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Dietary diversification and variations in the number of labrum sensilla in grasshoppers: Which came first?

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The diversity of the diet of grasshoppers (Acrididae, Orthoptera) is related to multiple factors, including the chemoreceptors on the antennae, palps and on the epipharyngeal face of the labrum. In the present study, we sought to understand the nature of the diet of 12 Moroccan acridian species and to try to relate various aspects of their diet to the number of labrum sensilla. If the effect of the labrum size on the number of sensilla is removed, four groups of species are recorded: (i) polyphagous species with a broad diet and numerous sensilla; (ii) polyphagous species with a graminivorous diet and numerous sensilla; (iii) oligophagous species feeding exclusively on Poaceae and with a medium number of sensilla; and (iv) strictly monophagous species feeding on a single plant species and with the smallest number of sensilla. These observations show the close relationship between the diet and the number of labrum sensilla. However, *Sphingonotus rubescens*, a polyphagous species, is an exception to this trend as it harbours a medium number of sensilla. We propose that the modification in the number of labrum sensilla is a result of a progressive adaptation to a different diet and does not represent its cause.

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1. Introduction

Grasshoppers (Orthoptera, Acrididae) are found mainly in open environments (Uvarov 1977) but prefer special microhabitats for shelter or trophic resources that may vary according to ecological factors considered at a larger scale, such as altitude (Boitier 2004). They are phytophagous and, depending on the breadth of their diets, can be classified as polyphagous, oligophagous, or monophagous (Kogan 1977; Dethier 1980; Chapman 1982). Polyphagous species feed on plant species belonging to several families or classes (Jolivet 1992, 1998), including Dicotyledones (Le Gall 1989). Oligophagous species feed on plants belonging to a single family or genus, and monophagous species feed on a single plant species (Nicole 2002).

This trophic variation can be influenced by several factors (Muralirangan and Muralirangan 1985) in terms of nutrient quality and taste or aspect. As a trophic source, experiments conducted by Raubenheimer and Simpson (2003) highlighted

the role of protein over carbohydrate ratios in the total amount of food consumed. Grasshoppers recognize their host plant by visual (Prokopy and Owens 1983), olfactory and gustatory (Finch 1980; Staedler 1982) or tactile stimuli (Le Gall 1989). It has been suggested that the structural and functional diversity of the chemoreceptors play a role in the food choice of grasshoppers (Haskell and Schoonhoven 1969; Sturckow 1970; Altner and Prillinger 1980). These chemoreceptors are located on the oral parts (Jin *et al.* 2006), the type A gustatory sensilla on the labrum (ElGhadraoui *et al.* 2002), and on the antennae (Chen and Kang 2000; Chen *et al.* 2003; Dumas *et al.* 2010). Chemosensory sensilla on palps and antennae express two classes of proteins involved in the recognition of odors and taste (Jin *et al.* 2005; Yu *et al.* 2009). The odorant binding proteins (OBP) correspond to c. 140-aa-long proteins, while chemosensory proteins (CSP) to c. 110-aa-long proteins. This last class of protein could play a role in both taste and odor detection, in contrast to OBP, specialized in odor detection (Angeli *et al.* 1999). Three sequences have been identified for

Keywords. Adaptation; diet; grasshopper; labrum sensilla

OBP and two for CSP in locusts but their involvement in sensitivity to chemical compounds are not yet characterized (Yu *et al.* 2011).

As for the mouthparts, according to Chapman and Thomas (1978), the epipharyngeal face of the labrum (the equivalent of upper lip for insects) bears four more or less symmetric groups of sensilla, homogeneous by their structure. Cook (1972) described each sensillum as a short cone lying in a circular pit of 7.5 µm diameter, surrounding five bipolar neurons. The four groups are named by their position: A1 sensilla in the proximal part of the labrum, A2 sensilla in the central part, A3 sensilla on the distal edge, and A10 sensilla on each side at a level between A1 and A2 sensilla (figure 1). No class of protein involved in taste has been yet identified in these sensilla, although the CSP are the best candidates (Angeli *et al.* 1999). Most direct information dealing with their physiology was obtained by removal experiments on *Schistocerca gregaria* (Haskell and Schoonhoven 1969). They showed that A3 sensilla can detect repellent molecules, whereas A1 and A2 were devoted to the detection of stimulating feeding molecules as sucrose.

In a previous study (ElGhadraoui *et al.* 2002), we showed that in *Doclostaurus* spp. (Gomphocerinae), the evolutionary change from oligophagy to polyphagy was linked to increase in the number of gustatory type A labrum sensilla. In the present study, we focus on the Acrididae, and especially the subfamily Oedipodinae, which has adopted a variety of diets (Gangwere and Morales-Agacino 1973). We will first quantify the connection between the number of gustatory sensilla on the epipharyngeal face of the labrum and the properties of the

food. Second, we will elucidate whether the change of diet precedes or follows the variation in the number of labrum sensilla.

