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A Meta-Analysis of Fecal Bacterial Diversity in Dogs

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메타분석을 통한 반려견 분변 박테리아 군집 조사

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Abstract In this study, a meta-analysis of fecal bacteria in dogs was conducted using 16S rRNA gene sequences that have been recovered from cloning and Sanger sequencing. For this meta-analysis, we retrieved all 16S rRNA gene sequences recovered from fecal bacteria in dogs in the RDP database (Release 11, Update 3). A total of 420 sequences were identified from the RDP database, 42 of which were also recovered from cultured isolates. The 420 sequences were assigned to five phyla, of which Firmicutes was the most predominant phylum, accounting for 55.2% of all 420 sequences. Bacteroidetes was the second most predominant phylum, accounting for 32.1% of the 420 sequences, followed by Actinobacteria (6.4%), Fusobacteria (3.8%), and Proteobacteria (2.4%). The genus *Bacteroides* within Bacteroidetes was the largest, representing 30.0% of all 420 sequences, while the putative genus *Clostridium* XI within Firmicutes was the second largest, representing 27.4% of all 420 sequences. A total of 82 operational taxonomic units (OTUs) that are putative species were identified from the retrieved sequences. The results of this study will improve understanding of the diversity of fecal bacteria in dogs and guide future studies on the health and well-being of dogs.

요 약 본 연구에서는 클로닝과 생어 염기서열 분석으로 획득된 16S rRNA 유전자 염기서열을 메타분석하여 반려견 분변 박테리아를 조사하였다. 이러한 메타분석을 위해서 RDP 데이터베이스(Release 11, Update 3)에 등록되어 있는 반려견 분변 박테리아 유래 16S rRNA 유전자 염기서열 검색하여 획득하였다. RDP 데이터베이스에서 총 420개의 반려견 분변 박테리아 유래 16S rRNA 유전자 염기서열이 확인되었고, 그 중에서 42개 유전자 염기서열이 배양가능한 박테리아에서 유래한 것으로 확인되었다. 이러한 420개의 유전자 염기서열은 박테리아 분류학상의 '문'(phylum)에서 총 5개(Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria)로 분류되었다. 그 중에서 Firmicutes가 가장 우점하는 '문'이었고, 총 420개 유전자 중에서 55.2%를 차지하였다. Bacteroidetes는 32.1%로 두 번째로 우점하는 '문'이였고, 다음으로 Actinobacteria(6.4%), Fusobacteria(3.8%), Proteobacteria(2.4%)가 우점하였다. 박테리아 분류학상의 '속'(genus)에서는 Bacteroidetes의 하위 단계인 *Bacteroides*가 가장 우점하였고 총 420개 유전자 중에서 30.0%를 차지하였다. 반면에 Firmicutes의 하위 단계인 *Clostridium* XI는 두 번째로 우점하는 '속'으로 총 420개 유전자 중에서 27.4%를 차지하였다. 추정상의 '종'(species)인 Operational taxonomic units의 수는 82개로 확인되었다. 본 연구의 결과는 반려견 분변 내 미생물 다양성을 이해하는데 도움을 줄 수 있을 것이고, 향후 반려견의 건강과 웰빙에 관한 연구에 활용될 수 있을 것이다.

Keywords : 16S rRNA gene sequences, dog, fecal bacteria, meta-analysis, RDP database

1. Introduction

As national income increases, pets have been recognized as companion animals in last 15 years ago in South Korea[1]. Companion animals positively affect our lives in terms of stress relief, social support, and treatment of disease[2]. Especially, dogs play an important role in improving our emotional stability and

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social interaction with other people[3]. In addition, dogs can make elderly people feel more relaxed in the era of aging[4]. Therefore, health state of dogs may be an important factor for our healthy lives due to interaction between humans and dogs.

Fecal bacterial communities can affect the health and well-being of dogs[5]. Middelbos et al.[6] investigated the fecal bacterial communities of dogs fed diets with or without dietary fiber, whereas Garcia-Mazcorro et al.[7] investigated the fecal bacterial communities of dogs fed diets with or without synbiotics. Beloshapka et al.[8] used next-generation pyrosequencing to examine the effect of inulin or yeast cell wall extracts on the fecal bacterial communities of dogs. These three studies showed that diet greatly affected the fecal bacterial communities of dogs.

