *Plantago maritima* and *Trichochin maritima* grazed by geese on Wadden Sea saltmarshes (Prop and Deerenberg 1991), the seagrasses *Zostera noltii* and *Zostera marina* that are heavily exploited by brent geese *Branta bernicla bernicla* in various estuaria (Ganter 2000), and the arctic salt marsh plants *Puccinellia phryganodes* and *Carex subspathacea* utilized by snow geese *Chen caerulescens* (Srivastava and Jefferies 1996). All these systems have in common that plants are exposed to frequent stressful conditions (due to seasonal freezing, flooding, drought or salinity), leading to the hypothesis that variation between plant adaptations to persist under these conditions may explain why some species in these ecosystems are super palatable.

Super-palatability is generally considered to be related to ecological stoichiometry and associated food quality (Sterner and Elser 2002). However, abiotic stress conditions, specifically cold, drought and salinity, force organisms to acquire different types of osmoregulatory mechanisms in order to persist (Koster and Lynch 1992, Munns 2002). Specific osmoregulatory mechanisms may explain the variation in palatability of plant species as food source for herbivores. We will explore the idea that super-palatability may be an 'evolutionary by-product' of plant adaptations, to drought, salinity or freezing through common principles of osmolyte-based stress adaptation.

Different plant species use different modes of osmotic adjustment to cope with the low water potential of the environment (either due to salinity, drought or freezing), which may differ in their effects on palatability. Species in saline conditions can be divided into salt-accumulators, which accumulate salts from their environment and either use those actively for osmotic adjustment or store the salts in salt bladders prior to excretion, and salt-excluders, which actively prevent salts from entering. Since osmotic adjustment by storing salts in the cytoplasm has detrimental effects on enzyme activity, salt-accumulating plant species utilize their vacuoles for compartmentalizing and storing of ions (Flowers and Colmer 2008, Munns 2002). Ions as K^+ and different organic compounds are stored in the cytoplasm to balance the high osmotic potential of the vacuoles (Munns 2002). Consumers living in a saline environment and feeding on saline prey have to regulate their osmotic value as well, for example by constantly excreting salts using salt glands (Gutierrez 2014, Stahl et al. 2002). This takes energy and has been shown to go at the expense of activity of the immune system (Gutierrez et al. 2013). In herring gulls and lesser black-backed gulls dehydration has been detected under highly saline conditions, despite the activity of the salt glands. Under such conditions compensation for water loss is necessary and can be achieved by drinking large amounts of (sea)water or, as juvenile gulls do, feeding on grass to obtain osmotically free water (Ensor and Phillips 1972). In order to avoid the costs of excreting or compensating for the large amounts of salts taken up, consumers in a saline environment are expected to avoid saline (salt-accumulating) food items, as was demonstrated in fish-eating pelicans (Troup and Dutka 2014), diving ducks (Nyström and Pehrsson 1988) and may also be the case for herbivores.

Compared to salt-accumulating species that regulate the osmotic value of the vacuole by storing Na^+ and Cl^-, salt-excluding plant species are generally perennial and show much lower salt concentrations, which will make them more attractive to herbivores (Munns 2002). Plants have evolved different strategies to exclude salts. Morphological adaptations, like leaf folding, trichomes, and stomatal adaptations can prevent water loss, which allows the species to take up less water, and hence salts, from the soil (Redondo-Gomez et al. 2007). Furthermore, salt can be physiologically excluded via biochemical adaptations to adjust the osmotic value of the cells. Organic compatible solutes can be stored in the cytosol and organelles to maintain a high osmotic potential inside the plant cells (Munns 2002, Flowers and Colmer 2008, Gil et al. 2013). Compatible solutes can be divided in two groups: ammonium-based components (e.g. amino acids, glycine betaine) (Gil et al. 2013) or polyhydroxylic compounds, like alcohols, polyols, sugars and the raffinose family of oligosaccharides (Gil et al. 2013). Many (but not all) of these solutes are easily digestible for herbivores, because of their simple structure, which reduces the costs of digesting these compounds (McWhorter et al. 2009). Apart from being less salty and hence less stress-inducing for herbivores under saline conditions, salt-excluders that are using these organic solutes can provide additional quality and hence could become super-palatable.

In this study we hypothesize that super-palatability can be a by-product of the nature of adaptation to salinity stress of specific plants growing on an island salt marsh in the Wadden Sea. All plant species growing on this salt marsh show salt accumulating or salt excluding adaptations (Minden et al. 2012). We investigate the osmoregulatory systems related to salt accumulation and salt exclusion, considering anion content (salt accumulating) and total amino acid and soluble sugar content (salt exclusion). We test whether the osmolyte used (anion content for salt-accumulators or total amino acid and soluble sugar content for salt excluders) forms a more suitable explanation for herbivore preference than the classical stoichiometric expectations (based on tissue nitrogen concentrations and acid detergent fibers). We focus on understanding the food choices of the migratory brent goose, an important herbivore in our study system (Kuijper and Bakker 2005) which was studied during spring staging in the Wadden Sea. Brent geese are highly selective herbivores, clearly preferring some plant species and avoiding others (Prop and Deerenberg 1991, Olff et al. 1997, van der Wal et al. 2000a). Grazing by the geese results in the loss of a large proportion of the biomass of preferred plant species (Prins et al. 1980, Prop and Deerenberg 1991) but is limited to early spring, when the geese are fattening up in the Wadden Sea before migrating to their breeding grounds in Siberia (Ganter 2000). The acquisition of high quality food resources on the salt marsh by this species is an essential condition (Clauss et al. 2013), since insufficient fattening up will decrease the probability of breeding success (Ebbinge and Spaans 1995).