2. Materials and methods

2.1 Studied species

The site of Al-Azagh in the Middle Atlas (Morocco) is one of the three gregarisation (swarming) areas of the Moroccan Locust *Doclostaurus maroccanus* (ElGhadraoui *et al.* 2003; Mokhlesse *et al.* 2007), where an interesting diversity of acridian species is associated with a diverse vegetation (Appendix Table 5). This station is located between Tazouta and Skoura (4°34' W, 33°38' NR) at 960 m A.S.L. We studied 12 grasshopper species collected during July 2008 and the Desert Locust (*Schistocerca gregaria*), provided by the Anti-Acridian National Centre of Agadir (Morocco). We analysed a total of 260 adults (i.e., 10 males and 10 females for each species). The studied species (table 1) belonged to three sub-families: the Barbary grasshopper *Calliptamus barbarus* (Calliptaminae); the Band-winged grasshopper *Oedipoda miniata mauritanica*, *Oedipoda fuscocincta fuscocincta*, *Oedaleus decorus*, *Sphingonotus azurescens*, the slender Blue-winged grasshopper *Sphingonotus coeruleans atlas*, *S. rubescens*, *S. lucasii*, *S. octofasciatus*, *S. finotianus*, *Paracinema tricolor bisignata* (Oedipodinae), the Moroccan locust *Doclostaurus maroccanus* (Gomphocerinae), and the desert Locust *Schistocerca gregaria* (Cyrtacanthacridinae). We adopted the tribe division of Oedipodinae defined in the Orthoptera Species File home page (osf2.orthoptera.org) and

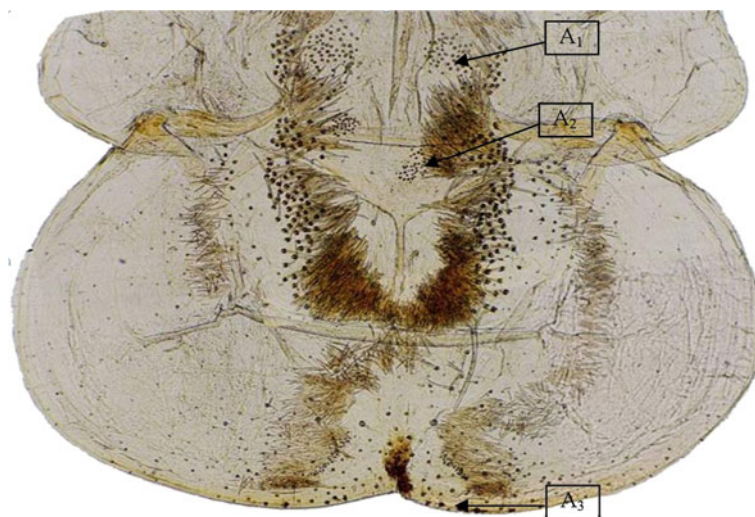


Figure 1. Arrangement of the various A-type sensilla on the epipharyngeal face of the labrum in *Oedaleus decorus*. The numerous hairs observed around the sensilla serve as wicks to conduct the liquids toward the base of the sensilla.

Table 1. Diet of the acridian species found in Al-Azaghar

Subfamilies	Tribes	Species	Diet (this work)	Literature
Oedipodinae	Epacromiini	<i>Paracinema tricolor bisignata</i> (Charpentier, 1825)	<i>Hordeum murinum</i>	Corsica: Cyperaceae (Boitier <i>et al.</i> 2006)
	Oedipodini	<i>Oedipoda miniata mauritanica</i> Lucas, 1846-1849	Poaceae ¹	
		<i>Oedipoda fuscocincta</i> Lucas, 1846-1849	Polyphagous ²	Spain: Polyphagous (Gangwere and Morales-Agacino 1973)
	Locustini	<i>Oedaleus decorus</i> (Germar, 1825)	<i>Hordeum murinum</i>	Spain : Poaceae (Gangwere and Morales-Agacino 1973)
	Sphingonotini	<i>Sphingonotus rubescens</i> (Walker, 1870)	Polyphagous ²	
		<i>Sphingonotus octofasciatus</i> (Serville, 1838)	<i>Hordeum murinum</i>	
		<i>Sphingonotus azurescens</i> (Rambur, 1838)	Poaceae ¹	
		<i>Sphingonotus coeruleans atlas</i> Chapman, 1938	Poaceae ¹	Spain: polyphagous (Gangwere and Morales-Agacino 1973)
		<i>Sphingonotus lucasii</i> Saussure, 1888	Poaceae ¹	
	Gomphocerinae	Doclostaurini	<i>Sphingonotus finotianus</i> (Saussure, 1885)	Poaceae ¹
<i>Doclostaurus maroccanus</i> (Thunberg, 1815)			Polyphagous ²	Morocco : polyphagous (Benhalima <i>et al.</i> 1984)
Calliptaminae	Calliptamini	<i>Calliptamus barbarus</i> (Costa, 1836)	Polyphagous ²	Algeria : polyphagous (Benzara <i>et al.</i> 2003)
Cyrtacan-thacidinae	Cyrtacanthacri-dini	<i>Schistocerca gregaria</i> (Forskål, 1775)	Polyphagous ²	Algeria: polyphagous (Guendouz-Benrima <i>et al.</i> 2010).

¹ Poaceae consumed: *Schismus barbarus*, *Echinaria capitata*, *Stipa retorta*, *Hordeum murinum*, and *Bromus rubens*.

² Plant families consumed: Poaceae, Caryophyllaceae, Borraginaceae, and Asteraceae.

in the work of (Fries *et al.* 2007) and (Hochkirch and Husemann 2008).

