



# Body size inequality in ground beetle (Coleoptera: Carabidae) assemblages as a potential method to monitor environmental impacts of transgenic crops

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**List of any nonstandard abbreviations**

GM: Genetically Modified  
GMOs: Genetically Modified Organisms  
PMEM: Post Market Environmental Monitoring  
GMCs: Genetically Modified Crops

**Keywords:** Lorenz asymmetry coefficient,  
Gini coefficient, monitoring, genetically modified crop,  
body length, inequality, MON810.

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## Abstract

**Background and Purpose:** Despite the obligatory post-market environmental monitoring of genetically modified (GM) crops in Europe, there are no available methodological guidelines or standards. Our aim was to examine the suitability of carabid body size inequality as a possible method for environmental monitoring.

**Material and Methods:** We used carabids collected by pitfall traps in both insect-resistant GM (producing a *Bacillus thuringiensis* toxin) and isogenic maize plots at Flakkebjerg (Denmark), within the framework of the AMIGA Project. The body size distribution was calculated using various measures of size inequality: the Lorenz curve, the Gini and the Lorenz asymmetry coefficients every month during the summer 2014.

**Results:** A total of 6339 carabids belonging to 38 species were captured and identified. The analysis detected a significant shift in size inequality between months, indicating the larger number of individuals of smaller-sized species later in the season, but no significant difference in inequality or mean body size was found between the assemblages in GM vs. isogenic maize plots.

**Conclusions:** We concluded that the evaluation of body size inequality was sensitive to subtle changes in the structure of the carabid assemblages, and this method had the potential to be used during monitoring of the unanticipated environmental effects of GM plants.

## INTRODUCTION

Genetically modified crops (GMCs) have been grown under field conditions since 1996 on a steadily increasing global area, with most of the growth restricted to certain regions (1). Despite almost twenty years of cultivation, the question whether the *Bt*-expressing and other pest-resistant GMCs lead to increased or reduced environmental and human safety remains a highly controversial issue among scientists and GM crop regulators (2–5).

On one hand, there are arguments that this new technology has an overall positive impact on the environment, agricultural production and human health (6, 7), but a scientific consensus has not yet been reached (4). On the other hand, a range of case-specific potential risks has been

identified at different levels of ecological complexity, for agricultural production systems and loss of „ecosystem services” (2–5). Apart from unwanted and unforeseen toxicological effects on so-called non-target organisms (NTOs) (2), ecosystem services could be affected (5) through indirect ecological interactions (8).

Thus, in addition to various pre-release biosafety tests, monitoring of the potential effects of GM organisms (GMOs) is important in order to detect effects that are not visible with short-time and small-scale experiments (9). For this reason, the EU Directive 2001/18/EEC affirms that Post Market Environmental Monitoring (PMEM) is a statutory requirement for the cultivation of GMCs in Europe (10). To date the European GMO regulatory system is one of most complete and articulate in the world (11), but there are no standardized approaches, methods or protocols in the European GMO monitoring guidance (12). The current AMIGA project (Assessing and Monitoring the Impacts of Genetically modified plants on Agro-ecosystems) is taking on these challenges (13). In particular, the aim of this project is to develop standards for effective PMEM designs for GMCs.

The most common error in monitoring is the lack of a match between the *indicator* and the *indicandum*, the phenomenon to be monitored or to be indicated (12). In this context, the *indicandum* is any substantial change in the status of the ecosystem in which the GMCs is grown.

As the indicator group, the ground beetles (Coleoptera: Carabidae) were selected. Carabids are a species-rich family of beetles, and many species are natural enemies of arthropods or weeds. Ground beetles are numerous and widespread in arable habitats all over the world (14) and are frequently used in environmental monitoring (15).

There can be various parameters (indices) to be used to detect the impact of the growing of a GMC on carabids. Most frequently, changes in the composition and abundance of the species are evaluated (15). This requires expertise to identify species, and this is not always available. An additional problem is also the difficulty of comparing various assemblages. When is, for example one assemblage significantly more diverse than another one, and what is the appropriate index to test this?

