Age-related mandible abrasion in the groundhopper *Tetrix tenuicornis* (Tetrigidae, Orthoptera)

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**Article Info**

**Article history:**
Received 24 September 2013
Received in revised form
16 February 2014
Accepted 18 February 2014

**Keywords:**
Exoskeleton
Morphology
Mechanical wear
Aging
Detritophagy
Bryophagy

**Abstract**

A study was conducted to determine whether the mandibles of the detrito-/bryophagous groundhopper *Tetrix tenuicornis* are subject to mechanical wear as a result of feeding, as is the case for grasshoppers that feed on silica-rich grasses. Abrasion was evaluated by measuring the length and width of the 3rd incisor and length of the 4th incisor in adults of different ages collected under natural conditions during one season. Although *T. tenuicornis* and other groundhoppers avoid feeding on grasses, we found that mandible abrasion increased with *T. tenuicornis* age. Age-related abrasion of the incisors of left and right mandibles was statistically significant in both sexes but the degree of abrasion was greater for females than males, apparently reflecting differences in the frequency and magnitude of feeding. Degree of abrasion also differed between right and left mandibles, probably because of differences in how each mandible is used during food processing. Abrasion of cuticular mandible structures may reduce the effectiveness of food processing late in the season.

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**1. Introduction**

The insect exoskeleton is composed of solid and elastic cuticle, which provides little resistance to mechanical abrasion (Vincent and Wegst, 2004; Gorb, 2005). Because the parts of the exoskeleton most affected by mechanical abrasion are the mandibles (Chapman, 1964), the highly stressed parts of mandibles are reinforced against wear by the presence of heavy metals such as zinc, manganese, or occasionally iron (Vincent and Wegst, 2004). Mandibles that function in mechanical grinding have been recorded for representatives of the Ephemeroptera, Isoptera, Orthoptera, Hemiptera, Lepidoptera, and Coleoptera (Brown, 1961; Chapman, 1964; Wallin, 1988; Köhler et al., 2000; Roitberg et al., 2005; Massey and Hartley, 2008; Raupp, 2008).

Grasshoppers, which can have a relatively long-lived adult stage (3–4 months), feed mostly on grasses, which are stiff and contain large amounts of silicates (Gangwere, 1960; Ingrisch and Köhler, 1998). The exception are groundhoppers of the genus *Tetrix*, which feed on mosses, lichens, algae, detritus, and only occasionally on young grass leaves (Kocárek et al., 2008, 2011). In the last months of grasshopper lives, their mandible structures may be sufficiently abraded so as to complicate food processing (Gangwere, 1960; Chapman, 1964). Age-related mandible abrasion has been documented in five species of grasshoppers and locusts: *Pezotettix giornae* (Rossi, 1794), *Locusta migratoria* (Linnaeus, 1758), *Schistocerca gregaria* (Forskal, 1775), *Nomadacris septemfasciata* (Serville, 1838), and *Chorthippus parallelus* (Zetterstedt, 1821) (Chapman, 1964; Köhler et al., 2000).

The focus of the current study, the groundhopper *Tetrix tenuicornis* (Sahlberg, 1893), feeds mostly on detritus and bryophytes and avoids grasses, as summarized by Ingrisch and Köhler (1998). The mandibles of *T. tenuicornis* are adapted to this type of food by having thin and sharp teeth and flat molar parts that have a file of parallel slats; these adaptations facilitate the trituration of soft tissues (Ingrisch and Köhler, 1998). The relationship between orthopteran mouthpart structure and diet has been known for years (e.g., Gangwere, 1965; Kaufmann, 1971; Paranjape and Bhalarao, 1985; Gangwere and Spiller, 1995; Kang et al., 1999).

To date, the grasshopper species that have been studied with respect to mandible abrasion are grass-feeding specialists, and high degrees of abrasion in incisor and molar areas were recorded for their mandibles in all cases. Mandible abrasion, however, has not been previously studied in orthopterans that feed on food other than grass. Our study determined whether orthopterans that feed on detritus and mosses experience similar mandible abrasion as those that feed on silica-rich grasses.

