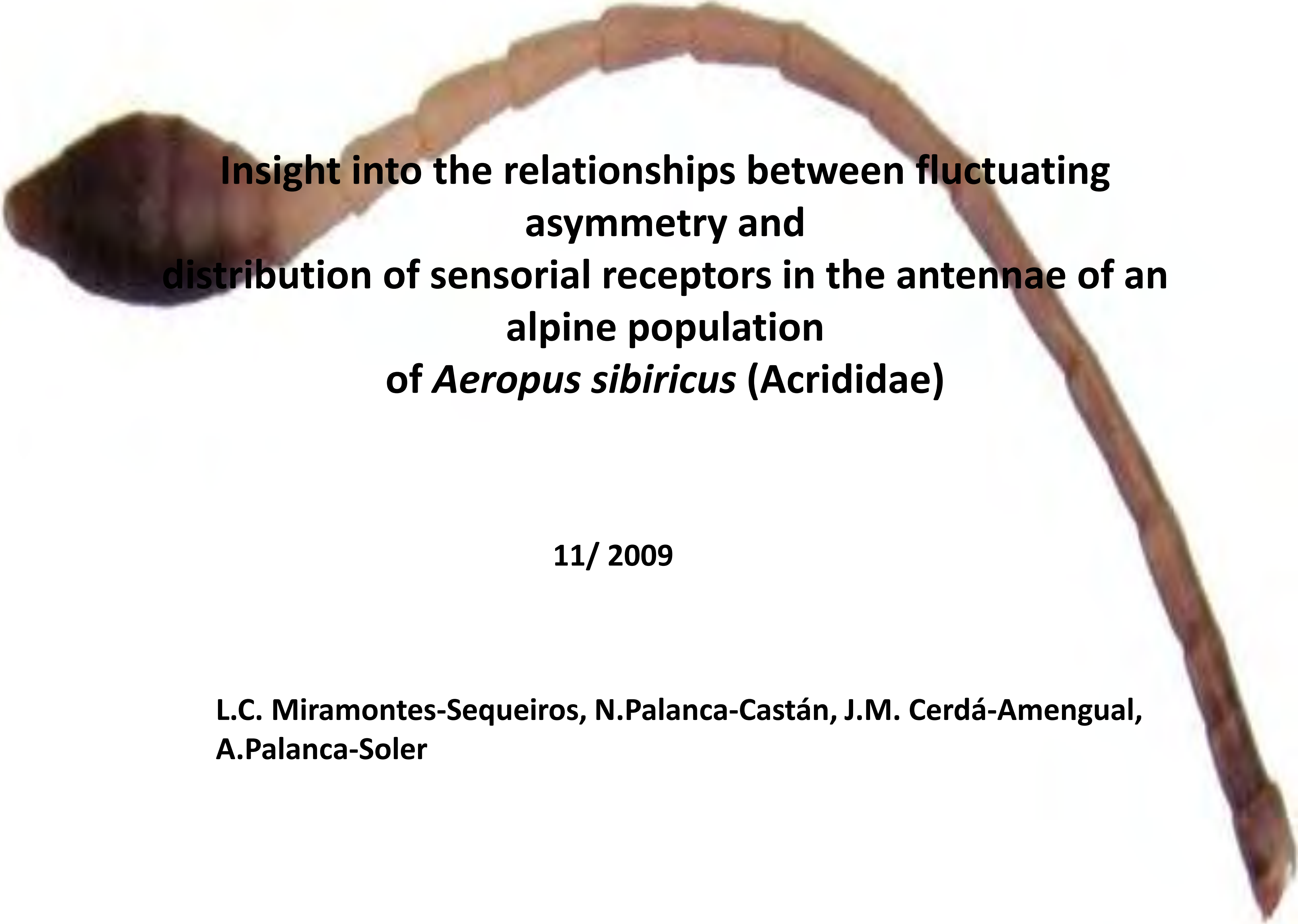

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Abstract

Developmental stability can be revealed by the individual variability, which refers to phenotypic differences among homologous structures and fluctuating asymmetry, non-directional differences between the left and the right side of bilateral structures. The objective of this study is to seek a relation between the number and variety of sensilla found on the different flagellar annuli the Acrididae family using previous studies as a base. Afterwards, the relation between them and the fluctuating asymmetry of each of the annuli on the antennae of an *Aeropus sibiricus* (a very abundant arctic-alpine grasshopper species) population will be assessed, as a method for the prediction of the adaptive value of each of the flagellar annuli throughout the family. Our results show a significant negative linear correlation between the probability of finding a high variety of sensilla and the fluctuating asymmetry. The middle flagellar annuli, specifically, present a low fluctuating asymmetry and a high probability of finding a high variety of sensilla when comparing them with the more distal and proximal annuli. It could be concluded that these middle flagellar annulli are adaptive characters, and thus are well-conserved and present a low fluctuating asymmetry. Further studies should address the relationship existing between the number and variety of sensilla and the adaptation capability of the population to its environment.

Keywords: Fluctutating asymmetry, anatomy, *Aeropus sibiricus,* adaptation, Antennae, Acrididae

Introduction

Stress is defined as any alteration of the normal environment that interrupts the steady state of an organism. The identification of stressed populations can be carried out through characteristics contributing to the fitness of the organism as: changes in community structure, diversity and species relative abundance and these changes occur relatively late after the environmental alteration (Clarke, 1993), survivorship, fecundity, reproduction success etc. This identification can be assessed by developmental stability, which is the ability of an organism to isolate its development from stressing environmental perturbations and to produce an ideal form in a certain environment (Leary & Allendorf, 1989).

Developmental stability can be revealed, for example, by the individual variability, which refers to phenotypic differences among homologous structures, and fluctuating asymmetry (FA) – non-directional differences between the left and the right side of bilateral structures. The latter is extensively used to assess developmental stability (Thoday, 1955, 1958; van Valen 1962).