Looking for an alternative, we considered body size, which is notably correlated with several biological traits (16), including dispersal capacity, reproduction rate and development time, and some potential indirect impacts (e.g. period of activity). Body size also influences ecological interactions (e.g. competition and habitat suitability), resource utilization and many other parameters (17). Therefore, changes in body size inequality in various assemblages can be a potentially useful parameter.

Szysko (18) hypothesised that during forest succession, the mean size of individual carabids would increase, because larger species appear later during the succession.

Both natural and anthropogenic disturbance has the potential to alter the inequality of body sizes, towards a dominance of smaller species in highly stressed habitats (17, 19–21). According to the „decreasing body size hypothesis”, a ground beetle assemblage under unfavourable environmental conditions will change so that small-size generalist and eurytopic species will increase their abundance, while large specialist species with poor dispersing ability will decrease (17).

We tested body size distribution of ground beetle assemblages as a possible monitoring method that could reflect any potential adverse effect of Cry1Ab toxin on the selected bioindicator, ground beetles. Here we show that several body size inequality measures are suitably sensitive to reflect changes in the assemblage during the season. They can also be statistically tested, and thus are useful for monitoring purposes.

## MATERIAL AND METHODS

### Study site

The field trial was carried out on the experimental farm of Flakkebjerg Research Centre (55° 19' 18.6" N 11° 23' 25.1" E; 30 m a.s.l.), in the western part of the island of Zealand (Denmark), from June to August 2014. Following the AMIGA protocol (13), a field of 0.5 ha was randomly divided into ten plots of Bt-maize (MON810) and ten plots of its parental isogenic line, serving as controls. Each plot measured 10x9 m and was surrounded by a 5 m strip of bare ground. The field was surrounded by barley. The ground was characterised by clayey soil, with 50 % of clay fraction, 45 % sand and 5 % humus (U. Pilegaard, Aarhus University, personal communication).

### Carabid collection methods

The ground beetles were sampled by pitfall traps (500 ml plastic cups of 10 cm diameter), filled with 100 ml of 70 % ethylene glycol as a killing-preserving agent and a drop of odourless detergent to reduce surface tension. The cups were placed in the middle of each plot, in order to avoid edge effects, and sunk into the ground, such that their rim was level with the soil surface. A straight plastic barrier connected two such traps, placed 1m from each other. Individual traps were covered by a 20x20 cm galvanised metal sheet, about 2 cm above the soil surface, in order to minimise the catch of undesired species (e.g. small vertebrates), potential debris and the accumulation of rainwater. Traps were open from June to August, one week per month (9 – 16 June, 7 – 14 July, 5 – 12 August). After every collection, we inactivated the pitfalls by plugging the traps with a plastic cover and pushing down the covers, to prevent additional captures during the three weeks of non-sampling. All captured carabids were identified to species using keys by Lindroth (22, 23) and

Hurka (24) plus a reference collection housed at the Department of Agroecology.

## Analysis of carabid body size distribution

Body size data for each species were taken from the literature (22, 23). We calculated the geometric mean of the minimum-maximum values, as in previous studies (17). The „decreasing body size hypothesis” was tested using the Lorenz curve (25), a traditional graphical measure describing inequality of body size pattern.

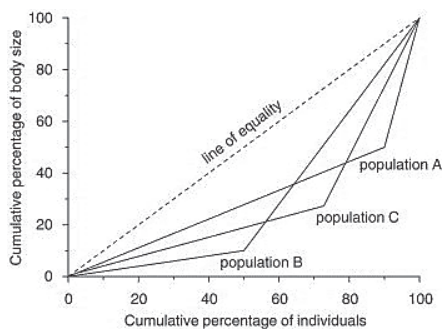
In this graphical approach, individuals are ranked by size and the cumulative proportion of carabid individuals is plotted against the corresponding cumulative proportion of their total size (17, 26). When all individuals are of the same size, the Lorenz curve follows the diagonal line, the „line of equality” (Fig. 1). Any difference in size forces the curve below this line.