2.2 Determination of the diet

To determine the diet of the studied species, we compared the plant fragment in the feces of the captured grasshoppers with the bank of plant epidermis in the reference collection prepared from the plant species collected at Al-Azaghar. This method has been employed in other studies of acridians (BenHalima *et al.* 1984; Damerdji *et al.* 2000; ElGhadraoui *et al.* 2002; Benfekih 2006; Benkenana *et al.* 2013). The grasshoppers were collected individually and placed in plastic tubes for 12 h, a time sufficient for the grasshoppers to empty their digestive tract. The obtained fecal samples were stored dry in Eppendorf tubes for further identification. The fecal samples were softened for 24 h in water supplemented with a surfactant and emulsifier (i.e., polysorbate 80 or 'tween 80') and then passed through a series of baths: 12% bleach (sodium hypochlorite) for 20 minutes to eliminate chlorophyll, distilled water to remove an excess of bleach,

and then 75% ethanol to dehydrate. The fecal samples were then placed between a glass slide and a cover slide and enclosed in liquefied glycerol gelatin. The plant epidermis fragments of the fecal samples were then examined under a light microscope and identified on the basis of (i) the shape, size, and the arrangement of the plant cells; (ii) the structure of the cell walls; and (iii) the length, arrangement, and structure of the stomata.

2.3 Sensilla counting

To count the number of labrum sensilla in the studied species, we used the technique employed by (ElGhadraoui *et al.* 2002) and (Picaud *et al.* 2003). This method consists in extracting the labrum from the grasshopper under a stereomicroscope, using a fine pair of forceps and a cutter or scissors. The isolated labrum is soaked in a 50% KOH solution in distilled water for 1–6 h depending on its size to lighten specimen and then rinsed in distilled water for 15 min. This procedure cleans the labrum and facilitates the counting of the sensilla. Each labrum was then placed

between a glass slide and a cover slide and fixed in liquefied glycerol gelatin. After the glycerol gelatin solidified at room temperature, the labrum was placed under the light microscope to count the sensilla A1, A2, A3, and A10 (Thomas 1996). The width of the labrum was measured under a stereomicroscope in order to control for the size of the labrum in the various species.

2.4 Statistical treatment

The regression lines between the number of sensilla and the width of the labrum were calculated using PAST 1.96 (Hammer *et al.* 2001). Regression residuals allowed the removal of effect of size on the sensilla numbers. If such a correction was not done, the species would be grouped according to their overall size, and not by their sensilla repertoire. The residuals have to be understood as sensilla numbers once the effect of size had been removed. To test if there is a significant difference in sensilla residuals according to diet, ANOVAs and Tukey's post hoc tests were conducted with SYSTAT 12.0 (SYSTAT 2007). To test the simultaneous effects of both diet and systematics, the General Linear Model (GLM) implemented in SYSTAT 12.0 (SYSTAT 2007) was used. This analysis was limited to Oedipodinae and Sphingonotini because both tribes comprise species with varied diets. To visualize the separation of groups of species according to their diet and sensilla configuration, a Principal Component Analysis (PCA) was conducted using PAST 1.96 (Hammer *et al.* 2001). The advantage of this multivariate analysis is to project on a two-dimensional graph the orientation of the residuals of the three groups of sensilla and the 13 species. If the envelopes gathering each group of species according to their diets are well separated, it will be possible to conclude that sensilla numbers are good discriminating parameters.

3. Results

3.1 Relationship between food mode and systematics

The study of the diet through a fecal analysis enabled us to distinguish three different types of phytophagy. Although oligophagy based on the consumption of Poaceae (true grasses) seems to be the most common type of diet, we found numerous variations depending on the species. As shown in table 1, there is no relationship between the diet and the systematic position of the grasshopper species. For example, within the genus *Sphingonotus*, there are polyphagous species (*S. rubescens*), oligophagous species (*Sphingonotus finotianus*, *S. azurescens*, *S. coeruleans*, and *S. lucasii*), and monophagous species (*S. octofasciatus*). Likewise, in the genus *Oedipoda*,

there is an oligophagous species (*O. miniata*) and a polyphagous species (*O. fuscocincta*).

3.2 Configuration of labrum sensilla

A10- type sensilla are absent in the majority of the species and are not considered here.

The mean numbers of the sensilla of the types A1, A2 and A3 in the females and males vary in parallel in the studied species (table 2). For the females and males of all the studied species, the number of A1-type sensilla is much greater than that of the A3-type sensilla, whereas the number of A2-type sensilla occupies an intermediate place. Four species (*Calliptamus barbarus*, *Oedipoda fuscocincta*, *Schistocerca gregaria*, and *Dociostaurus maroccanus*) possess a relatively high number of A1-type sensilla. The males and females of the other species have a lower number of sensilla. We checked whether there was an effect of size on the absolute numbers of sensilla by taking account the width of the labrum as a proxy for size. We obtained a linear relationship between the width of the labrum and the number of each type of sensilla (table 3), which was highly significant (N=260, Pearson $r=0.9$, 0.93 and 0.92 , respectively, $p<0.001$).