Ideally symmetrical structures represent an important comparison item for deviations (Palmer & Strobeck, 1986), and therefore, provide a convenient method for the study of the influencing factors (Palmer, 1994). Thus, the asymmetry of these morphological structures would indicate a tendency to deviate from the genetically programmed result, during development. The differences between the two sides measurements must be environmentally induced, and reflect the altering factors effect upon development (Waddington, 1942).

Aeropus sibiricus is a very abundant arctic-alpine grasshopper species where male can be easily identified by its modified fore tibiae (Fig.1). In this context we have studied the fluctuating asymmetry in the antenna of adult males (Fig.2) developed in their natural environment sited in a Pyrenean High Mountain population. In order to avoid both sexual dimorphism effect (Chen et al., 2003), and also the growth effect in the antenal sensilla distribution (Chapman, R.F. & Greenwood, M., 1986), only adult male specimens were studied. It is generally accepted that the sensilla on insect's antennae are not randomly distributed (Zacharuk, 1985). They play an important role in host orientation, food selection and oviposition site selection and their pattern may reflect the effect of many interacting selective pressures in which size of the individual, sex, developmental stages, feeding habits, habitats (Chapman, 1982; Chen et al., 2003), are of considerable significance. The sensilla are the insect antennae sensorial anatomical structures, their functions have been studied and described for many authors. The structural features of sensilla basiconica and sensilla trichodea are typical of olfactory receptors in general (Altner & Prillinger, 1980; Zacharuk, 1980; Ameismeier, 1987; Ochieng et al., 1996; Page, K.L. & Matheson, T., 2004). Functional properties of the two types of sensilla coeloconica have been shown to be chemosensitive, hygro-and thermostimulation (Boeckh, 1967; Steinbrecht, 1969; Kafka, 1970, 1971; Altner et al., 1977, 1981; Hansson et al., 1996). Sensilla chaetica probably function as taste/mechanoreceptors. Sensilla basiconica and coeloconica are normally distributed over the entire antennal flagellum, with a concentration in the middle segments (Chapman, 2002; Ochieng et al., 1998); sensilla trichodea have three areas of concentration on the 5th, 10th and 14th flagellar segments. Sensilla chaetica are most abundant on the terminal segment. The external morphology and distribution of sensilla on the antennae is very similar in acridid species (Slifer et al., 1959; Abushama, 1968; Greenwood & Chapman, 1984; Ochieng et al., 1998; Altner & Loftus, 1981; Ameismeier, 1987; Bland, 1982). The objective of this study is to seek a relation between variety of sensilla found on the flagellar annuli of 8 Acrididae species and the fluctuating asymmetry of each of them on *Aeropus sibiricus*, as a way of predicting the adaptive value of each of the flagellar annuli throughout the family.