## Gini coefficient

To quantify size inequality, the most common summary statistic is the Gini coefficient (27). If the data are ordered by increasing body size, the Gini coefficient is calculated as

$$G = \frac{\sum_{i=1}^n (2i-n-1)x_{[i]}}{n^2 \bar{x}} \quad (\text{Eq. 1})$$

where  $n$  is the number of individuals,  $x_{[i]}$  is the ordered body size of individuals  $i$  and  $\bar{x}$  is the mean body size (28). The Gini coefficient, calculated by the above equation should be multiplied with  $n/(n-1)$  to become an unbiased estimate (29). The Gini coefficient can then be thought of as the area that lies between the line of equality and the Lorenz curve, with coefficient values ranging from  $G = 0$  (complete equal distribution) to a theoretical maximum of  $G = 1$  (complete inequality) (28).



**Figure 1.** Lorenz curves of three hypothetical populations. All populations have the same Gini coefficient, but different Lorenz asymmetry coefficients ( $S$ ). In the case of the population A,  $S > 1$ , in the population B,  $S < 1$ , while in population C is symmetric ( $S = 1$ ). After Magura et al. (17)

## Lorenz asymmetry coefficient

This statistic does not contain all the information in the Lorenz curve (25), because different Lorenz curves can have the same Gini coefficient (as the three curves on Fig. 1. do). The Lorenz asymmetry coefficient ( $S$ ) can be calculated using the following equation (25):

$$S = F(\hat{x}) + L(\hat{x}) = \frac{m + \delta}{n} + \frac{L_m + \delta x'_{m+1}}{L_n} \quad (\text{Eq. 2})$$

where

$$\delta = \frac{\bar{x} - x'_m}{x'_{m+1} - x'_m}$$

$\bar{x}$  and  $n$  are the same as in Eq. 1,  $m$  is the number of individuals with a body size  $< \bar{x}$ ,  $L_m$  is the cumulative body size of individuals with a body size  $< \bar{x}$ , and  $L_n$  is the cumulative body size of all individuals.

When  $S = 1$ , the Lorenz curve is symmetric. If  $S > 1$ , the point where the tangent to the Lorenz curve is parallel with the line of equality is above the axis of symmetry, caused by the presence of large individuals (25). If  $S < 1$ , then that point falls below the axis of symmetry and the inequality is primarily due to the relatively large number of small individuals (Fig. 1). These indices were tested to evaluate changes in body size distribution of ground beetles along an urbanisation gradient (17), and the Lorenz curve performed best.

To compare the Gini and Lorenz asymmetry coefficients between GM and conventional maize crops, the normal distribution of data was tested by the Kolmogorov-Smirnov test and the Shapiro-Wilk normality test. Both proved that our data are normally distributed, so a linear mixed effects model for repeated measures data (lme) with plots as random effects was adopted. This model was chosen over more traditional approaches, such as repeated measures ANOVA, because of its ability to deal with missing values and pertinence in settings where repeated measurements are carried out on the same statistical units (30). The analyses were carried out using the R package (31), the internal packages „ineq” for the coefficient (32), „lattice” for graphs (33) and „nlme” (34) for statistical analysis.

## RESULTS

A total of 6339 carabids belonging to 38 species were identified (Table 1). The dominant species was the mixed feeder *Harpalus rufipes* (De Geer, 1774). Among the most abundant species, the genus *Bembidion* was well represented by three species, *B. lampros* (Herbst, 1784), *B. obtusum* (Audinet-Serville, 1821) and *B. properans* (Stephens 1828). There were some larger and common species such as *Anchomenus dorsalis* (Pontoppidan, 1763), *Stomis pumicatus* (Panzer 1796) and *Pterostichus melanarius* (Illiger 1798) and a small one, *Trechus quadristriatus* (Schrank 1781).