To eliminate the effect of size on the number of sensilla, we calculated the average residuals of the number of sensilla per species, using the regression lines indicated in table 3. This allowed us to show the interspecific variations of these chemoreceptor sensilla (figure 2). The studied species can be classified in four groups according to the average residuals of sensilla numbers (or corrected numbers): (1) Species with generally positive residuals for the three types of sensilla include *Calliptamus barbarus* and *Schistocerca gregaria*, *Dociostaurus maroccanus*, and *Oedipoda fuscocincta*; (2) species with negative residuals for the three types of sensilla include *Oedaleus decorus*, *Sphingonotus octofasciatus*, *S. azurescens*, and *Paracinema tricolor*; (3) species with residuals near the average for the three types of sensilla include *Oedipoda miniata*, *S. finotianus*, *S. coeruleans*, *S. rubescens*, and *S. lucasii*.

We checked whether the variation in the number of sensilla were linked to the diet by using one-way ANOVAs (figure 3) with the three phytophagous types (i.e. monophagous, oligophagous, and polyphagous). It appears that the polyphagous species have significantly more (Tukey's test, $p<0.01$) labrum sensilla, whatever the type, than monophagous species. The oligophagous species have an intermediate number of all sensilla types.

Within the Oedipodinae, we looked for any connections between the numbers of sensilla, systematic position of the grasshopper species and their diet, using the GLM implemented in SYSTAT 12 (SYSTAT 2007). Therefore, we restricted the data set to the only tribes comprising several species, i.e. Oedipodini and Sphingonotini, with 2

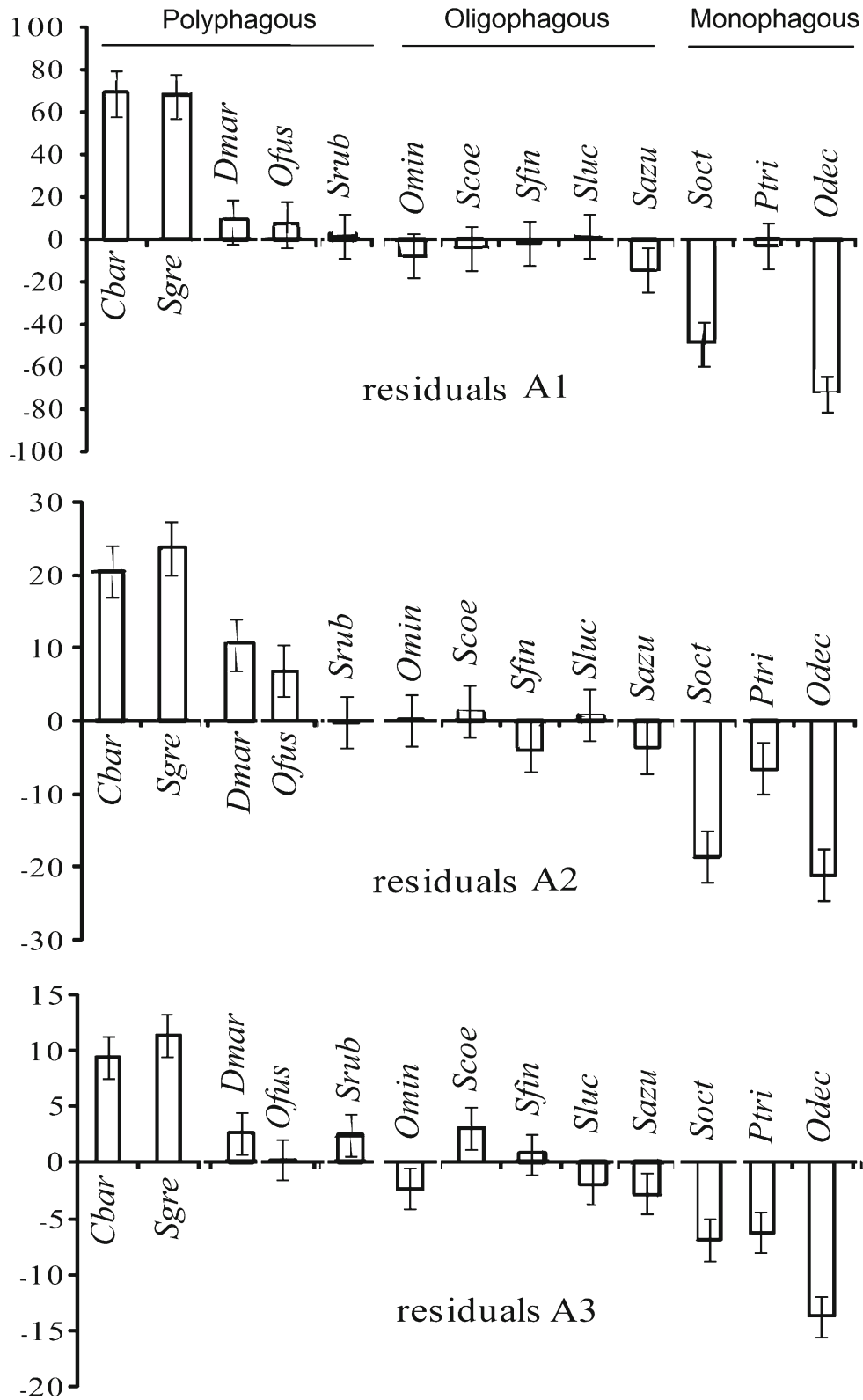


Figure 2. Mean residuals (\pm S.E.M.) of sensilla numbers in the studied species. Top: A1 residuals, middle: A2 residuals, bottom: A3 residuals. Cbar: *Calliptamus barbarus*, Sgre: *Schistocerca gregaria*, Srub: *S. rubescens*, Dmar: *Docioptaurus maroccanus*, Ofus: *Oedipoda fuscocincta*, Omin: *Oedipoda miniata*, Sazu: *Sphingonotus azurescens*, Scoe: *S. coeruleans*, Sfin: *S. finotianus*, Sluc: *S. lucasii*, Soct: *S. octafasciatus*, Ptri: *Paracinema tricolor*, Odec: *Oedaleus decorus*.