Material and Methods

Specimen collection and description of the location

All insects were field captured manually. Male adults of *Aeropus sibircus* were collected from a savage colony in the valley of post-glacial origin in Piedrafita Glaciar Cirque (figure 3), central Pyrenean mountains, Spain (42°49' N, 0°17' W, about 2200 m above sea level) in 2004. Riparian vegetation is poor and consists mainly of grasses and *Rhododendron* shrubs.

Measurements and statistics

Antennal flagellar annuli were numbered from distal to proximal, and we used only adult male specimens in order to avoid any variation in the number of annuli in any one developmental stage as well as the differences caused by sexual dimorphism. Because new annuli are added basally, numbering from the tip ensures homology between the more distal annuli of different individuals (Chapman & Greenwood, 1986). Statistical analyses were made using the SPSS15 Software Package (SPSS Inc.).

To assess the presence of FA between different flagellar annuli of the antennae of an *Aeropus sibiricus* population, we measured length and diameter of the different flagellar annuli from both right and left antennae. We used these values to calculate the total surface of each flagellar annulus and measure the differences between right (R) and left (L) flagellar annulus, transforming the absolute values into percentual values of R+L surface (a variable which we named V1) in order to remove counfounding effects of size variation (Palmer, 1994) such as increased variability with greater trait size (Lande, 1977) and artificial differences caused by non-random variations in size between groups. A K-S test was conducted after each of the variables was calculated to check if the values exhibit a normal distribution.

To validate the existence of the fluctuating asymmetry the data were submitted to a multiple-step analysis (Palmer & Strobeck, 2003). After we carried out a visual inspection of the scatter plots and a statistical confirmation by Grubb's test and Bonferroni correction to look for aberrant values on the measurement data, the surface absolute data and the percentual asymmetry data. Finally, we tested if the displayed asymmetry is fluctuating or of other type, by verifying the normal distribution of the asymmetries (visual inspection of frequency distribution plots, tests for skewness and kurtosis) and by verifying if the average asymmetry is 0 (one-sample t-test). We carried out Kendall and Friedman tests to verify that the values of each annulus were independent. After we used a T-test to eliminate the possibility of the presence of directional asymmetry in V1, and we found the mean to be not significantly different from 0, which implies the absence of directional asymmetry. After that, skewness and kurtosis tests were carried out for discarding the presence of measurement errors, which did not yield any significant result excepting one leptokurtic value, that was eliminated (14^{th} annulus). Using V1, we calculated V2, which equals the difference of (R-L) as a percentage of (R+L) in absolute values, and we used it to calculate the following FA index:

$$FA = \sum V2\_Annul\_n/N \quad \text{(Eq.1)}$$

Where, V2_Annul_n is the value of FA for a given annulus and N is the number of measured specimens.

We checked that FA was not correlated with the size of the annulus.