The specific aims of the present study were: (1) to assess the age-related abrasion rate of mandibles in the groundhopper *T.
2. Material and methods

2.1. Studied organism

The groundhopper *T. tenuicornis* is one of the most widespread species of its genus. The body length of adults ranges from 7 to 13 mm, and females are usually larger than males. This species is active from March to the end of October in Central Europe. It is most abundant in May and August, depending on climatic conditions (Kočárek et al., 2005; Baur et al., 2006). Like other species of groundhoppers, *T. tenuicornis* usually inhabits moist habitats (the embankments of water bodies or wet meadows) but also inhabits semidry or seasonally dry microhabitats. The cycle is univoltine, with the generation appearing in May; mating and oviposition occur at the beginning of summer, and nymphs of the next generation appear in summer and fall (Ingrisch and Köhler, 1998). The diet of *T. tenuicornis* includes detritus, mosses, algae, and small amounts of other substrates (Ingrisch and Köhler, 1998).

2.2. Material collection

Specimens of *T. tenuicornis* used for analysis of mandibles were collected with sweep nets along the banks of a flooded depression in a post-mining area near Karviná, Czech Republic (GPS: 49° 49′ 38.953″ N, 18° 33′ 13.567″ E), from 13 May to 20 June 2008. The specimens were collected on 13 May, 23 May, 2 June, 9 June, and 20 June, usually between 10:00 and 17:00 (when the groundhoppers were active).

Material for gut-content analysis was collected with sweep nets on 9 June 2009 at a former sedimentation pond in the post-mining area of the Frantisek mine, Prostřední Suchá, Czech Republic (GPS: 49° 48′ 28.2″ N, 18° 27′ 51.2″ E). The collected insects were placed in 70% ethanol.

2.3. Study of abrasion and analysis of alimentary tract contents

In the laboratory, both mandibles of each specimen were dissected and mounted horizontally on paper labels using methylcellulose glue. In total, mandibles of 44 specimens (23 males and 21 females) of *T. tenuicornis* were processed. The posterior sides of the mandibles were studied and photographed using an Olympus SZ51 binocular microscope with a mounted Olympus C3030 camera at 90× magnification. The length and basal width of the 3rd incisor (I3 in the following text) and the length of the 4th incisor (I4 in the following text) were measured using ATLAS image analysis software (Arsenal ver. 3.4.12.). Abrasion of selected mandibles was studied and documented by scanning electron microscope (SEM-JEOL JSM-6610LV, SEI, 30 kV, WD 22 mm, SS30, 45× magnification). The mandibles were sputter coated with gold (AUTOMATIC SPATTER COATER: JEOL JFC-1300). The terminology of mandible structures follows that of Gangwere (1960).

For gut-content analysis, the oesophagus, crop, and proventriculus were dissected from the abdominal cavity using a thin forceps. Permanent microscopic preparations of the alimentary tract contents were made using Hoyer’s solution (Anderson, 1954). For comparison and identification of tissue fragments in alimentary tract contents, permanent microscopic preparations of the leaves of each moss species taken from the same locality were made by the same method. A total of 20 specimens of *T. tenuicornis* (10 males and 10 females) were used for alimentary tract dissection and for analysis of food particles within alimentary tracts.

2.4. Statistical analyses

Mandible measurements (I3 length, I3 width, and I4 length) are presented as means ± standard error. According to the Shapiro–Wilk Normality Test all data were normally distributed. Differences between left and right mandibles of males and females and among mandibles from different collection dates were evaluated using linear models. The best combination factors were selected using the Akaike information criterion (AIC). Analysis of variance (ANOVA) was used to determine the effects of sex (male vs. female), mandible side (left vs. right), and sampling date (an indicator of specimen age) on mandible measurements. The abrasion rate was compared for the two sexes by plotting I3 length, I3 width, and I4 length on day of the year and comparing the slopes of the linear regressions. All analyses were performed using the R Ver. 2 statistical program (R Development Core Team, 2003). The significance level was α = 0.05 throughout.

BaDra 2.0 image analysis software (2011) was used to determine the proportions of detritus and moss in the permanent microscopic preparations of alimentary tract contents. Because the variance was large, the alimentary tract data were logarithmically transformed before evaluation with a generalized linear model (one-way ANOVA).