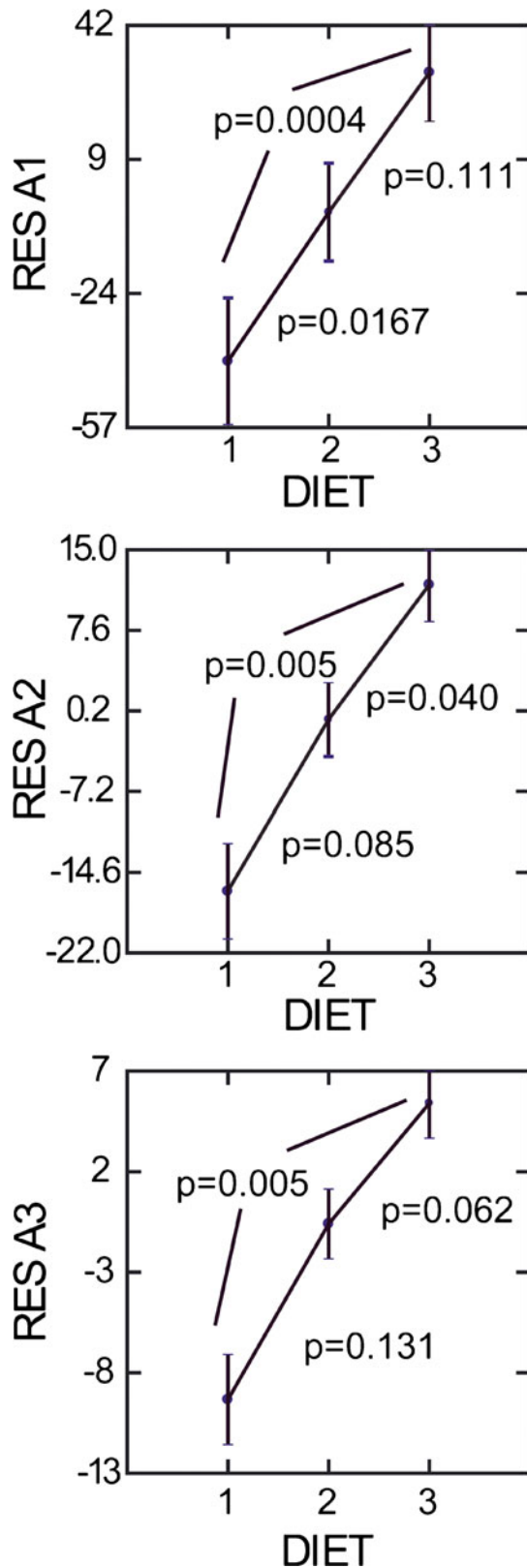


Figure 3. Variations in sensilla residuals according to diet (whole data set of species). 1: monophagous; 2: oligophagous; 3: polyphagous. The significance is given by Tukey's post hoc tests.

and 6 species respectively. Although only a few species were included in the analysis, the results gathered in table 4 indicate that an association exists between diet and the sensilla, irrespective of the type and tribe.

Assuming that there is a clear general connection between the diet and configuration of the labrum sensilla, we explored the individual variability in each species. The question being addressed is whether the modifications in the number of sensilla follow or precede a change in the diet during the evolution of a species. In other words, we wanted to know whether the interspecific differences in the numbers of the sensilla were the cause or the consequence of a dietary change. A global view of the distribution of the diet and sensilla of species is given by the PCA projection in figure 4. The three envelopes corresponding to the three diet categories are well separated with the polyphagous species situated at the right side of the plan and the monophagous species at the opposite side. The only exception to this pattern is represented by *Sphingonotus rubescens*, which is polyphagous and is situated within the range of oligophagous species. As illustrated in figure 2, in this species the residuals corresponding to A1-type and A2-type sensilla do not differ from zero, indicating that these sensilla did not adapt to a polyphagous diet. In contrast, the A3-type sensilla present a moderately positive residual, suggesting the possibility to an increasing recognition of deterrent molecules.

4. Discussion

Among the studied grasshopper species, four groups can be identified based on the number of sensilla on the labrum and the width of spectrum of consumed plants. (1) Polyphagous species with a broad dietary spectrum have a large number of A1-type on their labrum, such as *Calliptamus barbarus* and *Schistocerca gregaria*. (2) Polyphagous species with a broad dietary spectrum, but feeding preferentially on Poaceae, have a relatively high number of sensilla on their labrum, such as *Dociostaurus maroccanus* and *Oedipoda fuscocincta*. Based on its large number of sensilla *Dociostaurus maroccanus* is predicted to be more broadly polyphagous beyond being graminivorous (ElGhadraoui *et al.* 2002). The same could be said about *Oedipoda fuscocincta*, except that its A3-type sensilla number is more typical of oligophagous species. (3) Oligophagous species with a diet restricted to grasses (Poaceae), have intermediate numbers of the four types of sensilla (*Sphingonotus coeruleus*, *S. lucasii*, *S. azureus*, *P. finotianus*, and *Oedipoda miniata*). (4) Monophagous species harbor the smallest number of the three types of sensilla (*Oedaleus decorus*, *Paracrinema tricolor*, and *Sphingonotus octofasciatus*).