We expect that brent geese avoid plants in their diet that adapt to salinity through salt accumulation while preferring salt excluding plants, that adapt to salinity through accumulation of soluble organic osmolytes, in particular amino acids and sugars.

Material and methods

Sample collection field

The study area was located at the salt marsh of the island of Schiermonnikoog (53°29′02″N, 6°16′06″E). This is an important spring staging area for the brent goose (Ganter 2000), which is an important grazer of the salt marsh during that period (Kuijper and Bakker 2005). Brent geese preference for certain food plants over others has been well established. On Schiermonnikoog brent geese forage mostly on *Plantago maritima*, *Trichlogin maritima*, *Puccinellia maritima* and *Festuca rubra* (Prop and Deerenberg 1991). Other species, like *Artemisia maritima*, *Atriplex portucaloides, Elytrigia atherica*, *Limonium vulgare*, *Salicornia europaea* and *Sueda maritima* are largely avoided (van der Wal et al. 2000a, Bos et al. 2004). In order to compare these preferred and avoided species, samples of these species were collected between the

27 March and the 6 June 2013, the period when grazing brent geese are present (Ebbinge 1992).

Green plant material, mostly leaves, except for *Salicornia europaea*, for which the succulent stems were included,was collected in plots along four transects which were arranged perpendicular to the coastline along a gradient from lower to higher salt marsh, which are characterized by different plant communities. A single transect consisted of four plots at different elevations. The plots were distributed in a stratified random fashion, with the different vegetation zones along the elevation gradient as stratifying factor. Temporary exclosures of $1m^2$, which excluded both avian and rodent herbivores, were set up at the plot locations for two weeks, after which those were moved to a nearby new location. Since grazing pressure on the preferred plant species is high, the exclosures were necessary to target the biomass that is eaten by the brent geese. After the two weeks, half of the biomass of the exclosure was clipped off, separated by species and dried in the oven at 70°C to determine vegetation community composition. The other half was used to determine the chemical composition of preferred and avoided plant species. All species of which enough biomass for all the measurements could be collected were included in the analysis. Samples were taken to the lab to be freeze-dried and ground. Over four sampling rounds (every two weeks), a total of 116 samples of the four preferred (*Festuca rubra*: 22, *Plantago maritima*: 11, *Puccinellia maritima*: 7 and *Trichlogin maritima*: 4 samples) and six avoided (*Artemisia maritima*: 15, *Atriplex portucaloides*; 10, *Elytrigia atherica*: 5, *Limonium vulgare*: 29, *Salicornia europaea*: 3 and *Sueda maritima*: 9 samples) plant species were collected.

Droppings were collected every two weeks at fixed collection stations of 4 m^2 in the plot area (outside the exclosures) and dried in an oven at 70°C. Dropping biomass per area has been shown to be a good proxy for local brent geese abundance (Bedard and Gauthier 1986). Dropping biomass per area ($g\ m^{-2}$) was determined for every plot at every collection date.

Trait analysis

The freeze-dried and oven-dried material was analyzed for nitrogen and for acid detergent fiber (ADF) content, where the latter represents the indigestible part of the plant biomass, using a near infra-red spectrophotometer. In order to calibrate the NIR, 60 samples were analyzed chemically for %N using a CHNS elemental analyzer and 45 samples were chemically analyzed for ADF, using the procedure described by Vansoest and Wine (1967). The NIR calibration of %N had an R^2 of 0.93, the calibration of %ADF had an R^2 of 0.97. Furthermore, samples were chemically analyzed to determine the content of anions, amino acids and soluble sugars. Extracts were taken with a basis of water for anions and amino acids and with a basis of ethanol (80%) for soluble sugars. Anions (μmol per gram dry weight), in particular nitrate (NO_3^-), phosphate (PO_3^-), chloride (Cl^-) and sulphate (SO_4^{2-}), were determined using spectrophotometric methods on an automated segmented flow analyser system from Skalar. The concentrations were converted into mg per gram dry weight, after which the sum of the different anions was taken to obtain the total concentration of anions. Amino acids (μmol g^{-1} DW) were determined with a spectrophotometer using a ninhydrin color reaction (Shahbaz et al. 2010) and also converted into mg per gram dry weight. Soluble sugar content (mg g^{-1} DW) was also determined with a spectrophotometer using an anthrone color reaction (Fales 1951).

Statistical analysis

A principal component analysis was used to gain insight in the multivariate structure of the data. All samples on which all the chemical analyses were performed were included in the analysis. The first two principal components (PC 1 and PC 2) were used as species traits, which combine the chemical traits measured. The correlations between the measured chemical traits and the PCA scores were obtained using the Pearson correlation coefficients. Additionally, we performed a linear regression between anion and soluble sugar content and between anion and amino acid content to determine whether salt-accumulation and salt-exclusion were two distinct strategies.