Microbiologists have evaluated the composition of bacterial communities in an environmental sample using a traditional culture-based technique (ex.[9]). However, it became evident that most of bacteria could not be cultured in the lab due to the limitation of culture-based techniques[10].

The 16S rRNA gene is a part of genomic DNA of bacteria, and its sequences have changed very little because the evolution rate of the 16S rRNA gene is slow[11]. Since Woese and Fox[11] suggested the 16S rRNA gene as a phylogenetic marker for analysis of bacterial taxonomy, the composition of unculturable bacterial communities have been evaluated using 16S rRNA gene-based techniques.

The use of cloning and Sanger sequencing is one of 16S rRNA gene-based techniques to evaluate the composition of bacterial communities and can contribute to the identification of novel bacterial species, as described previously[12]. Previous individual studies (both published and unpublished) using this technique have evaluated the composition of fecal bacterial communities in dogs using cloning and Sanger sequencing. However, these individual studies using this technique might have been biased towards certain bacterial taxa due to PCR error[13]. To minimize this bias, Kim et al. [13] evaluated the composition of ruminal bacterial communities using a meta-analysis of all 16S rRNA gene sequences recovered from rumen bacteria.

In this study, we first performed a meta-analysis to provide a collective census of the fecal bacterial communities of dogs using 16S rRNA gene sequences obtained by Sanger sequencing from various studies.

2. Materials and Methods

All 16S rRNA gene sequences recovered from bacteria in the feces of dogs were retrieved from the RDP release 11 (Update 3) providing bacterial 16S rRNA gene sequences as suggested by Kim et al.[13]. The search terms used were ‘dog feces,’ ‘dog faeces,’ ‘canine feces,’ and ‘canine faeces’. Additionally, isolate sequences recovered from dog feces, which were not found in the RDP database, were manually retrieved from the American Type Culture Collection (ATCC), Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), and Japan Collection of Microorganisms (JCM). A taxonomic tree with Bergey’s taxonomy was constructed using the program

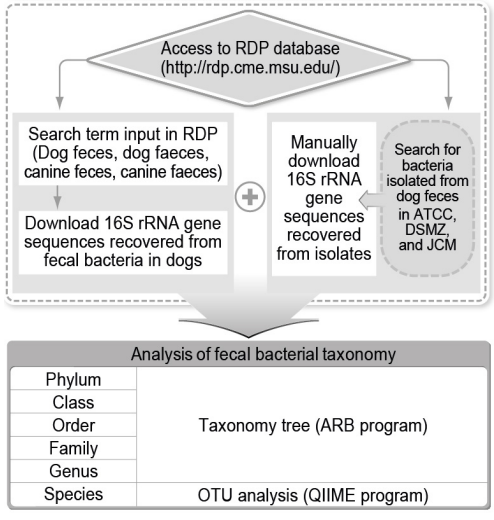

Fig. 1. A workflow for a meta-analysis of fecal bacterial diversity in dogs

ARB[14]. Operational taxonomic units (OTUs) that are defined as putative species were analyzed from sequences of the V2 to V3 regions using the QIIME software package 1.9.1[15]. A workflow for this meta-analysis is shown in Fig 1.

3. Results and Discussion

3.1 Data summary

A total of 420 sequences based on Sanger sequencing technology were obtained from six published and three unpublished studies. The 420 sequences including 28 isolate sequences were classified into five phyla, of which Firmicutes was the predominant phylum and accounted for 232 of the 420 sequences, followed by Bacteroidetes (135 sequences), Actinobacteria (27 sequences), Fusobacteria (16 sequences), and Proteobacteria (10 sequences) [Table 1]. These five taxa appear to be core phyla commonly found in dog feces[16]. From the sequences, 82 OTUs were identified.