Table 2. Variations in the numbers of sensilla in the different species

Species	Sex	LG		A1		A2		A3	
		Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
<i>C. barbarus</i>	Male	2.44	0.04	180.40	8.38	117.10	4.06	81.60	1.67
	Female	3.75	0.07	252.60	6.77	121.00	2.64	84.90	1.57
<i>O. decorus</i>	Male	3.42	0.08	135.40	7.78	96.90	3.69	71.00	2.76
	Female	4.44	0.06	146.20	6.06	103.60	3.04	75.20	2.86
<i>O. fuscocincta</i>	Male	3.14	0.06	186.90	12.17	116.60	5.77	80.90	1.07
	Female	4.04	0.07	200.00	8.55	121.10	3.01	82.70	1.79
<i>O. miniata maur.</i>	Male	2.76	0.05	131.70	4.65	93.90	2.51	70.70	2.78
	Female	3.52	0.08	155.60	8.31	105.90	3.67	73.80	3.13
<i>P. tricolor</i>	Male	2.05	0.05	67.90	0.92	63.60	0.98	51.00	0.42
	Female	2.60	0.05	101.70	1.21	78.50	1.02	60.30	0.79
<i>S. gregaria</i>	Male	5.26	0.08	401.00	2.46	197.10	3.39	121.60	2.15
	Female	5.90	0.09	417.10	1.76	182.90	9.83	128.90	4.09
<i>S. azurescens</i>	Male	2.31	0.03	93.40	2.02	78.50	1.43	62.60	2.20
	Female	3.02	0.05	106.10	2.93	88.00	2.44	66.20	2.13
<i>S. coerulans atlas</i>	Male	2.55	0.03	112.30	2.58	88.40	2.57	71.20	1.85
	Female	3.54	0.07	167.40	6.40	108.50	4.57	81.10	2.90
<i>S. finotianus</i>	Male	2.64	0.05	115.20	4.75	87.20	3.00	69.70	2.52
	Female	3.15	0.08	145.30	7.91	92.10	2.60	73.30	1.74
<i>S. lucasii</i>	Male	1.99	0.03	85.70	3.64	74.30	1.98	59.10	1.43
	Female	2.87	0.06	108.90	4.66	88.10	3.37	64.20	0.93
<i>S. octofasciatus</i>	Male	2.80	0.03	89.10	3.40	77.20	1.02	66.10	2.42
	Female	3.60	0.08	124.60	4.33	88.50	1.79	71.00	1.41
<i>S. rubescens</i>	Male	2.43	0.05	105.80	5.18	85.20	2.76	72.90	1.55
	Female	3.11	0.06	142.40	8.67	93.70	3.72	79.40	2.89
<i>D. maroccanus</i>	Male	2.84	0.11	121.6	5.97	95.20	2.15	50.6	3.70
	Female	3.25	0.09	139.6	8.82	117.6	10.53	68.0	6.30

LG=labrum width, in mm. For each mean, N=10 individuals.

4.1 Diet within Oedipodinae

Although several grasshopper species were studied here for the first time, our data on the Moroccan populations of the other species are largely confirmed by earlier studies on populations in the Mediterranean Basin (table 1). However, several variations could be observed, which may be due to local adaptations of different populations. For example,

Table 3. Relationships between sensilla numbers and labrum width (in mm)

Sensilla	Regression equations	r	p-values
A1	$y = 77.915x - 93.866$	0.90	$<10^{-30}$
A2	$y = 27.306x + 14.009$	0.93	$<10^{-30}$
A3	$y = 15.548x + 25.709$	0.92	$<10^{-30}$

Paracinema tricolor is oligophagous in Corsica (Boitier *et al.* 2006), but feeds only on *Hordeum murinum* in the Moroccan Middle Atlas, and *Sphingonotus coerulans* is polyphagous in Spain (Gangwere and Morales-Agacino 1973), but oligophagous in the Middle Atlas. Given the frequent oligophagy in the Oedipodinae, this dietary type could represent the ancestral feature for this subfamily. The two closest subfamilies within the Oedipodinae are the Gomphocerinae and Acridinae (Petit 2005; Chapco and Contreras 2011) and both are oligophagous on grass, feeding mainly on Poaceae and to a lesser extent on allied families, such as Joncaceae and Cyperaceae (ElGhadraoui *et al.* 2002). Moreover, most tribes within the Oedipodinae (sensu Fries *et al.* 2007) feed mainly on grass. In the Locustini, feeding on Poaceae was also observed in *Locusta migratoria* L. (Benfekih 2006) and *Gastrimargus* (Mestre 1988). In the sister tribe Acrotylini, most *Acrotylus* species consume Poaceae (Gangwere and Morales-Agacino 1973). In the

Table 4. Influence of systematic and diet on the residuals of labrum sensilla numbers using GLM

	A1 type residuals		A2 type residuals		A3 type residuals	
	Regression		Regression		Regression	
	Coefficient <i>p</i> -value		Coefficient <i>p</i> -value		Coefficient <i>p</i> -value	
Constant	-129.48	0.014	-34.69	0.067	-27.29	0.014
Systematics	11.96	0.179	1.379	0.689	3.09	0.116
Diet	35.24	0.005	12.34	0.012	6.89	0.007

Data set restricted to Oedipodini (2 species) and Sphingonotini (6 species).