For each plot (m^2) and collection date the content of every chemical element present was calculated (mg) using the average chemical trait values of each of the species and weighing for their relative contribution to total plot biomass. Similar weighted averages over all species per plot per collection date were calculated for PC 1 and PC2.

General linear mixed models were used in order to address the relation between the total dropping biomass per m^2 and the indices calculated above per plot. Records with no information on biomass of the samples of the target species were excluded. Since the same plots were measured several times, we included site and date (nested within site) as random effects. The fixed effects in the first set of models were the indices of the different chemical traits (N, ADF, sugars, amino acids, anions) and in the second set the indices based on the combined traits (PC 1 and PC 2). The most parsimonious model was selected using both forward and backward stepwise model selection based on model comparison using Akaike's information criterion (AIC). When the difference between the AIC-values of two models was within 2 points, models were considered the same and the most parsimonious model, with the least number of variables, was chosen. Marginal (percentage of the variance explained by fixed factors) and conditional (percentage of the variance explained by a combination of fixed and random factors) R^2_{GLMMs} were calculated using the method of Nakagawa and Schielzeth (2013).

One-sided t-tests were used to compare traits between a group with preferred plants and a group with avoided plant species. Whether plants were preferred or avoided was obtained from the literature (Olff et al. 1997, Prop and Deerenberg 1991, van der Wal et al. 2000b). For every species the average value per trait was determined. This value was included in the t-test. The number of samples in the preferred group was therefore four (*Plantago maritima*, *Trichlogin maritima*, *Puccinellia maritima* and *Festuca rubra*) and the number of samples in the avoided group was six (*Artemisia maritima*, *Atriplex portucaloides*, *Elytrigia atherica*, *Limonium vulgare*, *Salicornia europaea* and *Sueda maritima*). We used a one-sided t-test, because we had prior hypotheses on how the means of the distributions of the different traits should differ between the preferred and avoided groups of species. The expectation for nitrogen, amino acid and sugar content was that the preferred group would have a higher mean, whereas for ADF and anions we expected a lower mean for the preferred group in comparison to the avoided group.

Finally, to gain insight in differences in trait values between individual species, one-way ANOVAs were performed with species as predictor variable and the different traits as response. Some species were sampled much more often than others. To reduce this large difference in sample size between species, the average species value per plot over the different sampling rounds was used in the analysis, so maximal sample size per plant species was 16. Subsequently, Tukey HSD post hoc tests were performed to gain insight in which species differed from which.

Results

The first principal component (PC1) of the multivariate analysis of the trait variation among plant species explained 46.8 % of the variation while the second principal component (PC2) explained an additional 25.2%. PC1 was positively associated with sugar (Pearson correlation coefficient (r) = 0.8, $p < 0.001$) and ADF content (r = 0.8, $p < 0.001$) and negatively with anion (r = –0.6, $p < 0.001$),nitrogen (r = –0.7, $p < 0.001$) and amino acid (r = –0.4, $p < 0.001$) content, while PC2 was positively associated with amino acid (r = 0.8, $p < 0.001$) and nitrogen (r = 0.3, $p < 0.01$) content and negatively with anion content (r = –0.7, $p < 0.001$) (Fig. 1). The differences between plants with strategies of salt-accumulation, using anions, and salt-exclusion, using soluble sugars, were confirmed by the results of a linear regression, which shows a negative relation between anion and soluble sugar content (t-value = 5.5, $p < 0.001$). No correlation between anion and amino acid content was found.

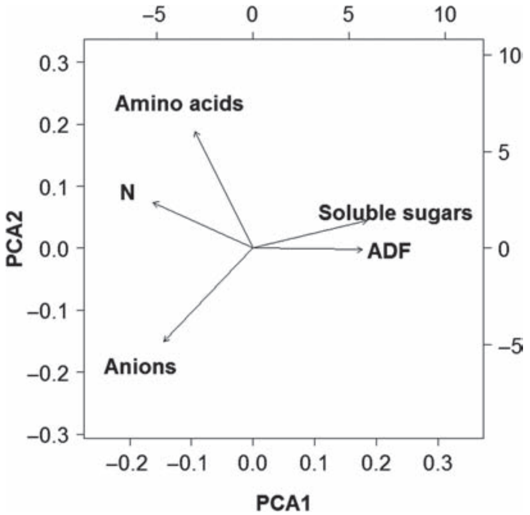

Figure 1. Results of a principal component analysis. The first two principal component axes, which explain 46.8% and 25.2% of the variation respectively, are plotted. The associations with the different traits are represented with red arrows.

Table 1. Most parsimonious general linear mixed models investigating how abundance-weighted chemical traits per plot (model 1) and abundance-weighted PCA scores of species per plot (model 2) respectively explain total dropping weight per plot. As plots were sampled multiple times, site and date (nested in site) were included in both models as random factors.