These eight dominant species made up 88.8% of the total numbers captured. The dominance was not different between the Bt- and isogenic maize plots. The GM plots had a higher number of species per month (mean = 22.33 and S.D. = 1.15) than the isogenic ones (mean = 20.67 and S.D. = 2.31). There were 12 singleton species, of which 4 were captured in the isogenic and 8 in the GM maize plots.

### Body size distribution

The species ranged in size from 3.13 mm to 23.29 mm (Table 1). The body size distribution profile (Fig. 2) did not show any obvious difference between assemblages in Bt- and isogenic maize plots. However, at the beginning of the season, the carabid assemblages had a lower mean body size (mean<sub>GM</sub> = 5.82, S.D. = 3.69 vs. mean<sub>ISO</sub> = 6.5, S.D. = 4.06), in particular by high numbers of *B. lampros* and *B. properans*. The only large species with high activity was *H. rufipes*. In July, medium sized species increased in activity density (mean<sub>GM</sub> = 7.86, S.D. = 3.96 vs. mean<sub>ISO</sub> = 8.09, S.D. = 4.05). In August, activity density of large species increased, further increasing the mean body size (mean<sub>GM</sub> = 10.12, S.D. = 4.35 vs. mean<sub>ISO</sub> = 9.77, S.D. = 4.34), in particular *H. rufipes*, *P. melanarius* and *P. niger*, and a decrease of the small species that were dominant in June.

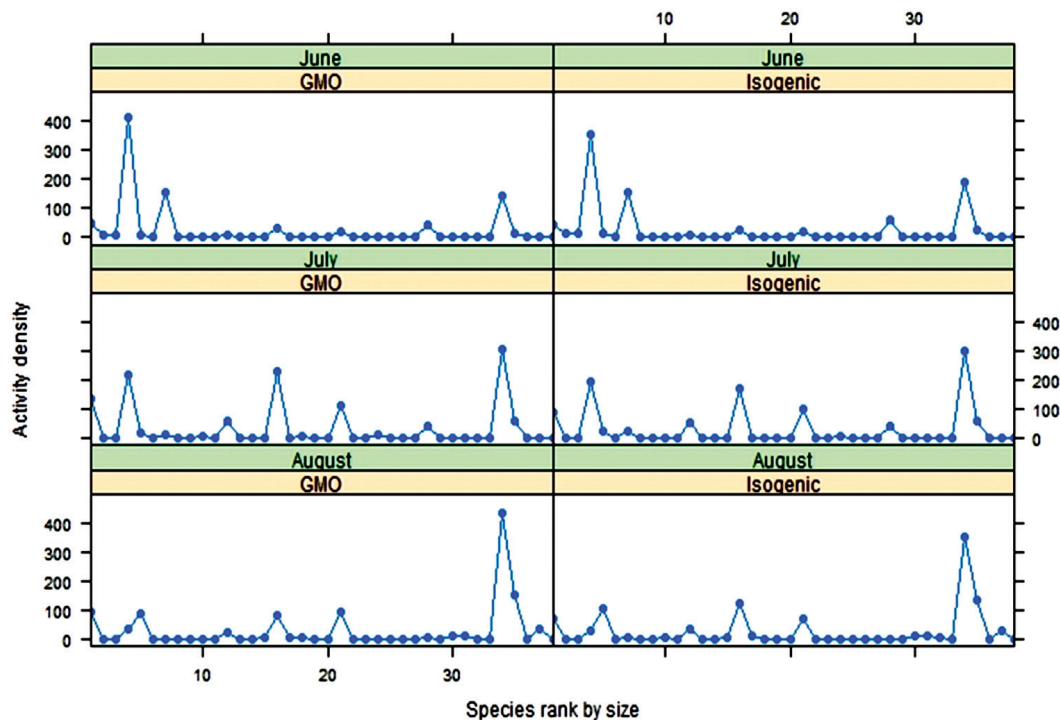
The Gini coefficient was generally low (Fig. 3), indicating two carabid assemblages with similar inequalities of body size. The coefficient was highest in the isogenic maize plots in June ( $G_{GM} = 0.28$ , S.D. = 0.05 vs.  $G_{ISO} =$