Once the index of FA was calculated we compared the values for each annulus with the stimated probability of sensilla heterogeneity (PSH).

$$\mathbf{PSH = 1 -} \left[\left(\left(\sum_{p=1}^{11} p\right)^2 \times (Np)\right) / \sum_{p=1}^{11} p^2\right] \quad \text{(Eq.2)}$$

Where, p equals the probability of finding a given sensilla, Np is the number of cases in which the probability of each of the 11 types of sensilla used in this study is different from 0. This probability was calculated from the data of presence/absence of 11 types of sensilla in 8 Acrididae species (Li et al., 2007). PSH value represents the probability of finding a high variety of sensilla types on a given annulus.

Results

Grubb's test and Bonferroni correction revealed four significant outliers. Therefore data from these four individuals were excluded from all the analyses. T-test pair of mean differences among R and L were not found to be significant ($p>0.05$). Thus Directional Asymmetry was discarted. After t-test (one sample) only four flagellar annuli exhibited signicant leptokurtosis of (R-L), and significant skewness was only found in flagellar annulus 14 which was excluded from the analysis. K-S test was conducted after each of the variables was calculated in the 13 remaining flagellar annuli, and in all cases the variables showed a normal distribution ($p>0.05$). The Kendall and Friedman tests results reveal that the means of the flagellar annuli surfaces (R+L) differ significantly ($p=0.000$), verifying that the values of each annulus were independent (Table 1, Fig.4). Therefore, we can say that there is no concordance between the specimens and the flagellomere surface values variation. The difference between FA did not depend on flagellar annuli surfice (Table 2).

The probability of finding certain type of sensilla in a given annulus of an Acrididae species can be calculated taking into account the pressence or absence of sensilla on each annulus, our study is based on data recorded in 8 Acrididade species (*Acrida cinerca, Chrysochraon dispar major, Euthystria lueifemora, Mongolotettix angustiseptus, Chrysacris changbaishanensis, Chrysacris jiamusi, Chrysacris heilongjiangensi*s, *Chrysacris liaoningensi*) by Na Li et al., (2007). In Table 3 we calculate the probability of finding each of the 11 types of sensilla for each flagellar annulus, and from 4th to 10th flagellar annuli there is a high probability of finding most types of sensillas. Flagellar annuli number 1, 2, 11, 12 and 13 will never present all types of sensillas.

Relationship between FA values of flagellar annuli and probability of finding a high variety of sensilla types in a given annuli (PSH) in sampling specimens of *Aeropus sibiricus* (Fig.5) shows that as the probability of finding a high variety of sensilla types on a flagellar annuli grows, as the FA decreases (Table 4).

Discussion

When the symmetry of a character is essential for the normal functioning of an organism, in such a way that the slightest alteration implies significant fitness costs, it can be expected that changes will be neutralized or channeled which implies that asymmetry on them is extremely uncommon (Thoday 1955, 1958; van Valen 1962, Palmer 1986). If we take in account the fact that fluctuating asymmetry has its origin in the impact of environmental factors during the development of the individual (Waddington 1942), which means that characters which show a low fluctuating asymmetry will be subject to selective pressures, of which can be deduced that this characters are somehow important for the adaptation of the population to its environment. This makes stable characters a realiable way of assessing the main environmental pressures that acting on a given population (Palmer 1994). On the other hand, some extremely labile characters, which need not a strict symmetry in order to carry out their function, may show high asymmetry levels under optimal conditions (Clarke 1995).

Our results, supported by the above mentioned studies, reveal that the middle flagellar annuli (4 to 10) exhibit a low fluctuating asymmetry, suggesting that the middle flagellar annuli of the antennae of *Aeropus sibiricus* are adaptive characters, and consequently well-conserved. On the contrary we found higher fluctuating asymmetry in the distal flagellar annuli, which is in concordance with the study of Chen et al., (2003) where less adaptative traits present a greater FA and were located in the more distal part of antennal flagellum, where olfactory perception is mainly accomplished. Several studies dealt with phenotipic variation in type of the sensilla and its distribution along the flagellar annuli among grasshoppers, and it has been recorded as arising from differences in crowding, food quality, and the odorous environment experienced by the insects (Chapman 2002; Ochieng 1998).