Variables	Estimate	SE	DF	t-value	p-value
(a) Model 1					
Intercept	2.507	2.052	29	1.222	ns
Total soluble sugars per plot (g/m^2)	0.451	0.194	29	2.326	< 0.05
(b) Model 2					
Intercept	3.642	1.811	29	2.011	ns
Abundance-weighted PC1 score all species	0.041	0.015	29	2.807	< 0.01

The most-parsimonious model explaining the weight of goose droppings per m^2 found at a location, with the chemical indices as predictors, only contained the soluble sugar content per m^2 (Table 1). Sugar content explained 10.5% of the variance, whereas the total model (including the random effects) explained 91.7% of the variance. Forward model selection showed that adding sugar content per plot to a model with only the random structure of date nested in site significantly improved the model (without sugar content: AIC = 341.6, with sugar content: AIC = 338.3). Adding additional variables did not significantly improve the model performance (Supplementary material Appendix 1, Table 1). All different possibilities of backward model selection revealed that removing variables does not negatively affect the model fit, as long as sugar content per m^2 remained in the model (Supplementary material Appendix 1 Table 2). The calculated pool of soluble sugar available per square meter plot significantly explained variation in the weight of the geese droppings found on the plot (Fig. 2a) in the form a positive linear relation.

The index based on the first principal component of the multivariate trait values (abundance-weighted average of PC1 species scores) was an important explanatory variable for the weight of the droppings per m^2 found at a location (Table 1). The most-parsimonious model based on the first two principal components did not include the second principal component (Supplementary material Appendix 1, Table 3). PC1 index per plot explained 16.4% of the variance, whereas the total model (including the random effects) explained 92.3% of the variance. Higher values for the PC 1 index, which suggest higher sugar and ADF concentration, positively influenced the weight of the droppings found (Fig. 2b).

Additionally, we performed a comparison between the trait values of plant species, which have been reported in previous studied to be either preferred or avoided by brent. Only soluble sugar content showed a significant difference between the preferred and avoided plant species (Table 2). The soluble sugar content was higher in preferred plants in comparison to avoided plants (t-value = –3.24, $p < 0.05$). Anion content showed an almost significant trend (t-value = 1.71, p = 0.06) of being higher in the avoided plants (Table 2).

One-way analyses of variance comparing traits among all plant species showed that only nitrogen content was not

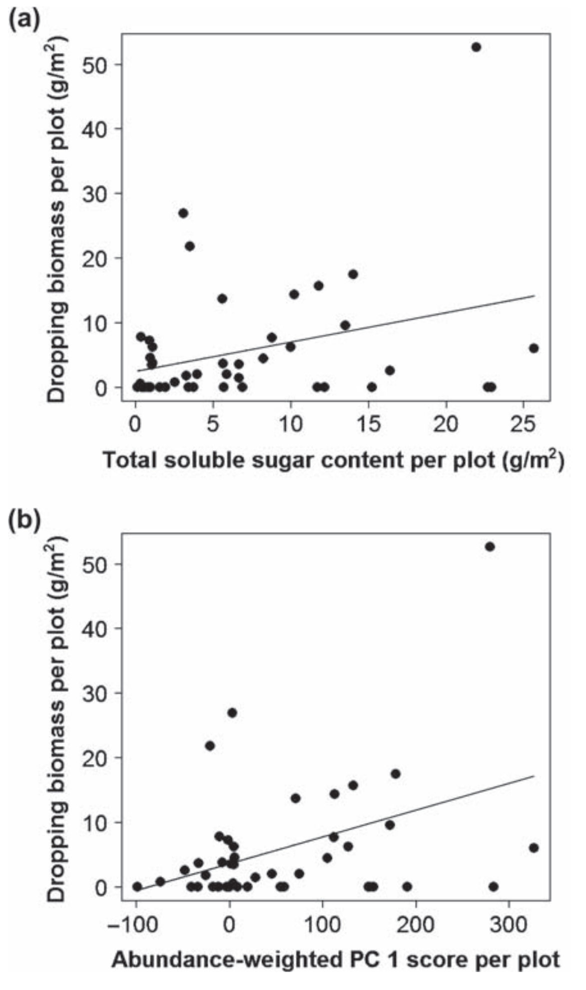

Figure 2. Model predictions most-parsimonious models. The points show the measured dropping weight values (g m^{-2}) for the total amount of soluble sugars per plot (g m^{-2}) (a) and the abundance-weighted PC1 scores over all species per plot (b). The lines in both figures show the model prediction based on the most-parsimonious model (Table 1).