0.3, S.D. = 0.02) and August ( $G_{GM} = 0.23$ , S.D. = 0.02 vs.  $G_{ISO} = 0.25$ , S.D. = 0.03), but similar to GMO in July ( $G_{GM} = 0.27$ , S.D. = 0.02 vs.  $G_{ISO} = 0.28$ , S.D. = 0.02), suggesting that body size inequality of carabid assemblages was slightly larger in the isogenic maize assemblages. The Gini coefficient indicated marginally significant ( $p < 0.1$ ) differences between the two treatments (Table 2). The Gini coefficient was significantly different ( $p < 0.0001$ ) between months.

The Lorenz asymmetry coefficients were generally similar between the two treatments, indicating two carabid assemblages with similar distribution of body size inequality. The coefficient was  $S < 1$  for both treatments over the whole season ( $S_{GM} = 0.95$ , S.D. = 0.29 vs.  $S_{ISO} = 0.92$ , S.D. = 0.2), suggesting that asymmetry was mostly caused by small species.

Looking at each month, the coefficient was decreasing in both the Bt- and isogenic maize plots (Fig. 4). It had values  $S > 1$  for June ( $S_{GM} = 1.18$ , S.D. = 0.25 vs.  $S_{ISO} = 1.06$ , S.D. = 0.18), suggesting the importance of larger individuals for the skewness of the Lorenz curve. In July, the value was very close to  $S = 1$  in both treatments ( $S_{GM} = 1.04$ , S.D. = 0.16 vs.  $S_{ISO} = 0.98$ , S.D. = 0.12). This is typical of a nearly symmetric Lorenz curve. In August, in both maize plots, Lorenz asymmetry coefficients were  $S < 1$  ( $S_{GM} = 0.64$ , S.D. = 0.13 vs.  $S_{ISO} = 0.74$ , S.D. = 0.14), indicating the contribution of small individuals to asymmetry.

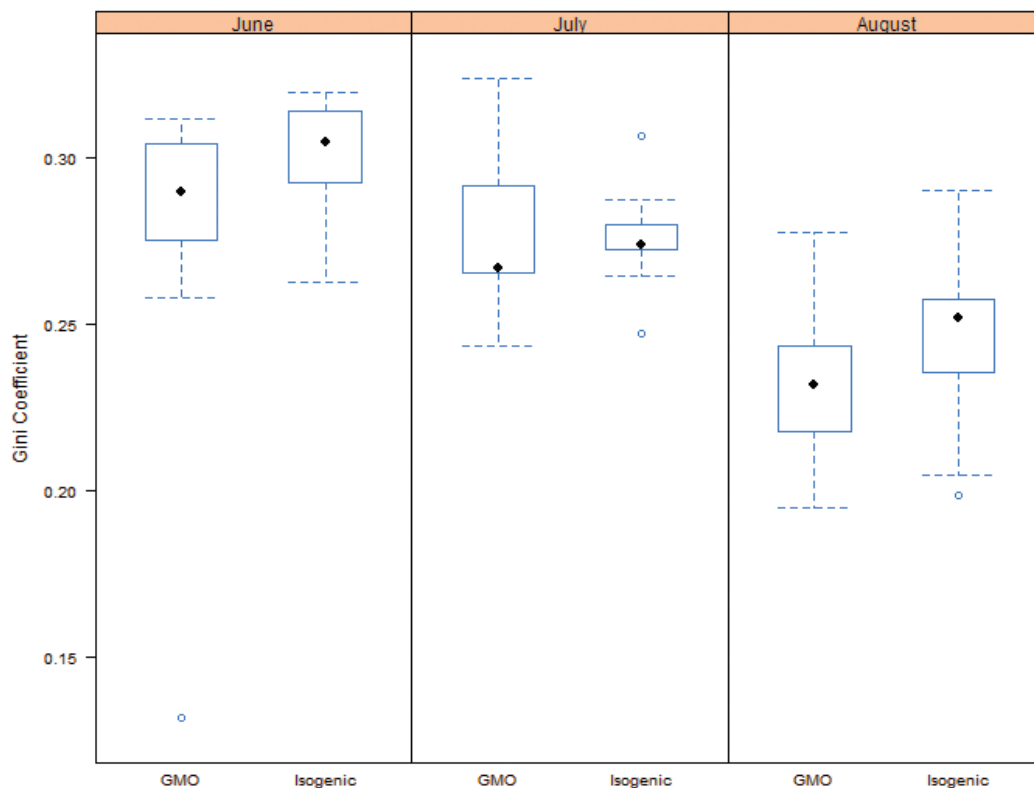
However, the differences in the Lorenz asymmetry coefficients among the studied areas were not significant