Oedipodini from Spain, *O. caerulescens* L. and *O. fuscocincta* feed on Dicotyledone and Poaceae, respectively. In the Epacromiini, *Aiolopus strepens* Latr. and *A. thalassinus* F. feed on grass (Gangwere and Morales-Agacino 1973), so does *Stethophyma grossum* (L.) (ElGhadraoui *et al.* 2002). In summary, it appears that the Oedipodini would be the only tribe predominantly polyphagous in contrast to the other tribes in which oligophagy seems to prevail. The interesting thing is that frequent reversals can be observed within a genus, and that plasticity can even affect a species according to the region where it is found. Nevertheless, we interpret polyphagy and monophagy in a few Sphingonotini species as a change from the fundamental oligophagy, and oligophagy as a change from polyphagy in Oedipodini.

The choice of a plant as a food by grasshoppers is related to its particular nutritional characteristics. However, the

physicochemical properties of plants, such as the hardness and the presence of hairs or denticles on the edges of the leaves, play an important role in food choice, as Bernays and Chapman (1970) have shown for *Chorthippus parallelus* Azam and Le Gall (1989) later confirmed. The food choice of acridians can be influenced by other factors, such as deterrent or stimulating molecules (Launois-Luong 1976; Otte and Joern 1977; Le Gall 1989), plant architecture and color (Picaud *et al.* 2002). Some of these features can be detected by sensory organs, such as olfactory or gustatory sensilla on the antennae (Chen *et al.* 2003), chemoreceptors on the palps (Jin *et al.* 2006), and sensilla on the labrum (Chapman 1982). A question arises as to how much of the configuration of the labrum sensilla is correlated with the dietary choice of the grasshoppers.

Our study shows that there is a close relationship between the diet and the number and types of sensilla on the labrum of grasshoppers. For this aim, it was necessary to remove the effect of size, as indicated by Chapman and Thomas (1978) and ElGhadraoui *et al.* (2002). The polyphagous species possess a greater number of sensilla than the oligophagous species and a fortiori than the monophagous species. Haskell and Schoonhoven (1969) showed that the A1-type and A2-type sensilla are sensitive to phagostimulant molecules, such as sugars, whereas the A3-type sensilla are sensitive to repulsive molecules. The Poaceae species possess fewer molecules of the secondary metabolism than the dicotyledonous species (Le Gall 1989). Furthermore, the alkaloids of the group of pyrrolizidines (Boppré *et al.* 1984), the quinolizidines (Picaud *et al.* 2003), the glucosides (Mainguet *et al.* 2000), and the tannins (Boppré and Fischer 1994), which are present mainly in Dicotyledones, are generally repulsive to grasshoppers.

During an evolutionary transition from oligophagy to polyphagy, which includes a dicotyledonous diet, a grasshopper faces a larger number of stimuli emanating from the surface of leaves (ElGhadraoui *et al.* 2002). Hence, a greater number and diversity of sensilla is selectively advantageous for a more diversified diet because it would

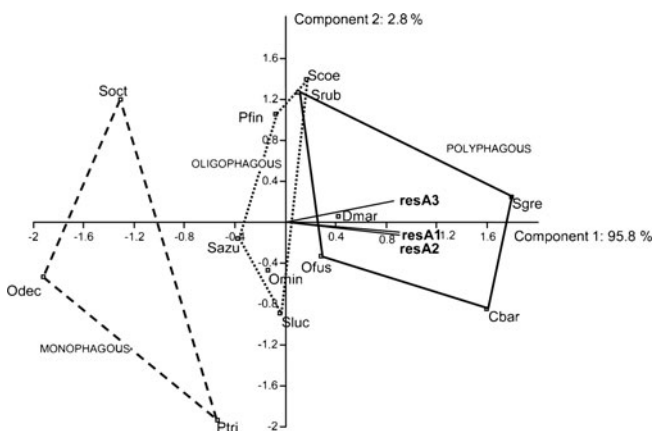


Figure 4. Ordination plan of the 2 first axes of PCA. Cbar: *Calliptamus barbarus*, Dmar: *Doclostaurus maroccanus*, Odec: *Oedaleus decorus*, Ofus: *Oedipoda fuscocincta*, Omin: *Oedipoda miniata*, Ptri: *Paracinema tricolor*, Sazu: *Sphingonotus azurescens*, Scoe: *S. coeruleans*, Pfin: *S. finotianus*, Soct: *S. octafasciatus*, Srub: *S. rubescens*, Sluc: *S. lucasii*, Sgre: *Schistocerca gregaria*.

allow the perception of more chemical molecules in plant tissues. This is probably the case for the Oedipodinae, in which *O. fuscocincta* has a greater number of A1-type and A2-type sensilla than oligophagous taxa. It is probably also the case for the very few non-grass-feeding Gomphocerinae species, such as *Dociostaurus maroccanus* (ElGhadraoui *et al.* 2002) and *Chorthippus binotatus* Charp. ssp. *binotatus* (Picaud *et al.* 1999).