In order to get insights into the relationship between fluctuating asymmetry and distribution of the sensorial receptors, we used data from other authors to calculated the probability of finding any type of sensilla (olfactory, chemosensitive, hygro- and thermostimulation, taste and mechano receptors) in each of the flagellar annuli in the antennae of the Acrididae family. These calculations resulted in the creation of an index we called PSH (Eq.2), which expresses probability of finding a high variety of sensilla in a given annulus.

The high PSH value appearing in Acrididae is in concordance with studies of distributions of sensillas observed over the entire antennal flagellum, which shows sensilla type variation and concentration in middle segments (Ochieng 1998; Na Li 2007; Chapman 2002; Chen et al., 2003). Therefore, considering that the PSH value is inversely proportional to the fluctuating asymmetry, these flagellar annuli should represent adaptative anatomical structures.

Taking into account above mentioned criteria for the non-labile adaptive characters and considering the PSH value we found for the Acrididae family; we could say that the more adaptive flagellar annuli in *Aeropus sibiricus* would be those in which the possibility of finding any type of sensilla is greater, while the more homogeneous flagellar annuli would be less adaptive and thus would present greater FA.

We could ask ourselves about the possible ways in which FA acts to provoke changes in the adaptive characteristics of the flagellar annuli. These mechanisms would be somehow implied in the evolution of higher than species taxa. If we universalize these conclusions we could say that FA is directly implied in the evolutive mechanisms of the anatomic features in the animal kingdom.

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Figure1. …grasshopper species where male can be easily identified by its modified fore tibiae