**Figure 2.** Trend of activity density of the carabid species captured, comparing treatments and months. Species (x-axis) are arranged according to increasing body size.

**Table 1.** Total number of ground beetles individuals captured over three months. Species are arranged by increasing body size. The body size is a geometric mean calculated from, and nomenclature follows Lindroth (22, 23).

Species	Body size (mm)	Number of individuals captured						Total
		June		July		August		
		GMO	Isogenic	GMO	Isogenic	GMO	Isogenic	
<i>Bembidion obtusum</i>	3.13	51	42	137	91	96	70	487
<i>Bembidion quadrimaculatum</i>	3.13	10	11	3	0	0	0	24
<i>Acupalpus meridianus</i>	3.54	8	12	0	3	0	0	23
<i>Bembidion lampros</i>	3.63	414	357	218	198	39	30	1256
<i>Trechus quadristriatus</i>	3.74	7	12	18	23	90	107	257
<i>Trechus secalis</i>	3.74	0	0	1	0	0	0	1
<i>Bembidion properans</i>	3.83	152	155	15	24	3	6	355
<i>Notiophilus aestuans</i>	4.69	1	2	0	0	0	0	3
<i>Bradycellus verbasci</i>	4.84	0	0	0	0	1	0	1
<i>Demetrias atricapillus</i>	5.02	4	0	6	3	4	7	24
<i>Paradromius linearis</i>	5.14	0	0	0	0	0	1	1
<i>Bembidion tetracolum</i>	5.47	7	5	63	56	26	35	192
<i>Notiophilus biguttatus</i>	5.48	0	0	0	0	1	0	1
<i>Oxypselaphus obscurus</i>	5.74	0	0	0	1	0	0	1
<i>Clivina fossor</i>	5.98	1	1	0	1	5	5	13
<i>Anchomenus dorsalis</i>	7.01	32	27	231	174	81	125	670
<i>Synuchus vivalis</i>	7.14	0	0	7	4	6	4	21
<i>Loricera pilicornis</i>	7.14	0	0	1	0	6	11	18
<i>Calathus melanocephalus</i>	7.27	0	0	1	1	1	2	5
<i>Amara aenea</i>	7.39	1	0	0	0	0	0	1
<i>Stomis pumicatus</i>	7.51	18	22	114	100	98	71	423
<i>Amara apricaria</i>	7.65	0	0	1	0	0	0	1
<i>Ophonus rufibarbis</i>	7.67	0	0	0	1	0	0	1
<i>Agonum muelleri</i>	8.27	1	2	12	6	4	3	28
<i>Amara consularis</i>	8.67	0	1	2	2	0	0	5
<i>Harpalus tardus</i>	9.61	1	0	0	0	0	0	1
<i>Calathus erratus</i>	10.01	1	0	0	0	0	0	1
<i>Harpalus affinis</i>	10.10	44	63	44	45	5	2	203
<i>Poecilus cupreus</i>	10.48	1	1	0	0	0	0	2
<i>Harpalus melancholicus</i>	10.49	0	1	0	0	13	13	27
<i>Nebria brevicollis</i>	11.83	0	0	0	0	14	12	26
<i>Calathus fuscipes</i>	12.00	0	0	1	2	1	5	9
<i>Amara aulica</i>	12.54	0	0	1	0	1	3	5
<i>Harpalus rufipes</i>	12.92	142	187	309	299	437	355	1729
<i>Pterostichus melanarius</i>	14.70	13	27	61	60	154	138	453
<i>Dolichus halensis</i>	16.43	0	0	1	0	0	0	1
<i>Pterostichus niger</i>	17.54	1	0	3	1	35	29	69
<i>Carabus nemoralis</i>	23.92	0	0	0	1	0	0	1
Total number of individuals captured		910	928	1250	1096	1121	1034	<b>6339</b>
Total number of species captured		21	18	23	22	23	22	