During an evolutionary transition from oligophagy to monophagy, however, the specialized diet of a grasshopper species is related to a reduction in the number of labrum sensilla, such as in *Sphingonotus octofasciatus*. This interpretation is supported by Chapman and Thomas (1978), who showed that the smallest numbers of A1-type sensilla and, to a lesser extent, of A3-type sensilla, were found in specialist Gomphocerinae species, such as *Anablepia* ssp. and *Xenocheila* ssp.

Nevertheless, the number of labrum sensilla alone is not sufficient to infer the diet for all grasshopper species we studied. For example, *Sphingonotus rubescens* is a species that can feed on several plant families, but whose number of sensilla (but not of A3-type sensilla) resembles that of oligophagous species. This suggests that the dietary change during evolution may precede the modification of the number and types of sensilla on the labrum. Similarly, in their study of the *Chorthippus* subgenus *Glyptobothrus*, (Picaud *et al.* 1999) found that the polyphagous *C. binotatus* ssp. *moralesi*, a Pyrenean endemic grasshopper, did not differ from the oligophagous congeneric species by the configuration of its sensilla. In contrast, the subspecies *C. b. binotatus* feeds exclusively on the leaves and flowers of Fabaceae bushes of the genus *Ulex*. It may be that these subspecies were incorrectly assigned to the same species, but an examination of their songs by Ragge and Reynolds (1998) confirms their conspecificity. On the basis of the entirety of our data, we would like to propose that the labrum sensilla progressively reach an 'optimal' configuration. So the problem of diet change would be pushed further and the first change could depend on a modification of physiological properties of sensilla rather than their number.

In contrast to other groups of grasshoppers, the Oedipodinae can change their diet at the level of species, and it appears that these changes are frequent events. This group is an interesting model for trying to understand the driving forces that lead to dietary changes and their underlying and causative mechanisms. Using this model, we were able to show that the number and types of labrum sensilla is closely linked to the diet and, more interestingly, we were able to infer that the number of labrum sensilla are probably an adaptation to the diet and not the cause of a change in diet. Comparable studies should be undertaken on antenna sensilla to test our hypothesis.

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Appendix

Table 5. Most frequent plant species recorded in Al-Azaghar area

Families	Species
<i>Gymnospermae</i>	<i>Juniperus phoenicea</i>
<i>Angiospermae</i>	<i>Poaceae</i>
	<i>Schismus barbarus</i>
	<i>Echinaria capitata</i>
	<i>Stipa retorta</i>
	<i>Hordeum murinum</i>
	<i>Bromus rubens</i>
	<i>Caryophyllaceae</i>
	<i>Petrorrhagia illyrica</i> subsp. <i>angustifolia</i> (= <i>Tunica illyrica</i> subsp. <i>angustifolia</i>)
	<i>Paronychia capitata</i> subsp. <i>capitata</i>
	<i>Herniaria cinerea</i> (= <i>H. lenticulata</i>)
	<i>Vaccaria pyramidata</i>
	<i>Ranunculaceae</i>
	<i>Delphinium nanum</i>
	<i>Resedaceae</i>
	<i>Reseda decursiva</i> (= <i>R. alba</i> subsp. <i>decursiva</i>)
	<i>Santalaceae</i>
	<i>Thesium humile</i>
	<i>Frankeniaceae</i>
	<i>Frankenia corymbosa</i>
	<i>Cistaceae</i>
	<i>Helianthemum papillare</i>
	<i>Rhamnaceae</i>
	<i>Zizyphus lotus</i>
	<i>Linaceae</i>
	<i>Linum tenue</i>
	<i>Convolvulaceae</i>
	<i>Convolvulus</i> sp.
	<i>Brassicaceae</i>
	<i>Eruca sativa</i>
	<i>Euphorbiaceae</i>
	<i>Euphorbia chamaesyce</i>
	<i>Borraginaceae</i>
	<i>Echium horridum</i>
	<i>Leguminosae</i>
	<i>Anthyllis tetraphylla</i>
	<i>Ebenus pinnata</i>
	<i>Trifolium stellatum</i>
	<i>Lamiaceae</i>
	<i>Ajuga iva</i>
	<i>Teucrium pollium</i>
	<i>Plantaginaceae</i>
	<i>Plantago ovata</i>

Table 5. (continued)

Families	Species
	<i>Oleaceae</i>
	<i>Olea oleaster</i>
	<i>Plumbaginaceae</i>
	<i>Limonium echioides</i>
	<i>Dipsacaceae</i>
	<i>Scabiosa stellata</i>
	<i>Asteraceae</i>
	<i>Atractyllis cancellata</i>
	<i>Centaurea melitensis</i>
	<i>Centaurea sulfurea</i>
	<i>Reichardia tingitana</i> subsp. <i>discolor</i>
	<i>Hedypnois cretica</i> (= <i>H. polymorpha</i>)
	<i>Leontodon mulleri</i>
	<i>Hypochoeris glabra</i>
	<i>Scorzonera laciniata</i>
	<i>Lasiopogon muscoides</i>
	<i>Micropus supinus</i>
	<i>Filago germanica</i>
	<i>Asteriscus pygmaeus</i>