3. Results

3.1. Mandibular morphology and abrasion in *T. tenuicornis*

Each of the two mandibles of *T. tenuicornis* consists of two functional parts: the incisor area and the molar area (Fig. 1). The incisor area is equipped with four slender and sharp teeth, of which I3 is the longest on both the right and left mandibles (Table 1). The molar part of the left mandible consists of two central teeth and a flat molar ridge; the molar part of the right mandible consists of the molar ridge and one weak tooth. The two central teeth on the left mandible are sharper and more conical than the single central tooth on the right mandible. The molar ridge is strongly concave on the left mandible but is convex on the right mandible. The molar ridge of the left mandible forms a biting area with parallel slats; such slats are not evident on the molar ridge of the right mandible. The left molar ridge has 11.7 ± 1.1 slats (minimum 10, maximum 14). For both sexes, right mandibles are larger than left mandibles, and both right and left mandibles are larger in females than males (Table 1).

Mandible abrasion was indicated by the linear decline in I3 length, I3 width, and I4 length with specimen age (which was inferred from the sampling day) (Fig. 2). Incisors of left and right mandibles were distinctly longer and sharper on females collected early in the season than late in the season (Fig. 3).

According to ANOVAs, I3 length, I4 length, and I3 width of the mandibles were significantly affected by sex, mandible side, and day of the year (Table 2). For the left mandible, I3 length decreased with age (as indicated by day of the year) for both females and males (Fig. 2A). For the right mandible, I3 length decreased with age for females but not for males (Fig. 2B). For the left mandible, I4 length decreased with age for both females and males (Fig. 2C). For the right mandible, I4 length decreased with age for females but increased with age for males (Fig. 2D). For the left mandible, I3 width decreased with age for both females and males (Fig. 2E). For the right mandible, I3 length decreased with age for females but not for males (Fig. 2F). The similarity slopes of the line between sexes for the left mandible were 92% for I3 length, 88% for I4 length, and 31% for I3 width and for the right mandible were similarity slopes of the line 1% for I3 length and 84% for I3 width.
3.2. Food composition

The alimentary tracts of *T. tenuicornis* specimens contained detritus, moss phylloids, gemmae, and rhizoids, fungal spores, cyanobacterial fibres (order Oscillatoriales), mineral particles, and antennae, cuticles, and setae of invertebrates. The dominant component was detritus, which is defined as soil with unidentified decomposed organic matter. Across the two sexes, detritus accounted for 83.7% and moss 16.3% of the total quantity of food in the alimentary tract. The detritus/moss ratio was 3.35 in females and 6.87 in males (Fig. 4). Five moss species were found to be components of the groundhopper diet at the studied locality: *Barbula convoluta* Hedw., *Brachythecium albicans* (Hedw.) Schimp., *Bryum caespiticium* Hedw., *Campylopus introflexus* (Hedw.) Brid., and *Ceratodon purpureus* (Hedw.) Brid. No fragments of grass were found in the digestive tracts of the studied individuals. The proportions of dominant food components differed between the sexes, with females consuming a larger proportion of moss ($F = 3.27, p = 0.08$) and males consuming a larger proportion of detritus ($F = 5.68, p = 0.03$) (Fig. 4).

4. Discussion

As indicated by the linear decline in I3 length, I3 width, and I4 length with specimen age, mandible abrasion was detected in the groundhopper *T. tenuicornis*. Similar mandible abrasion was

<table>
<thead>
<tr>
<th>Sex</th>
<th>Statistic</th>
<th>Left mandible</th>
<th>Right mandible</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I3 length</td>
<td>I3 width</td>
</tr>
<tr>
<td>Male</td>
<td>Mean</td>
<td>73.7 ± 11.5</td>
<td>68.3 ± 9.0</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>44.3</td>
<td>53.1</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>98.5</td>
<td>86.0</td>
</tr>
<tr>
<td>Female</td>
<td>Mean</td>
<td>88.8 ± 13.3</td>
<td>80.7 ± 10.7</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>61.5</td>
<td>55.7</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>119.3</td>
<td>102.4</td>
</tr>
</tbody>
</table>
I3 length, I4 length, and I3 width declined linearly with age in both sexes of *Tetrix tenuicornis*, with the exception of the right mandibles of males. The regression showed that the length and the width of incisors were good indicators of the abrasion of the whole mandible. Given that mandible abrasion increased with ground-hopper age, such abrasion could be used to estimate ground-hopper age and such estimation could be used to assess evolutionary processes in natural populations, life table parameters, or age-related life history changes. For example, Reinhardt et al. (2007) estimated the age of *C. parallelus* females based on mandibles abrasion.