**Figure 3.** Variation of the Gini coefficient between GM and isogenic maize plots over three summer months, in Flakkebjerg, 2014. The black dot indicates the median, the box marks the central quartile; ranges are denoted by broken lines.

(Table 2). The only highly significant effect ( $p < 0.0001$ ) was the seasonality („month”) as in the Gini coefficient values.

**DISCUSSION**

In the present case study on the impacts of Bt-(MON810) maize on agro-ecosystem, we had two main goals. The first was to produce a basic checklist of carabid species in the agro-ecosystem in Flakkebjerg (Denmark)

as a basis for a more effective PMEM. The second main goal was the assessment of the potential of the size distribution asymmetry as a parameter in monitoring the effects of GMCs on selected NTOs, the ground beetles.

The „decreasing body size hypothesis” has been extensively tested in studies on carabid fauna (17, 19, 21), but so far not in relation to GM environmental effects. The results suggested no effect of the maize crop, whether GM or its isogenic equivalent, on carabid body size inequality.

**Table 2.** Analysis with a linear mixed effect model for Gini and Lorenz asymmetry coefficient on treatment (GMO vs. isogenic) and month (June vs. July vs. August) for carabid body size inequality. Plots are the random effects.

	numDF	denDF	F-value	p-value
<b>Gini coefficient</b>				
Month	2	36	14.401	<0.0001
Treatment	1	18	3.086	0.096*
Month: Treatment	2	36	0.725	0.491
<b>Lorenz asymmetry coefficient</b>				
Month	2	36	34.102	<0.0001
Treatment	1	18	0.468	0.503
Month: Treatment	2	36	2.105	0.137

\*Possible biological ( $p < 0.10$ ) but not significant ( $p < 0.05$ ) treatment effect.

However, an increasing body size trend from June to August was evident, and both indices reflected the increasing activity density of larger species as the season progressed and according to the different phenology of the dominant species (spring or autumn breeders).

Analysing the Lorenz curve, no significant differences in inequality were found between Bt- and non-Bt maize fields. The only marginally significant differences were obtained by the use of the Gini coefficient. This parameter suggested that body size inequality of carabid assemblages was slightly larger in isogenic maize assemblages respect to the GM one.

