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The use of the Rényi scalable diversity index to assess diversity trends in comparative and monitoring studies of effects of transgenic crops

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Abstract:【Background】One of the standard parameters to assess the impact of transgenic plants on ecological communities is the evaluation or comparison of diversity. Diversity can be described using many indices, but their interpretation is not straightforward, and different indices have different strengths and weaknesses. However, there are modern biodiversity methods that describe diversity relations in more sophisticated ways. The intent of this paper is to introduce the application scalable diversity index families (Rényi-diversity) to biosafety studies. 【Method】The scalable one-parametric Rényi-diversity index family includes several well-known diversity indices as special cases, but provides a complex assessment of diversity relations between several assemblages. After the introduction of the equation, we demonstrate the suitability of this method by comparing the censused spider fauna on two cultivars of transgenic Bt-cotton fields and compared to non-transgenic cotton fields under conventional or integrated (IPM) management in Hebei Province, north central China. 【Result】The diversity relationships demonstrated all possible interaction types: an unequivocal ordering, as well as different potential relationships between two spider assemblages. Among the sample fields, the Rényi index profile showed that the spider diversity in the Chinese Zhongmian 30 transgenic Bt-cotton was unequivocally the highest. The other three assemblages could not be unequivocally ordered: considering the rare species, the most diverse was the Bt-cultivar Monsanto 33B, followed by the IPM and the conventional fields. When common species had more weight, the most diverse assemblage was the IPM field, followed by conventional and the Monsanto 33B Bt-cotton one. 【Conclusion and significance】The suggested new method can provide a synthetic, multi-faceted assessment of the assemblages, and this allows a context-dependent evaluation of the diversity effects of various management actions. This method overcomes the traditional shortcomings of single-number diversity indices, and while considers several of them, it binds them into a single, coherent conceptual framework.

Key words: Bt-cotton; scalable diversity index; pesticide effect; biosafety evaluation; biodiversity comparison; Rényi diversity index

Rényi 多样性指数曲线在评价和监测转基因作物对生物多样性影响中的应用

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摘要:【背景】多样性比较是评价转基因作物对生物群落影响的标准参数之一。以往用于描述多样性的指数很多,但这些指数各有优缺点,生物学解释不全面。运用现代生物多样性表示方法,能够描述更复杂情况下的多样性关系。本文主要介绍了 Rényi 多样性指数曲线在生物安全研究中的应用。【方法】Rényi 多样性指数曲线与以往常用的一维多样性指数不同,当其等级参数为某些特定值时代表了几个著名的多样性指数,该方法可较为确切地评价复杂的生物群落的多样性。本文在介绍其计算公式后,通过比较河北省 2 种转 Bt 基因棉田(孟山都 33B 和中棉 30,均未施农药)以及常规管理(施用农药)和综合治理(IPM,施用农药和释放赤眼蜂相结合)条件下非转基因棉田的蜘蛛群落,证明了该方法的适用性。【结果】Rényi 多样性指数曲线分析表明了所有可能的互作类型:1 个明确的多样性排序以及 2 类棉田蜘蛛群落间不同的潜在关系。Rényi 指数分析表明,在供试棉田中,中棉 30 棉田的蜘蛛多样性指数最高,其他 3 种棉田蜘蛛的多样性高低则难以一概而论。蜘蛛稀有种的多样性以孟山都 33B 棉田最高,IPM 和常规棉田次之;蜘蛛常见种的多样性以 IPM 棉田最高,常规棉田和孟山都 33B 棉田次之。【结论与意义】Rényi 多样性指数可用于生物群落多样性的综合评价,并可用于评价不同管理措施

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对生物多样性的影响。该方法弥补了仅用单一数量参数评价多样性的传统方法的缺陷,将不同参数整合成一个具有内在关联的概念框架。