The length of the 3rd incisor seems to be a very good indicator of abrasion in ground-hopper mandibles. Dependence of abrasion on the age of the individual was evident for I3 and I4 length and I3 width of the left mandible. This relationship, however, was not always significant for the right mandible. The results indicate that the left and right mandibles of *T. tenuicornis* ground-hoppers abrade at different rates, perhaps because of how the ground-hoppers gnaw food. Because the mandibles overlap during chewing and because the right mandible is beneath the left one, contact with the consumed food is longer for the left than for the right mandible.

There is greater abrasion of the left mandible’s outer side by the right mandible, which is underneath it while chewing. In the processing of food by the mandibles, not only is incisor length decreased, but all contact surfaces of the mandible are abraded, as evidenced by the reduction in the incisor width. While other authors described abrasion of the right mandible in other grasshoppers (*Chapman, 1964; Köhler et al., 2000*), those authors unfortunately did not mention abrasion of the left mandible, and so we cannot compare the abrasion to the left mandible of *T. tenuicornis* with that occurring in other species. In the case of *T. tenuicornis* in the current study, the abrasion rate of the right mandible was higher in females than in males while the abrasion rate of left mandible was similar in females and males. A question remains as to why the I4 length of the right mandible in males increased with age even as the length of this incisor on the left mandible decreased.

The rate of abrasion differed between female and male *T. tenuicornis*. Greater wear of mandibles in females than in males correlates with increased feeding activity of females, as demonstrated in several studies on various species of *Tetrix* (Kočárek et al., 2008, 2011). Females consume more food than males not only because they are larger but also because they require energy and materials to develop and lay eggs. The males, in comparison,
...mandible movement apparently have a great influence on the degree of abrasion. According to Köhler et al. (2000), the slope of the linear regression of I2 length (in μm) of the right mandible on age (in days) was \(-0.022\) for females and males of *L. migratoria*. In the current study of *T. tenuicornis*, the abrasion rates of I3 and I4 length of right mandibles were higher in both females and males than those reported for *L. migratoria* by Köhler et al. (2000), but *L. migratoria* fed only on Chinese cabbage in the latter study, which was conducted in the laboratory. The relatively soft tissues of Chinese cabbage apparently resulted in only a slow rate of mandible abrasion. Given the character of the *T. tenuicornis* food sources, we had assumed that the mandibles of *T. tenuicornis* would exhibit less abrasion than those of other species that feed on harder food. The structures of the mandible by the groundhoppers are very special. As indicated earlier, the mandible of the adult *T. tenuicornis* has a molar ridge with slats. Isely (1944) described three categories of mandibles in grasshoppers (Caelifera) according to general structure and associated diet. These categories, which are still used today, are graminivorous, forbivorous, and herbivorous. Kaufmann (1965) distinguished another specialized type of mandible pair in the family Tetrigidae and named it the bryovorous type. Later, Aguirre et al. (1987) classified this type of mandible pair as the grater type. In the current study, we show that the interior part of the mandibles consists of two structurally distinct areas (incisor and molar area). The incisor area consists of a set of four long teeth (among which the second and third are the longest). The molar area consists of about ten slats. According to Pananje (1985), the molar area of the mandibles is used for triturating the fragile tissues of mosses and algae, while the teeth of the incisor area are used for cutting pieces from larger items in the diet.