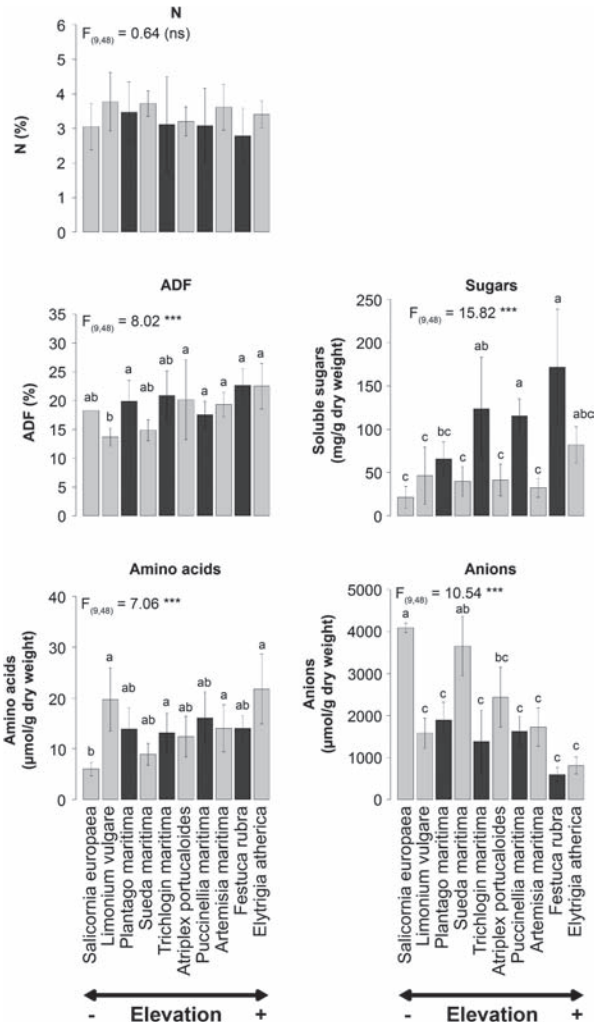

Figure 3. Chemical trait values per species. Species are ordered along the x-axis according to their maximal abundance along the elevation gradient. Dark bars represent the preferred species, whereas light bars represent the avoided species. The results (F-value, degrees of freedom and level of significance: $p < 0.001$***) of the one-way ANOVAs are given in the upright corner of every graph. Significant differences between species, resulting from a Tukey HSD test, are indicated by letters: bars with the same letter are significantly the same.

significantly different between species, whereas all other traits showed significant differences (Fig. 3). ADF content was lowest in *Limonium vulgare. Sueda maritima* and *Salicornia europaea* also have low ADF values. Soluble sugar content was high for *Festuca rubra* and *Puccinellia maritima*, followed by *Trichlogin maritima*, *Elytrigia atherica* and *Plantago maritima*. Amino acid content was higher in *Artemisia maritima*, *Elytrigia atherica*, *Limonium vulgare* and *Trichlogin maritima* and lower in *Salicornia europaea*. Anion content was high in *Salicornia europaea*, *Sueda maritima* and *Atriplex portucaloides*.

Table 2. Results of one-sided t-tests comparing chemical trait values of preferred and avoided plants. The 'Expectation preferred' column shows whether the preferred plants are expected to have a higher value than the avoided plants (+) or a lower value (–). These hypotheses are tested in the one-sided t-tests. The mean values for the preferred and avoided groups are shown, as well as the t-value and level of significance ($p < 0.1$, * $p < 0.05$) resulting from the one-sided t-tests.

Trait	Expectation preferred	Mean preferred	Mean avoided	t-value and significance
N (%)	+	3.11	3.46	ns
ADF (%)	–	20.2	18.1	ns
Sugars (mg g^{-1} DW)	+	119	44	−3.24*
Amino acids (µmol g^{-1} DW)	+	14.3	13.8	ns
Anions (µmol g^{-1} DW)	–	1376	2383	1.71

Discussion

We find that the type of osmoregulatory system of different salt marsh plant species is a good predictor for the variation in the preference of herbivorous brent geese to use the plants as food. Brent geese show a strong preference for plant species that seem to osmoregulate through maintaining high contents of soluble sugars in their cells, and prefer locations where the relative abundances of such plant species are highest. Salt accumulating species, which store salt ions in their vacuoles, are instead largely avoided by brent geese. Classical ecological stoichiometry-based measures (nitrogen and acid detergent fiber (ADF) contents) are not related to either brent goose preference or grazing site selection. These findings suggest that soluble sugars instead of nitrogen or protein content play a key role in the selection of food resources by spring staging brent geese in this ecosystem.

The importance of proteins versus energy in the diet of herbivores is hard to detect, since nitrogen and carbon levels are generally correlated (Klaassen and Nolet 2008). Surprisingly, all plant species in our study showed similar nitrogen levels. This is in contrast to other studies on the stoichiometry of salt marsh plants, which did detect species differences in nitrogen levels (Minden and Kleyer 2014, Sammul et al. 2012). We think that our nitrogen levels are similar between species, because all our samples contained young tissues, which generally show high nitrogen contents (Fryxell 1991, vandeKoppel et al. 1996, Augustine and McNaughton 1998), even though further research is needed to confirm that this lowers the differences in nitrogen content between salt marsh species. However, the lack of species differences in nitrogen levels enables us to detect that carbon-based energy-rich sugars seem to be more important in the diet of spring staging brent geese than proteins. Sugars are a source of energy and fat, which has also been shown to be important for passerines, that switch from a protein-rich insectivorous diet to a sugar-rich frugivorous diet prior to migration (Bairlein 2002). Our results complement to this and suggest that focusing on sugars, instead of on proteins, might lead to better understanding why particular plant species are highly preferred by small grazing herbivores, like geese.