Table 1. Taxa of fecal bacteria in dogs

Phylum	no. of sequences	no. of isolate sequences	Proportion
Firmicutes	232	19	55.2%
Bacteroidetes	135	9	32.1%
Actinobacteria	27	0	6.4%
Fusobacteria	16	0	3.8%
Proteobacteria	10	0	2.4%
Total	420	28	100%

3.2 Firmicutes

The phylum Firmicutes was represented by 12 genera, of which putative *Clostridium* XI was the largest genus, represented by 115 sequences including 11 isolate sequences [Fig 2 and 3]. The 115 *Clostridium* XI sequences clustered into 10 OTUs, of which three included sequence(s) recovered from *Clostridium* isolates. One OTU was represented by 94 sequences, including eight sequences recovered from *Clostridium hiranonis* isolates (unpublished study). *C. hiranonis* seems to be common in dog feces[16]. Another OTU was represented by two sequences recovered from *Clostridium difficile* isolates (unpublished study). Wetterwik et al.[17] suggested that *C. difficile* was toxigenic in dogs with diarrhea but non-toxigenic in healthy dogs, suggesting that *C. difficile* strains have different roles in the fecal microbial ecosystem in dogs. *Blautia*, the second largest genus within Firmicutes, was represented by 37 sequences, including two isolate sequences. The 37 *Blautia* sequences clustered into six OTUs, one of which was represented by sequences recovered from *Blautia glucerasea* isolates[18]. *Blautia* seems to be common in heathy dogs, but it decreased in dogs with diarrhea[19] and may play a role in producing short-chain fatty acids[20]. Putative genus *Clostridium sensu stricto* was represented by 17 sequences, including one isolate sequence recovered from *Clostridium colicanis*. The 17 sequences clustered into three OTUs, one of which was dominant and represented by 14 sequences that did not include any sequence recovered from a cultured isolate. Species corresponding to this OTU will need to be isolated and characterized in the future. *Allobaculum* comprised eight sequences, with seven clustered into one OTU, including a sequence recovered from *Allobaculum stercoricanis*. *Allobaculum* may play a role in producing short-chain fatty acids[20]. *Enterococcus* was represented by two sequences recovered from *Enterococcus canintestini* and *Enterococcus faecium* isolates, whereas *Streptococcus* and *Lactococcus* were represented by one sequence each recovered from *Streptococcus alactolyticus* and *Lactococcus lactis*, respectively. No sequences of the remaining genera (*Ruminococcus*, *Butyricicoccus*, putative *Clostridium* XVIII, and putative *Clostridium* XIVa) were recovered from the isolates. The 10 *Ruminococcus* sequences clustered into six OTUs. Handl et al.[21] reported that *Ruminococcus* is thought to be prevalent in dog feces. Unclassified Lachnospiraceae, unclassified Ruminococcaceae,

and unclassified Peptostreptococcaceae were represented by 22, 9, and 1, sequences, respectively. The unclassified Lachnospiraceae and Ruminococcaceae sequences clustered into 12 and 4 OTUs, respectively. The unknown species corresponding to these 16 OTUs need to be isolated and characterized in the future.

3.3 Bacteroidetes

The phylum Bacteroidetes was represented by 135 sequences that were assigned to the genera *Bacteroides* (126 sequences) and *Prevotella* (nine sequences) [Fig 2]. These two genera were not represented by any sequence recovered from isolates. *Bacteroides* and *Prevotella* clustered into 17 and 4 OTUs, respectively. Handl et al.[21] postulated that the combined abundance of *Prevotella* and *Bacteroides* is inversely related to the abundance of Fusobacteria due to competition for the same niche. *Bacteroides* is associated with diarrhea in dogs[22].

3.4 Proteobacteria

The phylum Proteobacteria was represented by 10 sequences that were assigned to *Plesiomonas* (four sequences), *Yersinia* (three sequences), *Campylobacter* (two sequences), and *Sutterella* (one sequence) [Fig 2]. *Plesiomonas* included four sequences recovered from *Plesiomonas shigelloides* isolates, whereas *Yersinia* included three sequences recovered from *Yersinia rohdei* isolates [Fig 3]. *Sutterella* was represented by one sequence recovered from the *Sutterella stercoricanis* type strain[23].