Figure2 …. antenna of adult males

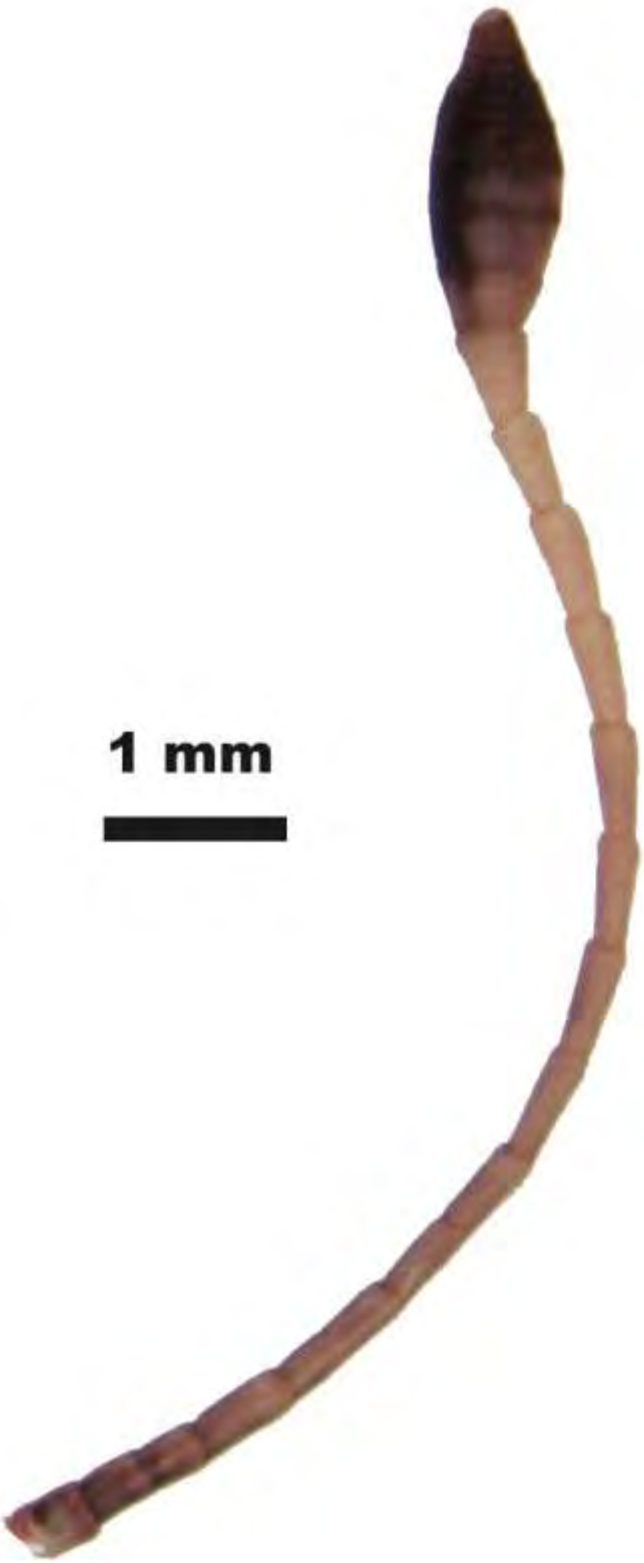

Figure 3 ……valley of post-glacial origin in Piedrafita Glaciar..

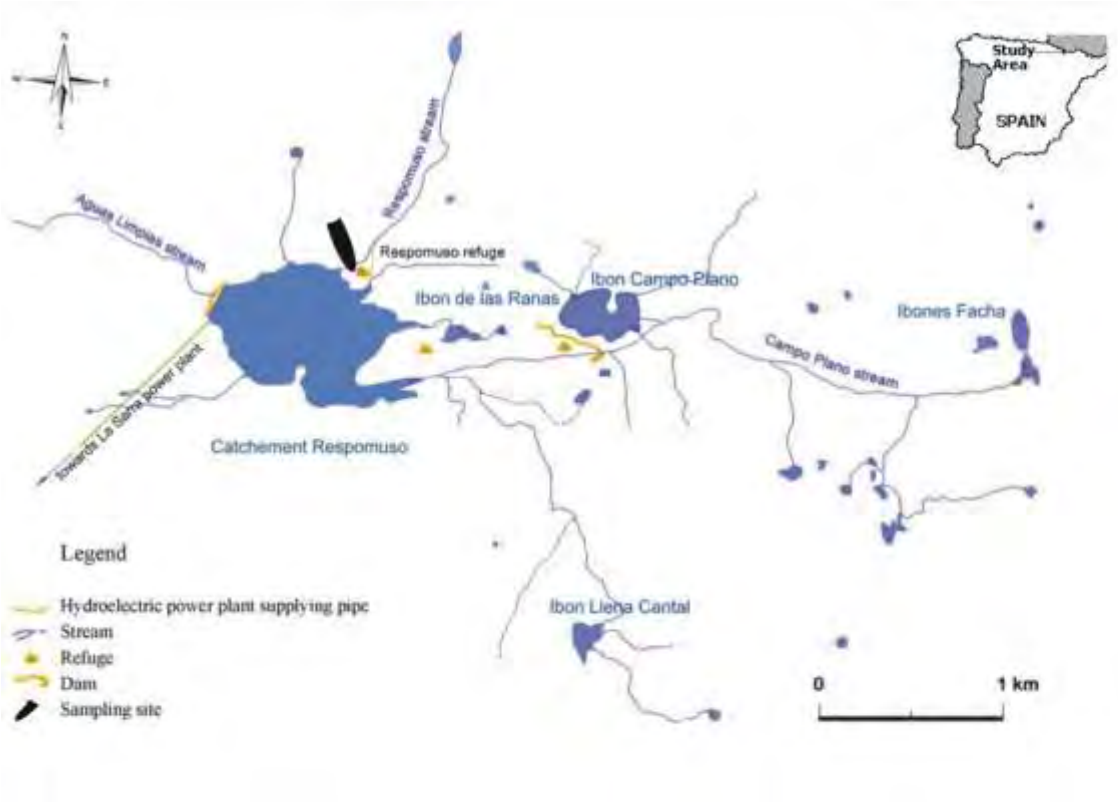

Figure4. Fluctuating asymmetry values (with confidence level 95%) in the flagellar annuli of *Aeropus sibiricus* sampled.