We observe that brent geese spent most time grazing (with dropping biomass per m^2 as a proxy) in plant communities that contain many salt excluding plants with high soluble sugar contents. However, *Elytrigia atherica*, a salt excluding plant with relatively high soluble sugar content, is known to be largely avoided by small herbivores on the salt marsh (Olff et al. 1997, Bos et al. 2002). Adult plants are of low quality, with a low nitrogen and high carbon content, a high leaf dry matter content and a low specific leaf area (Minden et al. 2012, Minden and Kleyer 2014). We do however not detect this in our measures of *Elytrigia atherica*, since we have only sampled the young fresh leaves in the early growing season. Plant tissue of a young ontogenetic stage is generally more palatable than older plant tissue (Augustine and McNaughton 1998). The plant maturation hypothesis (Fryxell 1991, vandeKoppel et al. 1996) focusses on the buildup of structural material in older plant tissue; thereby the stoichiometry of the plant changes into less favorable, with fewer nutrients per unit of structural carbon (Sterner and Elser 2002, Klaassen and Nolet 2008). This process is accelerated when nutrients are withdrawn from aboveground tissues prior to a harsh season (winter of drought) (Yuan and Chen 2009). *Elytrigia atherica* resorbs its nutrients prior to winter, leaving large stands of tall highly unpalatable dead biomass. This material will have high ADF and low nutrient contents. The young leaves of *Elytrigia atherica* we have collected, were intermingled with the dead standing biomass. Based on stoichiometry and osmoregulation, brent geese should be able to feed on the fresh leaves of *Elytrigia atherica* during their spring staging on the salt marsh. We think that the reason why *Elytrigia atherica* is avoided, is that the older ontogenetic stages, in particular the low-quality standing dead biomass (Olff et al. 1997), are still present, which makes selection of the fresh green leaves a difficult undertaking (cf. Artemisia in van der Wal et al. 2000b). Additionally the tall vegetation on the higher salt marsh reduce the birds' abilities to see risks, thereby further decreasing the preference of brent geese for higher salt marshes (Inger et al. 2006).

The super-palatable plant species on the salt marsh also likely undergo a changed stoichiometric quality over ontogenetic stages. During the growing season their protein content strongly declines (Prop and Deerenberg 1991). Brent geese are therefore, during early spring, consuming plant material with the highest protein contents (Prop and Deerenberg 1991). However, also with respect to osmoregulation plant quality is expected to decline from young to older ontogenetic stages. In salt excluding plants, which exclude most but not all salts, Na^+ and Cl^- are gradually building up in older leaves (Munns 2002). Sample sizes in this study are too small to detect any patterns over the growing season. However, diving ducks feeding on mussels select the smallest (youngest) mussels which are least saline (Nyström and Pehrsson 1988, Nyström et al. 1991). Such a within prey species selection could also be made by herbivores feeding on plants under saline conditions. An interesting hypothesis is therefore that young leaves, which have no buildup of salts yet, are more palatable than older leaves, thereby complementing the patterns found in nutrient-based explanations for declining food quality with leaf age. In addition, the inundation frequency of the lower salt marsh by sea water is the lowest of the year during the months of April and May due to solar-lunar annual tidal cycles (Olff and Howison unpubl.), which may further contribute to relatively favourable low salinity levels in key food plant species during this key staging period for the brent geese.

Since brent geese are migratory, they are only feeding on salt marsh plants during early spring (Ganter 2000). After that the growing season still lasts long enough for the super-palatable plants to complete a full life cycle and refill their (taproot-based) carbohydrate storages. In fact, species like *Plantago maritima* and *Trichlogin maritima* only become a visible component in the vegetation after the brent geese have left and this is due to high grazing pressure since biomass of these species does build up earlier when geese are excluded (Fokkema, de Boer, van der Jeugd, Nolet, Dokter, de Kok and Olff unpubl.). Similar super-palatable plants are fed upon by migratory geese also in other migratory systems (Prop and Deerenberg 1991, Srivastava and Jefferies 1996, Ganter 2000). Furthermore wildebeest on the Serengeti Plains are only consuming the super-palatable grasses in this system during a specific (wet in this case) season (Bell 1971). This may suggest that super-palatable species can only persist under seasonal, as opposed to continuous, herbivory. The same may hold for specialized insect species that only feed upon plant material during part of their ontogeny. During caterpillar peaks, super-palatable plants can be overexploited (Wesolowski and Rowinski 2008), but continue growing after the peaks have subsided. In conclusion, super-palatable plants may only evolve and persist in ecosystems if they are only utilized this way by herbivores during part of the year, and especially only during part of their main growing season.

Super-palatability of plants caused by osmoregulation is not only expected in response to salinity, but also in response to extreme drought, where plants show similar adaptations to conserve their water balance (Munns 2002). In fact, the same species can occur under different stress conditions, like *Festuca rubra*, which can tolerate both salinity on the salt marsh and drought in dry grasslands. Furthermore, plants in arctic or high altitude ecosystems accumulate compatible organic solutes as cryoprotectants in their cytoplasm in order to prevent their cell content from freezing (Koster and Lynch 1992). Under conditions of drought or cold we would therefore expect similar effects of adaptations to abiotic stress on plant–herbivore interactions.