关键词: Bt 棉; 可扩展多样性指数; 农药效应; 生物安全评价; 生物多样性比较; Rényi 多样性指数

Transgenic crops may have significant environmental impacts (Wolfenbarger & Phifer, 2000). Consequently, commercial cultivation of such crops is conditional on an environmental risk assessment, during which the risk to the environment is assessed. The pre-release risk assessment regulations currently vary by country. Different countries have different regulations, and these sometimes contain inconsistencies and insufficiently supported assumptions (National Research Council, 2002). An emerging additional regulation, already codified by the European Community (directive 2001/18/EC), is the post-release monitoring of the transgenic plants, but its methodology is not yet fully developed (National Research Council, 2002). The evaluation of biodiversity changes is often part of such an assessment (Chen *et al.*, 2011; Liu *et al.*, 2011).

Agriculture crucially depends on ecological services (MEA, 2005; Tilman *et al.*, 2002), and more so in developing than developed countries (Mertz *et al.*, 2007). The effect of transgenic crops on ecosystem services was suggested as a conceptual framework to structure and unify the otherwise fragmented concerns about "non-target effects" (Lövei, 2001). One of the important biological services is natural pest control (MEA, 2005), increasingly used and not necessarily in good condition world-wide (Carpenter *et al.*, 2009). Agricultural habitats can also be significant in supporting biodiversity, especially in heavily cultivated areas (see, for example, Duelli *et al.*, 1999; Mészáros, 1984), yet we do not have a general understanding of the level of biodiversity that can be supported by an agricultural landscape (Daily, 1999) and which part of this is important in beneficial ecological functions. A diverse array of natural enemies is thought to boost the biological control of pests (Crowder *et al.*, 2010).

Biodiversity is often evaluated in biosafety studies (e. g. Dillon & Sharma, 2013; Liu *et al.*, 2011). Our aim was to increase the sophistication of assessing the impacts of transgenic plants on biodiversity. To analyse the possible differences in biodiversity, we suggest that the method of scalable diversity profiles (Rényi, 1961; Tóthmérész, 1995) can be very useful to detect the impact of different management regimes, including transgenic crops. This method is linked to a generalisation of the one-parameter diversity index families, developed by the Hungarian mathematician Alfred Rényi (Rényi, 1961), and allows a more comprehensive evaluation than traditional one-dimensional diversity indices (Lövei, 2005; Tóthmérész, 1995). In China, this method has been rarely used to compare the above-ground arthropod community structures with different crop management patterns (but see Guo *et al.*, 2007, 2009). Even these papers lack a detailed description of the general features of the method. After discussing these details, we use an example from China to illustrate how diversity comparisons can be made using the Rényi-diversity index.

MATERIAL AND METHODS

The Rényi diversity index and its features

The Rényi diversity, $HR(a)$, was first suggested by Hungarian mathematician Alfred Rényi (Rényi, 1961), in the form:

$$HR(a) = \frac{1}{1-a}\log\sum_{i=1}^{S} p_i^a$$

where p_i is the relative abundance of the i-th species, and S is the total number of species in the sample, and a is a scale parameter. The scale parameter, a is a mathematical abstraction and has no direct biological meaning. The equation is interpreted for the range $a \geq 0$, $a \neq 1$. Four special scale parameter values merit extra consideration:

(ⅰ) when the scale parameter $a = 0$, the value of the Rényi diversity is the logarithm of the number of species of the community; $HR(0) = \log S$. In this case the method is extremely sensitive to the contribution of the rare species to the diversity of the assemblage.

(ⅱ) When the scale parameter, a approaches 1 (it cannot take the exact value: $a \neq 1$, see above), the Rényi diversity gives the value of the Shannon diversity index. In this case the diversity is sensitive to the rare species, although not so extremely as for $a=0$.

(ⅲ) at $a=2$, the Rényi diversity is related to the quadratic or Simpson diversity. In this case the method is more sensitive to the frequent species than to the rare ones.