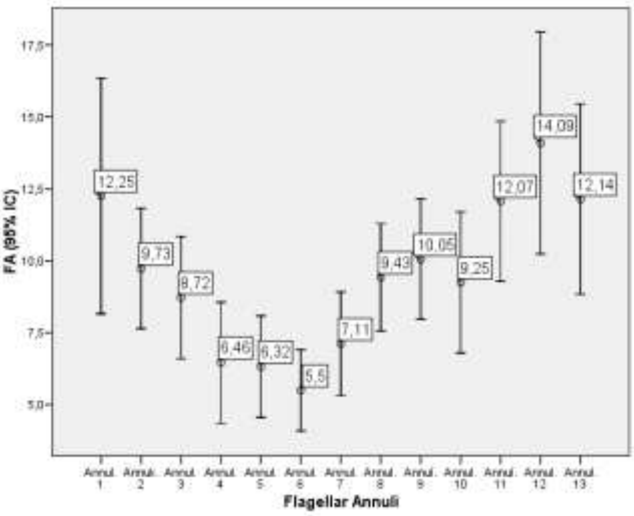

Figure5. Relationship between fluctuating asymmetry (FA) values of flagellar annuli and probability of finding a high variety of sensilla in a given annulus (PSH) in sampling specimens of *Aeropus sibiricus* (r=-0.836, P=0.000).

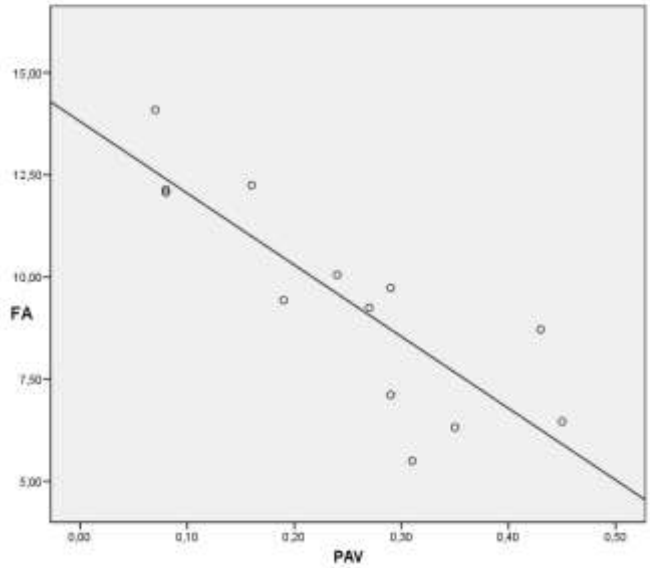

Table 1. Descriptive statistics of the fluctuating asymmetry values in the flagellar annulus of *Aeropus sibiricus* sampled.

Flagellar annulus	FA		Confidence interval for FA with confidence level 95%		Variance	Standar deviation	Asymmetry		Kurtosis	
		Standar error	Upper limit	Lower limit				Standar error		Standar error
1	12.7822	2.11968	8.5247	17.0397	229.144	15.13751	1.320	.333	.737	.656
2	10.2892	1.07632	8.1274	12.4511	59.081	7.68645	.939	.333	.552	.656
3	9.0355	1.08704	6.8521	11.2188	60.265	7.76302	.879	.333	.059	.656
4	6.8334	1.05566	4.7131	8.9538	56.835	7.53889	1.486	.333	2.234	.656
5	6.4198	.86015	4.6921	8.1474	37.733	6.14269	.934	.333	.219	.656
6	6.0765	.78454	4.5007	7.6523	31.391	5.60272	.928	.333	.341	.656
7	7.2335	.88445	5.4571	9.0100	39.895	6.31624	.657	.333	-.200	.656
8	9.4409	.93325	7.5665	11.3154	44.419	6.66473	.726	.333	.123	.656
9	10.1073	1.02491	8.0487	12.1659	53.573	7.31935	.403	.333	-.546	.656
10	10.1766	1.36870	7.4275	12.9257	95.541	9.77450	1.836	.333	4.571	.656
11	12.7500	1.42981	9.8781	15.6218	104.263	10.21091	1.180	.333	1.142	.656
12	14.0470	1.87053	10.2899	17.8040	178.444	13.35828	1.202	.333	1.066	.656
13	12.6200	1.61695	9.3723	15.8678	133.342	11.54737	1.194	.333	1.161	.656