The nature of responses of herbivores to plant osmoregulation can be contingent on the type of resource limitation of herbivores. Unlike herbivores in environments with high soil salinity, herbivores in savannas can be limited by essential minerals as sodium (McNaughton 1988) while energy can be available in surplus in these tropical environments. Herbivores are therefore expected to actively select the plant species that are storing compartmentalized ions in their vacuoles, making plant that are adapted to drought through salt accumulation more attractive than plant that are adapted through accumulation of organic solutes. Evidence can be found in super-palatable grasses on dry grazing lawns in South African savannas which accumulate high concentrations of sodium in their tissues and are preferred by white rhino (Veldhuis et al. 2014). Similarly, wildebeest highly prefer grasses on the halophytic dry grasslands of the Serengeti plains which contain large amounts of calcium and magnesium (McNaughton 1988). These minerals are required by female wildebeest, that are lactating during the period they occupy the Serengeti plains (McNaughton 1988).

Finally, osmoregulation-based herbivore preferences are not only expected in saline or dry environments, but also in cold ecosystems. In Arctic wetlands, lesser snow geese have been shown to strongly prefer plants that contain the highest amounts of soluble sugars (Jefferies and Edwards 2008). These soluble sugars are likely to be used as protection against cold and/or salinity in these systems (Koster and Lynch 1992, Munns 2002, Flowers and Colmer 2008).

In summary, we suggest that osmoregulation is important in explaining variation in attractiveness of plants to herbivores under different conditions, including salinity, drought and cold with important food web and ecosystem consequences. Super-palatability in these systems may rather be an evolutionary by-product of the osmoregulatory plant adaptations instead of reflecting direct plant–herbivore coevolution.

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References

Augustine, D. J. and McNaughton, S. J. 1998. Ungulate effects on the functional species composition of plant communities: herbivore selectivity and plant tolerance. – J. Wildl. Manage. 62: 1165–1183.

Bairlein, F. 2002. How to get fat: nutritional mechanisms of seasonal fat accumulation in migratory songbirds. – Naturwissenschaften 89: 1–10.

Bedard, J. and Gauthier, G. 1986. Assessment of fecal output in geese. – J. Appl. Ecol. 23: 77–90.

Bell, R. H. V. 1971. Grazing ecosystem in Serengeti. – Sci. Am. 225: 86–93.

Bos, D. et al. 2002. Long-term vegetation changes in experimentally grazed and ungrazed back-barrier marshes in the Wadden Sea. – Appl. Veg. Sci. 5: 45–54.

Bos, D. et al. 2004. Dark-bellied Brent geese aggregate to cope with increased levels of primary production. – Oikos 107: 485–496.

Clauss, M. et al. 2013. Herbivory and body size: allometries of diet quality and gastrointestinal physiology, and implications for herbivore ecology and dinosaur gigantism. – Plos ONE 8: e68714.

Crawley, M. J. 1986. Herbivory: the dynamics of animal–plant interactions. – Blackwell.

Ebbinge, B. S. 1992. Regulation of numbers of dark-bellied brent geese *Branta bernicla-bernicla* on spring staging sites. – Ardea 80: 203–228.

Ebbinge, B. S. and Spaans, B. 1995. The importance of body reserves accumulated in spring staging areas in the temperate zone for breeding in dark-bellied brent geese *Branta bernicla-bernicla* in the high arctic. – J. Avian Biol. 26: 105–113.

Ensor, D. M. and Phillips, J. G. 1972. Effect of age and environment on extrarenal salt excretion in juvenile gulls (*Larus argentatus* and *L. Fuscus)*. – J. Zool. 168: 119–126.

Fales, F. W. 1951. The assimilation and degradation of carbohydrates by yeast cells. – J. Biol. Chem. 193: 113–124.

Flowers, T. J. and Colmer, T. D. 2008. Salinity tolerance in halophytes. – New Phytol. 179: 945–963.

Fryxell, J. M. 1991. Forage quality and aggregation by large herbivores. – Am. Nat. 138: 478–498.

Ganter, B. 2000. Seagrass *Zostera* spp. as food for brent geese *Branta bernicla*: an overview. – Helgoland Mar. Res. 54: 63–70.

Gil, R. et al. 2013. Are soluble carbohydrates ecologically relevant for salt tolerance in halophytes? – Funct. Plant Biol. 40: 805–818.

Gutierrez, J. S. 2014. Living in environments with contrasting salinities: a review of physiological and behavioural responses in waterbirds. – Ardeola 61: 233–256.

Gutierrez, J. S. et al. 2013. Effects of salinity on the immune response of an 'osmotic generalist' bird. – Oecologia 171: 61–69.

Herms, D. A. and Mattson, W. J. 1992. The dilemma of plants: to grow or defend. – Q. Rev. Biol. 67: 283–335.

Inger, R. et al. 2006. Prey choice affects the trade-off balance between predation and starvation in an avian herbivore. – Anim. Behav. 71: 1335–1341.

Jefferies, R. L. and Edwards, K. A. 2008. Soluble carbohydrate content of shoots of Arctic wetland plants that are consumed by lesser snow geese. – Botany 86: 995–1001.