(ⅳ) When the value of the scale parameter is large (formally $a \to +\infty$), the value of the Rényi diversity is closely related to the relative abundance of the most common species. This is the logarithm of the reciprocal value of the so-called Berger-Parker or dominance index (Southwood & Henderson, 2000).

Thus it can be seen that the generalised Rényi diversity index is sensitive to the rare species for small values of the scale parameter (close to 0), whereas it is sensitive to the abundant species for larger values of the scale parameter. Diversity profiles can be calculated by several packages, including the DivOrd package (Tóthmérész, 1993), the R package *vegan* (Oksanen *et al.*, 2012), and the BiodiversityR package (Kindt, 2011).

Because the diversity profile is a monotonously decreasing curve, the relationship between two such profiles (i. e. two assemblages or communities, whose diversity is to be compared) can be of three types:

1. An unequivocal ordering of two assemblages occurs if the diversity profiles of the two assemblages to be compared do not cross each other at any point. For the assemblage represented by the upper profile, we can, in common words, claim that this assemblage is "more diverse" than the other one.

2. Due to the mentioned monotony, two profiles can cross each other once or twice. This depends on the rate of decline of the profile. This rate of decline is related to the evenness of the assemblage: a more even assemblage displays a more gradual, less steep decline. It often occurs that one assemblage starts out more diverse than the other, meaning higher diversity for rare species, but at one point, as emphasis gradually shifts towards the more common species, the lines cross. This means that for common species, the other assemblage is more diverse. The situation represents a not unequivocal ordering.

3. The occurrence of two crossings may indicate a stressed, species-poor and low density assemblage, because this assumes that a diversity profile starts low (low species richness) but ends up not so dominated by the most common species (low dominance index). This can occur when the evenness is high — a species- and individual-poor assemblage will, due to statistical constraints, have a high evenness, and thus its diversity profile will decline at low rate.

The diversity profiles of the assemblages to be compared are presented graphically, and analysed verbally. Only in the case of unequivocal ordering (see above) can one assemblage or community be declared to be "more diverse" than another, thus a precise description should usually accompany the graphical presentation of the diversity profiles.

Field example

In order to illustrate the use of the method, we selected a spider survey from China (Liu *et al.*, 2004). We present some experimental detail below — but we stress that the census results serve only an illustrative purpose.

Study area

The study site was at the Nan-Pi Agricultural Research Station, CAAS Institute of Plant Protection, Hebei Province, north central China (38°00′N, 116°70′E). In 1998, this station had a total of 15 ha of experimental fields planted in cotton as well as a range of other cultivated crops and trees. The field census was done on a series of 0.3 ha cotton plots separated by a non-cultivated strip of 10 ~ 50 m. Four different management regimes were compared:

1. Conventionally managed cotton with pesticide treatments (Conventional plot). This plot was planted with the locally developed cv. Xinxi-82. There were five insecticide treatments per season, on 25 June, 3, 7, 25 July, 6 August 1998. For sprayings, different insecticides were used. They were, in the sequence of the treatments: 60% methamidophos EC, 50% parathion-methyl EC, 20% esfenvalerate EC, and 10% cypermethrin EC on the last two occasions.

2. Cotton under an experimental, integrated pest management regime (IPM plot). This field, also planted with cv. Xinxi-82, received only two insecticide treatments, with the same mixture as the conven-

tional field, on 30 June and 27 July 1998. The egg parasitoid *Trichogramma chilonis* was released twice during the second generation of *Helicoverpa armigera*, 4 times during the third generation, and 3 times during the fourth generation. The parasitoid was released at a density of 180,000 ~ 210,000 wasps/ha at one time.

3. Bt-transgenic cotton, cv. Monsanto 33B (33B plot). This cv. expresses the *Cry*1A(c) endotoxin gene from *Bacillus thuringiensis* and is toxic to the cotton bollworm, *H. armigera*, and several other species of Lepidoptera (Perlak *et al.*, 1990). There was no pesticide treatment in this plot.