3.5 Other phyla

The phylum Actinobacteria was represented by 27 sequences that were assigned to the genera *Bifidobacterium* (seven sequences), *Collinsella* (17 sequences), and *Slackia* (three sequences) [Fig 2]. *Slackia* included a sequence recovered from an asaccharolytic *Slackia faecicanis* isolate[24] [Fig 3]. *Slackia* may be culturable, but it plays a minor role in

Fig. 2. A taxonomic tree showing the genera of fecal bacteria in dogs. A total of 420 sequences were classified to 22 genera including putative genera. The numbers in rectangular bars mean the number of 16S rRNA gene sequences.

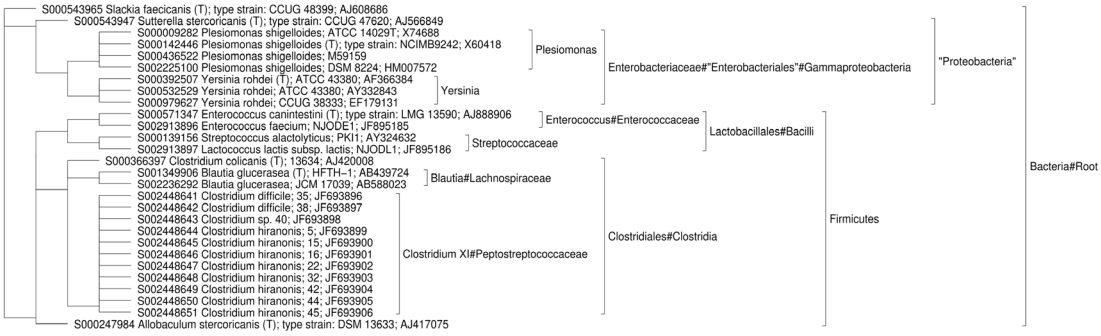

Fig. 3. A taxonomic tree showing the genera isolated from dog feces. In total, 28 isolate sequences were recovered from dog feces and accounted for 6.7% of all the 420 sequences.

the fecal microbial ecosystem in dogs due to its low abundance. The phylum Fusobacteria was represented by 16 sequences that were assigned to *Fusobacterium* (13 sequences) and *Cetobacterium* (three sequences). Fusobacteria produces volatile fatty acids, such as butyrate and acetate, via fermentation of carbohydrates and amino acids[25]. *Fusobacterium* was the predominant genus in the phylum Fusobacteria in dogs, as described previously[16].

4. Conclusion

This study presents a collective view of the fecal bacterial diversity in dogs using 16S rRNA gene sequences obtained by Sanger sequencing. The 5 phyla that are Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria and Proteobacteria appeared to be ubiquitous in dog feces irrespective of diets that greatly affect the composition of fecal bacterial communities in dogs. This result corroborates the findings of previous studies[6-8, 16], indicating that these 5 phyla may play a central role in maintaining the health and well-being of dogs. This meta-analysis of global bacterial diversity in dog feces will guide future studies and help develop analytics tools.

References

[1] Y. H. Shin, “Improvement in a matter for the proper popularization of companion animal-culture in Korea”, *Proceedings of the KAIS Fall Conference*, pp. 702 - 704, 2014.

[2] Y. H. Shin, “Analysis of the Pet and Companion Animal Management of University(College) Students in Korea”, *Proceedings of the KAIS Fall Conference*, pp. 826 - 828, 2014.

[3] A. R. Han, “The relationship between attitude toward pets and elementary school student’s empathy ability”, *Journal of the Korea Academia-Industrial cooperation Society*, vol. 17, no. 2, pp. 570 - 578, 2016.
DOI: http://doi.org/10.5762/KAIS.2016.17.2.570

[4] S. E. Kim, Y. H. Shin, “Analysis of the companion dog preference and rearing characteristic by family income levels”, *Proceedings of the KAIS Fall Conference*, pp. 829 - 831, 2014.