Table 2. Descriptive statistics of flagellar annuli total surfices (R+L) and FA in *Aeropus sibiricus*.

Flagellar annuli	Mean surfice R+L		Standar Deviation	FA
		Standar error		
Annulus_1	3314.9956	128.08259	975.44794	12.25
Annulus_2	487.2730	17.68159	134.65897	9.73
Annulus _3	470.6983	13.63912	103.87242	8.72
Annulus _4	530.9846	15.32736	116.72973	6.46
Annulus _5	483.3804	15.43432	118.55327	6.32
Annulus _6	444.8293	14.57682	111.96669	5.5
Annulus _7	407.4872	12.90165	98.25606	7.11
Annulus _8	411.7179	14.05181	105.15412	9.43
Annulus _9	370.6517	13.13112	97.38301	10.05
Annulus _10	356.5134	13.86827	101.91053	9.25
Annulus _11	319.1633	13.11231	96.35543	12.07
Annulus _12	407.4772	18.21291	133.83702	14.09
Annulus _13	318.3891	12.18383	87.85888	12.14

Table3. Probabilityof finding each of the 11 types of sensilia for each flagellar annulus (based on data from the study of flagellar sensilla distribution of 8 species by Na Li et al. 2007).

Flagellar Annuli	**Tricoide**		**Chaetic**		**Basiconic**					**Coleoconic**	**Cavity**
	Type 1	**Type 2**	**Type1**	**Type 2**	**Type 1**	**Type 2**	**Type 3**	**Type 4**	**Type 5**		
1	0.500	0.375	0	1.000	0	0	0	0	0	0	0
2	0.500	0.375	0.125	1.000	0	0	0	0	0	0	0
3	0.375	0.375	0.125	1.000	0.125	0	0	0	0	0	0.125
4	0.375	0.375	0.125	1.000	0.250	0.125	0.125	0.125	0.125	0.125	0.250
5	0.375	0.375	0.375	1.000	0.250	0.125	0.125	0.125	0.125	0.375	0.750
6	0.250	0.250	0.375	1.000	0.625	0.250	0.250	0.125	0.125	0.500	0.875
7	0.125	0.250	0	1.000	0.875	0.375	0.250	0.125	0.375	0.625	0.875
8	0.250	0.125	0	1.000	1.000	0.625	0.750	0.375	0.375	0.750	0.875
9	0.125	0.125	0.250	1.000	1.000	0.875	1.000	0.375	0.375	0.875	0.875
10	0.125	0	0.250	1.000	1.000	0.750	1.000	0.375	0.375	1.000	1.000
11	0	0	0	1.000	1.000	1.000	1.000	0.500	0.375	1.000	1.000
12	0	0	0	1.000	1.000	0.875	1.000	0.500	0.375	1.000	1.000
13	0	0	0	1.000	1.000	1.000	1.000	0.500	0.375	1.000	1.000

Table 4. Probability of Sensilla heterogeneity (PSH) calculation in Acrididade and fluctuating asymmetry (FA) values for flagellar annuli in sampling specimens of *Aeropus sibiricus*.

Flagellar annuli	**FA**	**PSH**
1	12.25	0.16
2	9.73	0.29
3	8.72	0.43
4	6.46	0.45
5	6.32	0.35
6	5.50	0.31
7	7.11	0.29
8	9.43	0.19
9	10.05	0.24
10	9.25	0.27
11	12.07	0.08
12	14.09	0.07