4. Chinese Bt-transgenic cotton, cv. Zhongmian 30 (Zhongmian 30 plot). This is a Chinese-developed line of cotton, containing the *Cry*1Ab gene, also toxic to Lepidoptera. There was no pesticide treatment in this plot, either.

This design was unreplicated but under the logistical and land constraints, it was decided that smaller plots would be unrealistic as they would be too much influenced by spill-over effects from neighbouring areas (Holt, 1985). This is also the reason why we refrained from the statistical evaluation of the patterns. In the neighbouring farming areas, cotton is grown on even smaller plots, the average family land being in the range of 0.5 ha and supporting several crops (G. L. Lövei, personal observation).

Survey method

Starting in early June 1998, a visual inspection of 10 (until 17 July) or 5 (22 July ~ end of September) plants at 10 locations per plot (total of 50 ~ 100 plants) was done every 5 days to the end of September 1998. The locations were regularly distributed within the field, and the plants around them were randomly chosen, and labelled. All censuses were done on the same selected plants. During census, the observer counted and identified all spiders seen on the plants and on the ground. Unidentifiable adult spiders were collected and taken to the laboratory for rearing and identification. The taxonomy followed Zhao (1995) and Platnick (2003). Voucher specimens are deposited in the CAAS Institute of Agro-Environment & Sustainable Development, Beijing, China.

RESULTS

The empirical example: diversity comparison of four spider assemblages

Assemblage composition — A total of 5,605 individuals, belonging to 16 identified and 13 unidentified species, were observed during the sampling season. The most species-rich was the Zhongmian 30 Bt-cotton plot, followed by the Monsanto 33B, the IPM plot and finally, the conventionally managed plot (Table 1). The 33B Bt-cotton plot had the highest total number of spiders observed, followed by the other Bt-cotton cultivar, Zhongmian 30. In the conventional plot, only about one-fourth of the numbers found in the Bt-cotton plots were present (Table 1).

Table 1 The list of common spiders observed in four different types of cotton fields at Nan-Pi Agricultural Research Station, Hebei Province, China, during the growing season, early June ~ late September 1998

Family	Species name	Total no. of spiders observed in cotton fields under			
		Conventional management	IPM management	Bt-cotton	
				Monsanto 33B	Zhongmian 30
Lycosidae	*Pardosa astrigera* L. Koch	220	324	333	448
Linyphiidae	*Hylyphantes graminicola* Sundevall	92	179	371	331
	Ummeliata insecticeps (Boes. et Str.)		10	16	15
Dictynidae	*Dictyna arundinacea* (L.)	54	66	176	135
Theridiidae	*Achaearanea tepidariorum* (L. Koch)	72	433	1068	658
Philodromidae	*Thanatus formicinus* (Clerck)	14	11	33	46
Araneidae	*Neoscona nautica* (L. Koch)	16	8	52	61
Salticidae	*Evarcha albaria* (L. Koch)		1	15	9
Thomisidae	*Misumenops tricuspidatus* (F.)	18	9	21	55
	Xysticus atrimaculatus Boes. et Str.	2		40	36
Clubionidae	*Clubiona kurilensis* Boes. et Str.	10	5	15	19
Total no. of species		12	14	20	25
Total no. of families		8	9	11	11
Total no. of individuals		514	1071	2163	1857

Diversity & diversity ordering — The rank-abundance curve indicated that the two Bt-cotton plots had about equal diversity (Fig. 1) and they were the two most diverse assemblages. The conventional plot and the IPM plot curves indicated lower diversity, the latter having a "longer tail", indicating more species present (Fig. 1).