[5] K. S. Swanson, S. E. Dowd, J. S. Suchodolski, I. S. Middelbos, B. M. Vester, K. A. Barry, K. E. Nelson, M. Torralba, B. Henrissat, P. M. Coutinho, I. K. Cann, B. A. White, G.C. Jr. Fahey, “Phylogenetic and gene-centric metagenomics of the canine intestinal microbiome reveals similarities with humans and mice”, *ISME Journal*, vol. 5, no. 4, pp. 639 - 649, 2011.
DOI: http://doi.org/10.1038/ismej.2010.162

[6] I. S. Middelbos, B. M. Vester Boler, A. Qu, B. A. White, K. S. Swanson, G. C. Jr. Fahey, “Phylogenetic characterization of fecal microbial communities of dogs fed diets with or without supplemental dietary fiber using 454 pyrosequencing”, *PLoS One*, vol. 5, no. 3, p. e9768, 2010.
DOI: http://doi.org/10.1371/journal.pone.0009768

[7] J. F. Garcia-Mazcorro, D. J. Lanerie, S. E. Dowd, C. G. Paddock, N. Grützner, J. M. Steiner, R. Ivanek, J. S. Suchodolski, “Effect of a multi-species synbiotic formulation on fecal bacterial microbiota of healthy cats and dogs as evaluated by pyrosequencing”, *FEMS Microbiology Ecology*, vol. 78, no. 3, pp. 542 - 554, 2011.
DOI: http://doi.org/10.1111/j.1574-6941.2011.01185.x

[8] A. N. Beloshapka, S. E. Dowd, J. S. Suchodolski, J. M. Steiner, L. Duclos, K. S. Swanson, "Fecal microbial communities of healthy adult dogs fed raw meat-based diets with or without inulin or yeast cell wall extracts as assessed by 454 pyrosequencing", *FEMS Microbiology Ecology*, vol. 84, no. 3, pp. 532 - 541, 2013.
DOI: http://doi.org/10.1111/1574-6941

[9] K. D. Yook, J. S. Park, "The prevalence of extended spectrum B-lactamase type produced by clinical isolates of *Escherichia coli* from chungcheong area", *Journal of the Korea Academia-Industrial cooperation Society*, vol. 15, no. 4, pp. 2295-2302, 2014.
DOI: http://doi.org/10.5762/KAIS.2014.15.4.2295

[10] P. Hugenholtz, "Exploring prokaryotic diversity in the genomic era", *Genome biology*, vol. 3, no. 2, pp. reviews0003.1 - reviews0003.8, 2002.

[11] C. R. Woese, G. E. Fox. "Phylogenetic structure of the prokaryotic domain: the primary kingdoms", *Proceedings of the National Academy of Sciences of the United States of America*, vol. 74, no. 11, pp. 5088-5090, 1977.

[12] M. Kim, M. Morrison, Z. Yu, "Phylogenetic diversity of bacterial communities in bovine rumen as affected by diets and microenvironments", *Folia Microbiologica*, vol. 56, no. 5, pp. 453 - 458, 2011.
DOI: http://doi.org/10.1007/s12223-011-0066-5

[13] M. Kim, M. Morrison M, Z. Yu, "Status of the phylogenetic diversity census of ruminal microbiomes", *FEMS Microbiology Ecology*, vol. 76, no. 1, pp. 49-63, 2011.
DOI: http://doi.org/10.1111/j.1574-6941.2010.01029.x

[14] W. Ludwig, O. Strunk, R. Westram, L. Richter, H. Meier, Yadhukumar, A. Buchner, T. Lai, S. Steppi, G. Jobb, W. Förster, I. Brettske, S. Gerber, A. W. Ginhart, O. Gross, S. Grumann, S. Hermann, R. Jost, A. König, T. Liss, R. Lüssmann, M. May, B. Nonhoff, B. Reichel, R. Strehlow, A. Stamatakis, N. Stuckmann, A. Vilbig, M. Lenke, T. Ludwig, A. Bode, K. H. Schleifer, "ARB: a software environment for sequence data", *Nucleic Acids Research*, vol. 32, no. 4, pp. 1363 - 1371, 2004.
DOI: http://doi.org/10.1093/nar/gkh293

[15] J. G. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Pena, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozu-pone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yat-sunenko, J. Zaneveld, R. Knight, "QIIME allows analysis of high-throughput community sequencing data", *Nature Methods*, vol. 7, no. 5, pp. 335 - 336, 2010.
DOI: http://doi.org/10.1038/nmeth.f.303