Klaassen, M. and Nolet, B. A. 2008. Stoichiometry of endothermy: shifting the quest from nitrogen to carbon. – Ecol. Lett. 11: 785–792.

Koster, K. L. and Lynch, D. V. 1992. Solute accumulation and compartmentation during the cold acclimation of puma rye. – Plant Physiol. 98: 108–113.

Kuijper, D. P. J. and Bakker, J. P. 2005. Top–down control of small herbivores on salt-marsh vegetation along a productivity gradient. – Ecology 86: 914–923.

McNaughton, S. J. 1983. Serengeti grassland ecology: the role of composite environmental factors and contingency in community organization. – Ecol. Monogr. 53: 291–320.

McNaughton, S. J. 1988. Mineral nutrition and spatial concentrations of African ungulates. – Nature 334: 343–345.

McWhorter, T. J. et al. 2009. The integration of digestion and osmoregulation in the avian gut. – Biol. Rev. 84: 533–565.

Minden, V. and Kleyer, M. 2014. Internal and external regulation of plant organ stoichiometry. – Plant Biol. 16: 897–907.

Minden, V. et al. 2012. Plant trait–environment relationships in salt marshes: deviations from predictions by ecological concepts. – Persp. Plant Ecol. Evol. Syst. 14: 183–192.

Munns, R. 2002. Comparative physiology of salt and water stress. – Plant Cell Environ. 25: 239–250.

Nakagawa, S. and Schielzeth, H. 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. – Meth. Ecol. Evol. 4: 133–142.

Nyström, K. G. K. and Pehrsson, O. 1988. Salinity as a constraint affecting food and habitat choice of mussel-feeding diving ducks. – Ibis 130: 94–110.

Nyström, K. G. K. et al. 1991. Food of juvenile common eiders (*Somateria mollissima*) in areas of high and low salinity. – Auk 108: 250–256.

Olff, H. et al. 1997. Vegetation succession and herbivory in a salt marsh: changes induced by sea level rise and silt deposition along an elevational gradient. – J. Ecol. 85: 799–814.

Prins, H. H. T. et al. 1980. The interaction of brent geese *Branta bernicla* and sea plantain *Plantago maritima* during spring staging: field observations and experiments. – Acta Bot. Neerl. 29: 585–596.

Prop, J. and Deerenberg, C. 1991. Spring staging in brent geese *Branta bernicla-bernicla*: feeding constraints and the impact of diet on the accumulation of body reserves. – Oecologia 87: 19–28.

Redondo-Gomez, S. et al. 2007. Growth and photosynthetic responses to salinity of the salt-marsh shrub *Atriplex portulacoides*. – Ann. Bot. 100: 555–563.

Sammul, M. et al. 2012. Biomass accumulation during reed encroachment reduces efficiency of restoration of Baltic coastal grasslands. – Appl. Veg. Sci. 15: 219–230.

Shahbaz, M. et al. 2010. Copper exposure interferes with the regulation of the uptake, distribution and metabolism of sulfate in Chinese cabbage. – J. Plant Physiol. 167: 438–446.

Srivastava, D. S. and Jefferies, R. L. 1996. A positive feedback: herbivory, plant growth, salinity, and the desertification of an arctic salt-marsh. – J. Ecol. 84: 31–42.

Stahl, J. et al. 2002. Foraging along a salinity gradient: the effect of tidal inundation on site choice by dark-bellied brent geese *Branta bernicla* and barnacle geese *Branta leucopsis*. – Ardea 90: 201–212.

Sterner, R. W. and Elser, J. J. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. – Princeton Univ. Press.

Troup, G. and Dutka, T. L. 2014. Osmotic concentration of prey affects food discrimination behaviour in the Australian pelican. – J. Zool. 294: 170–179.

van der Wal, R. et al. 2000a. Are spring staging brent geese evicted by vegetation succession? – Ecography 23: 60–69.

van der Wal, R. et al. 2000b. On facilitation between herbivores: how brent geese profit from brown hares. – Ecology 81: 969–980.

vandeKoppel, J. et al. 1996. Patterns of herbivory along a productivity gradient: an empirical and theoretical investigation. – Ecology 77: 736–745.

Vandermeijden, E. et al. 1988. Defense and regrowth, alternative plant strategies in the struggle against herbivores. – Oikos 51: 355–363.

Vansoest, P. J. and Wine, R. H. 1967. Use of detergents in analysis of fibrous feeds: determination of plant cell wall constituents. – J. Ass. Off. Anal. Chem. 50: 50–55.

Veldhuis, M. P. et al. 2014. A novel mechanism for grazing lawn formation: large herbivore-induced modification of the plant–soil water balance. – J. Ecol. 102: 1506–1517.

Wesolowski, T. and Rowinski, P. 2008. Late leaf development in pedunculate oak *Quercus robur*: an antiherbivore defence? – Scand. J. For. Res. 23: 386–394.

Yuan, Z. Y. Y. and Chen, H. Y. H. 2009. Global trends in senesced-leaf nitrogen and phosphorus. – Global Ecol. Biogeogr. 18: 532–542.

Supplementary material (available online as Appendix oik.02757 at <www.oikosjournal.org/readers/appendix>). Appendix 1.