The diversity ordering showed a more complete but more complex picture. The spider assemblage in the cv. Zhongmian 30 was unequivocally the most diverse as its respective diversity profile did not cross any of the other profiles at any value of the scale parameter (Fig. 2). The other three assemblages could not be unequivocally ordered. At small values of the scale parameter, the IPM and conventional plots supported a less diverse assemblage than the Monsanto Bt-cotton cv. 33B but the situation changed and the 33B curve descended to remain the lowest when the scale parameter value was $a > 1.6$. The profile of the pesticide-treated, conventional field crossed the other two curves at about $a = 0.4 \sim 0.6$ and remained the highest until ca. $a = 4.1$ (Fig. 2).

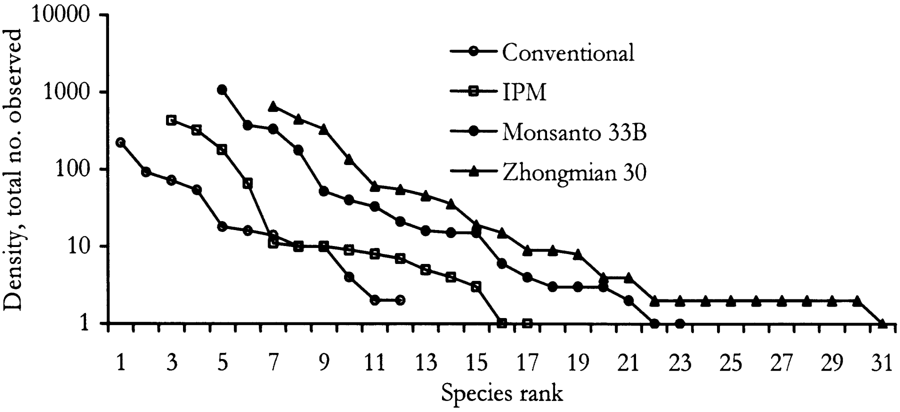

Fig. 1 Rank-abundance curves of the four spider assemblages in transgenic Bt- and non-transgenic cotton fields at Nan-Pi Research Station, Hebei Province, north central China, in 1998

To decrease overlap, the starting positions of the individual curves are displaced by two ranks.

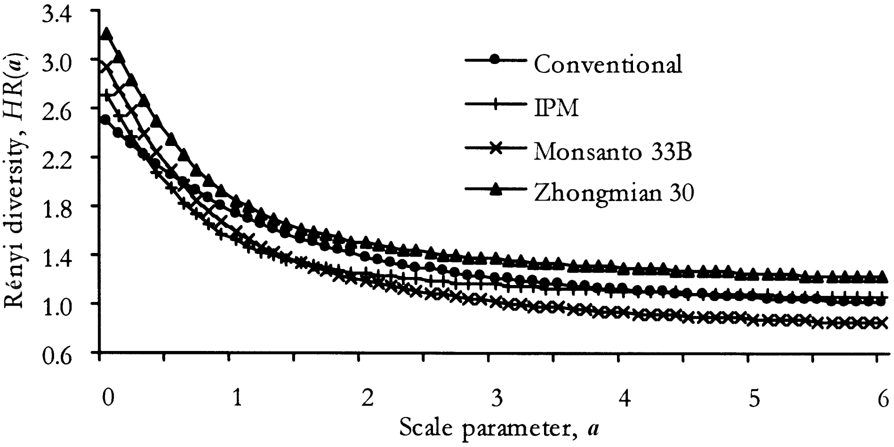

Fig. 2 The Rényi diversity profiles of the four spider assemblages studied at Nan-Pi Research station, Hebei Province, north central China, in 1998

The relative positions of the four profiles did not change when $a > 6$, so only the $0 < a < 6$ interval is pictured.

DISCUSSION

The spider fauna encountered at Nan-Pi was not particularly species-rich. A survey of cotton plants in South Africa (van den Berg *et al.*, 1990) detected 76 spider species. The known fauna of cotton fields in Arkansas, USA is 189 species (Heiss *et al.*, 1988) although this cannot be directly compared to our survey because that list was compiled using different methods and a wider spatial and temporal scale. In Australia, the species richness is close to ours (25 species, Bishop, 1980). An extensive survey in China (Qu *et al.*, 1986) found 61 species, but only 30 species were found in cotton fields in Wuhan Province, Southern China (Li & Zhao, 1993).