[16] K. R. Kerr, G. Forster, S. E. Dowd, E. P. Ryan, K. S. Swanson, "Effects of dietary cooked navy bean on the fecal microbiome of healthy companion dogs", *PLoS One*, vol. 8, no. 9, p. e74998, 2013.
DOI: http://doi.org/10.1371/journal.pone.0074998

[17] K. J. Wetterwik, G. Trowald-Wigh, L. L. Fernström, K. Krovacek, "Clostridium difficile in faeces from healthy dogs and dogs with diarrhea", *Acta Veterinaria Scandinavica*, vol. 55, p. 23, 2013.
DOI: http://doi.org/10.1186/1751-0147-55-23

[18] H. Furuya, Y. Ide, M. Hamamoto, N. Asanuma, T. Hino, "Isolation of a novel bacterium, *Blautia glucerasei* sp. nov., hydrolyzing plant glucosylceramide to ceramide", *Archives of Microbiology*, vol. 192, no. 5, pp. 365 - 372, 2010.
DOI: http://doi.org/10.1007/s00203-010-0566-8

[19] J. S. Suchodolski, M. E. Markel, J. F. Garcia-Mazcorro, S. Unterer, R. M. Heilmann, S. E. Dowd, P. Kachroo, I. Ivanov, Y. Minamoto, E. M. Dillman, J. M. Steiner, A. K. Cook, L. Toresson, "The fecal microbiome in dogs with acute diarrhea and idiopathic inflammatory bowel disease", *PLoS One*, vol. 7, no. 12, pp. e51907, 2012.
DOI: http://doi.org/10.1371/journal.pone.0051907

[20] X. Zhang, Y. Zhao, M. Zhang, X. Pang, J. Xu, C. Kang, M. Li, C. Zhang, Z. Zhang, Y. Zhang, X. Li, G. Ning, L. Zhao, "Structural changes of gut microbiota during berberine-mediated prevention of obesity and insulin resistance in high-fat diet-fed rats", *PLoS One*, vol. 7, no. 8, p. e42529, 2012.
DOI: http://doi.org/10.1371/journal.pone.0042529

[21] D. Hand, C. Wallis, A. Colyer, C. W. Penn, "Pyrosequencing the canine faecal microbiota: breadth and depth of biodiversity", *PLoS One*, vol. 8, no. 1, p. e53115, 2013.
DOI: http://doi.org/10.1371/journal.pone.0053115

[22] J. Jia, N. Frantz, C. Khoo, G. R. Gibson, R. A. Rastall, A. L. McCartney, "Investigation of the faecal microbiota associated with canine chronic diarrhea", *FEMS Microbiology Ecology*, vol. 71, no. 2, pp. 304-312, 2010.
DOI: http://doi.org/10.1111/j.1574-6941.2009.00812.x

[23] H. L. Greetham, M. D. Collins, G. R. Gibson, C. Giffard, E. Falsen, P. A. Lawson, "Sutterella stercoricanis sp. nov., isolated from canine faeces", *International Journal of Systematic and Evolutionary Microbiology*, vol. 54, pp. 1581 - 1584, 2004.
DOI: http://doi.org/10.1099/ijs.0.63098-0

[24] P. A. Lawson, H. L. Greetham, G. R. Gibson, C. Giffard, E. Falsen, M. D. Collins, "*Slackia faecicanis* sp. nov., isolated from canine faeces", *International Journal of Systematic and Evolutionary Microbiology*, vol. 55, pp. 1243 - 1246, 2005.
DOI: http://doi.org/10.1099/ijs.0.63531-0

[25] S. Handl, S. E. Dowd, J. F. Garcia-Mazcorro, J. M. Steiner, J. S. Suchodolski, "Massive parallel 16S rRNA gene pyrosequencing reveals highly diverse fecal bacterial and fungal communities in healthy dogs and cats", *FEMS Microbiology Ecology*, vol. 76, no. 2, pp. 301 - 310, 2011.
DOI: http://doi.org/10.1111/j.1574-6941.2011.01058.x

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