In accordance with our initial assumptions, the food of the *T. tenuicornis* groundhoppers at the monitored location consisted primarily of detritus (undiately soil with decomposed organic matter). Various parts of mosses formed the next most abundant component. In the *T. tenuicornis* digestive tract, we also found pollen grains, fungal hyphae, algae, mineral particles, and even body parts of various invertebrates. A similar food composition has been described for other species of this family (e.g., Hodgson, 1963; Bhalerao et al., 1987; Reynolds et al., 1988; Hochkirch et al., 2000, 2007; Kočár et al., 2008, 2011). Age-related mandible abrasion has been studied in five species of grasshoppers and locusts, all of which are grass-feeding specialists (Chapman, 1964; Köhler et al., 2000). Abrasion of bryovorous (grater-type) mandibles is investigated for the first time in this paper. Generally, the main component of the groundhopper diet is vegetation (leaves, seeds, stems, blooms, fruits, etc.) (Gangwere, 1991). Plants exhibit several defenses against herbivorous insects and other herbivores, and these include secondary chemicals and physical defenses, such as leaf toughness and pubescence (Miura and Ohsaki, 2004). Some plant tissues also contain silicates, which contribute to the hardness of leaves and make them difficult for herbivores to consume (Bernays and Chapman, 1978; Miura and Ohsaki, 2004; Markham et al., 2006). Chewing tough, fibrous, silica-containing tissues leads to mechanical wear of the mandibles (Chapman, 1964). It follows that mandible wear would be a useful measure of adult age in grass-feeding Gomphocerinae in the field, although the wear also depends greatly on the specific composition of the diet (Isely, 1944; Gangwere, 1960; Köhler et al., 2000).

We assume that the abrasion of mandibles is mostly affected by inorganic particles contained in the detritus. However, it will be necessary to test this assumption experimentally. Groundhoppers probably scrape algae and microorganisms from the surfaces of sand particles, and such particles may be an important source of abrasion. In conclusion, even though the groundhopper *T. tenuicornis* feeds mainly on detritus and mosses, the foods consumed lead to the wearing of the mandible parts. As noted, mandible abrasion was greater in females than in males, presumably because females have a greater requirement for energy and materials than males. Finally, the wearing down of the mandible's

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**Table 2**

Effects of *Tetrix tenuicornis* sex (male vs. female), mandible side (left vs. right), and day of year (an indicator of groundhopper age) on lengths and widths of 3rd incisors (I3) and lengths of 4th incisors (I4) as indicated by analyses of variance (ANOVA). For each characteristic, there were 85 degrees of freedom.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sex</th>
<th>Mandible side</th>
<th>Day of the year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-value</td>
<td>p-value</td>
<td>F-value</td>
</tr>
<tr>
<td>I3 length</td>
<td>45.09</td>
<td>&lt;0.01</td>
<td>32.92</td>
</tr>
<tr>
<td>I3 width</td>
<td>61.48</td>
<td>&lt;0.01</td>
<td>61.48</td>
</tr>
<tr>
<td>I4 length</td>
<td>14.16</td>
<td>&lt;0.01</td>
<td>14.08</td>
</tr>
</tbody>
</table>

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**Fig. 3.** Ventral view of A) non-abraded mandibles and B) abraded mandibles of a female *Tetrix tenuicornis* (SEM micrographs). Mandibles in A and B were dissected from females collected on 13 May and 20 June, respectively.
cuticular structures probably reduces the efficiency of food processing late in the season and could lead to groundhopper starvation. In the current research, the abrasion of T. tenuicornis mandibles was studied under natural conditions without control of food composition or specimen age. Further study under laboratory conditions is necessary to assess the degree of abrasion caused by individual food components.

Acknowledgements

This research was supported by an Institutional Research Support grant from the University of Ostrava (reg. no. SGS21/PF/2013) and by the Institute of Environmental Technologies — Ostrava (CZ.1.05/2.1.00/03.0100 and ED 2.1.00/03.0100), funded jointly by the EU Operational Program ‘Research and Development for Innovations’. The authors thank Dr. Bruce Jaffee (USA) for linguistic and editorial improvements.

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Fig. 4. Percentage of detritus and moss relative to all food components in all individuals in females and males of T. tenuicornis as determined by gut-content analysis. Detritus (unidentified soil with decomposed organic matter) followed by moss were the dominant food components. The box plots show the median (50th percentile), 25th and 75th quartiles, error bars show 10th and 90th percentiles and filled symbols show outliers.