The diversity comparison of the four treatments indicated that only one of the assemblages, the one living in the Chinese Bt-cotton cv. Zhongmian 30, can be considered unequivocally more diverse than the others. The diversity profile for the Bt-cotton cv. 33B, surprisingly, indicated the least diverse spider assemblage for most of the range. This was probably caused by unidentified conditions that made this crop a very favourable habitat for one theridiid species, *A. tepidariorum*. This was the most common species overall, and nearly half of all specimens observed were found in this cotton cultivar. The diversity under scale parameters sensitive to medium-rare species showed that the conventional, pesticide-treated field had the second most diverse assemblage. Evaluating diversity based on only the frequently used Shannon-diversity (near scale parameter $a = 1$, see Material and Methods section) would have given a similar result that would not be representative of the total impact on diversity. The diversity in this region of the scale parameter is influenced by the combined effect of species number and their relative density. In the conventional plot, the very low densities of even the common species increased the evenness of the assemblage, thus inflating its diversity. The impact of restricted pesticide use on spider diversity in relation to the Bt-cotton cultivars is better reflected in the run of the IPM profile over a wider interval of the scale parameter values. This also indicates that spraying indeed was harmful for the diversity of the spider assemblage (Fig. 2).

The density differences for spiders active on plants, however, probably reflect real differences as they are directly comparable. The effect of frequent pesticide spraying in the conventional plot was evident on the density and diversity of spider assemblage in conventionally managed cotton. This was the only treatment where *P. astrigera*, a wolf spider, was the most common species. Plant-living spider densities were drastically reduced. Pesticide sprayings, especially early in the season, disrupt the beneficial arthropod assemblage, and they fail to recover during the season (Hagerty *et al.*, 2000). In our experiments, the Bt-plot had no insecticide treatments. This, however, is not the usual practice. The number of insecticide sprayings on Bt-cotton in China is much reduced, especially early in the season, but not completely stopped (Huang *et al.*, 2002). Therefore we expect that the composition and dynamics of the spider assemblage in Bt-cotton could be closer to the "IPM assemblage" than in our experiments reported here. The large differences among plots were most likely due to the degree of pesticide application and less likely due to the cotton genotype. A similar trend was reported from cotton fields in the U. S. A. (Hagerty *et al.*, 2000).

There were no noticeable differences in the surrounding crops around the different plots, nor a clear gradient in slope or soil type. If the differences encountered were caused only by differences in the pesticide treatments, the two Bt-crops, having no pesticide treatments, should show only minor differences. This was clearly not so, and thus the causes that generated differences in the spider assemblages cannot entirely be apportioned to differences in pesticide treatments and agronomy.

Due to the census technique used, the presence of wolf spiders that are mostly active on the ground was very probably underestimated. Only one species, *P. astrigera*, was identified, although that was a very common one. A better understanding of the ground-active fauna would require soil sampling or fenced pitfall trapping (Lövei & Sunderland, 1996) or D-vac + hand count (Greenstone, 2001; Sunderland & Topping, 1992) that can provide real density data.

Overall, the use of the Rényi diversity profiles allows the evaluation of diversity in a more articulated way than the use of the various diversity indices in isolation. The use of the individual indices nearly always gives conflicting results, which are difficult to reconcile (Tóthmérész, 1995). The Rényi diversity family index and the graphical evaluation method offers a possibility to understand the validity of the indication by single indices, and can be reconciled by specifying the range over which one particular relationship holds, and allows a biological interpretation of diversity by placing various weights on the different abudance catgegories (rare vs. medium-rare vs. common species).

We would recommend the more extended use of this method, as it is eminently suitable for a complex assessment on diversity of any management regime, especially in agriculture and conservation biology.

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