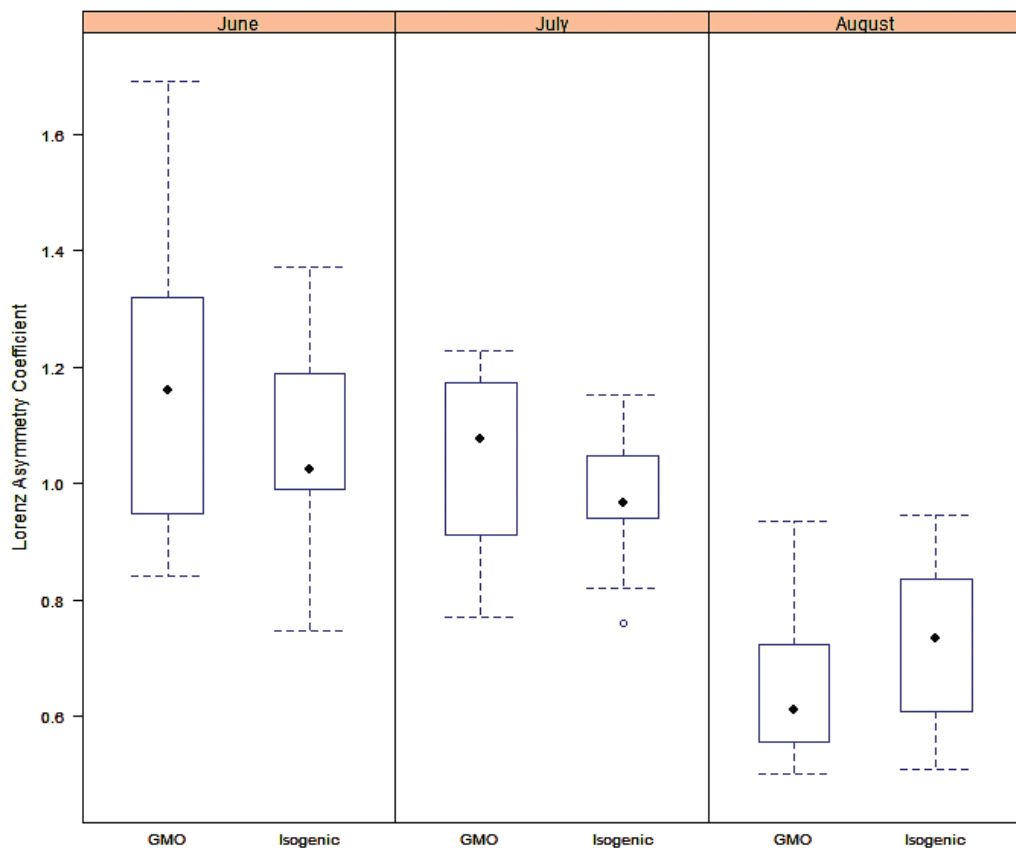
Several papers used the Gini coefficient to measure inequality in size or biomass, but the biological interpretation of skewness of a distribution is difficult (25) and it is referred to the total amount of size inequality. Nevertheless, it may be useful to look at not only the overall degree of inequality between treatments, but also how this inequality is distributed. The Gini coefficient does not have the power to distinguish what causes the deviation from perfect evenness (25).

As in the case of examining the effect of urbanisation-related disturbance on ground beetle assemblages in Hungary (17), the inequality in carabid body size was much

easier to biologically interpret using the Lorenz asymmetry coefficient. It was transparent that the skewness of Lorenz curve was sensitive to the seasonal changes of body size classes and not the treatments. Magura et al. (17) as well as earlier studies considered the whole year or season together, while we looked at a finer level of analysis, which proved fruitful. We were able to detect a significant impact of the season on body size asymmetry in carabid assemblages, indicating the sensitivity of the method for monitoring purposes.

We conclude that these methods, and in particular the Lorenz asymmetry coefficient, were indeed sensitive to subtle seasonal changes in the structure of the carabid assemblages, and they indicated no important differences between the structural composition, body size inequality and diversity of ground beetles in GM (MON810) vs. isogenic maize in Denmark at this spatial scale.

Work at species level was important to better discriminate the phenology that in this case study mainly indicated seasonal variations, and exclude the risk for some species to be suppressed by Bt maize and replaced by other less sensitive species. Furthermore, surveys covering the whole activity season are necessary, in order to completely investigate the seasonal variation of ground beetles.



**Figure 4.** Variation (presented as Box-plots) of Lorenz asymmetry coefficients between GM and isogenic plots over three summer months, in Flakkebjerg, 2014. The black dot indicates the median, the box marks the central quartile; ranges are denoted by broken lines.

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