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The symbiotic intestinal ciliates and the evolution of their hosts

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Abstract

The evolution of sophisticated differentiations of the gastro-intestinal tract enabled herbivorous mammals to digest dietary cellulose and hemicellulose with the aid of a complex anaerobic microbiota. Distinctive symbiotic ciliates, which are unique to this habitat, are the largest representatives of this microbial community. Analyses of a total of 484 different 18S rRNA genes show that extremely complex, but related ciliate communities can occur in the rumen of cattle, sheep, goats and red deer (301 sequences). The communities in the hindgut of equids (*Equus caballus, Equus quagga*), and elephants (*Elephas maximus, Loxodonta africanus*; 162 sequences), which are clearly distinct from the ruminant ciliate biota, exhibit a much higher diversity than anticipated on the basis of their morphology. All these ciliates from the gastro-intestinal tract constitute a monophyletic group, which consists of two major taxa, i.e. Vestibuliferida and Entodiniomorphida. The ciliates from the evolutionarily older hindgut fermenters exhibit a clustering that is specific for higher taxa of their hosts, as extant species of horse and zebra on the one hand, and Africa and Indian elephant on the other hand, share related ciliates. The evolutionary younger ruminants altogether share the various entodiniomorphs and the vestibuliferids from ruminants.

Keywords: Caecum/colon; Ciliates; Community analysis; Gastro-intestinal tract; Rumen; 18S rDNA

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Introduction

Ruminants constitute a remarkably flourishing taxon of large, herbivorous animals, which encompasses several hundred species and subspecies including important farm animals such as cattle, sheep and goats (Grzimek 1979). Their evolutionary success has been attributed to the fact that they possess a highly specialised foregut differentiation, the rumen (Langer 1988), which allows an efficient fermentative degradation of high fibre plant diets with the aid of an extremely diverse microbiota consisting of archaea, eubacteria, anaerobic fungi and ciliates (Hungate 1966; Kittelmann et al. 2013). Hindgut fermenters, such as horse, zebra, and elephant, host microbiota of similar complexity in their caecum/colon (Janis 1976). However, the hindgut fermenters, which evolved and radiated early in the Eocene to Miocene, appear to have been replaced by the later radiating ruminants in most non-extreme environments in the recent evolutionary past. Today only a few species of horses, donkeys and zebras, two tapir species, a few rhinoceroses and three elephant species contrast with a diversity of more than a hundred of ruminant species with several hundreds of subspecies, which radiated recently from the Pliocene/Pleistocene on (Cifelli 1981; Grzimek 1979; Hassanin and Douzery 2003; Janis 1976; Springer et al. 2003).

Symbiotic ciliated protozoa are found in the fermenting compartments of the gastro-intestinal tract (GIT) of both ruminants and hindgut fermenters, but the ciliates and potentially also their metabolic capacities are rather different (Williams and Coleman 1992). A total of more than 200 morphospecies of rumen ciliates from the various ruminant hosts have been identified (Williams and Coleman 1992), and about 100 species of ciliates from the hindgut of the horse, elephant and rhinoceros have been described morphologically (Adam 1951; Hsiung 1930; Ito et al. 2011; Obanda et al. 2007; Strelkov 1939; Timoshenko and Imai 1995; van Hoven et al. 1988). The number of ciliate morphospecies in ruminants has been reported to be very variable, and most of them have been found in different (ruminant) host species (e.g. Kittelmann and Janssen 2011). However, rumen ciliates do not belong to the characteristic microbiota of hindgut-fermenting hosts and, vice versa, “hindgut” ciliates are rarely found in the rumen (Clarke 1964; Dehority 1986, 1987), potentially arguing for a certain host-specificity at higher taxonomic levels (Cameron and O’Donoghue 2004; Imai 1998; Ito et al. 2011; Obanda et al. 2007; Timoshenko and Imai 1995; van Hoven et al. 1988; Williams and Coleman 1992). This might in part be caused by the manner of infection with ciliates. While ruminants can be infected throughout their lifetime by contact with other ruminants, horses are preferentially infected as young foals by coprophagy (Egan et al. 2010).

Notably, the presence of gut ciliates is not essential for the survival of their hosts. “Defaunated” animals, e.g. ruminants whose ciliate microbiota has been removed experimentally, or those which have been reared in the absence of any ciliate species, perform quite normally under laboratory conditions. Moreover, captivity, domestication, feed and husbandry of the various hosts are known to influence the composition of the ciliate communities in the GIT (Kittelmann and Janssen 2011). Moreover, zebras and some elephants living in zoos appeared to lack intestinal ciliates. Notably, there was in these animals no evidence for a replacement of the “original”, innate ciliate community by ciliates commonly found in domestic horses or ruminants.

However, the most serious limitation for an assessment of the diversity of gut ciliates and, consequently their particular function in the GIT, is the limited number of distinct morphological traits, the enormous morphological plasticity of ciliates (Dehority 1994), and, lastly, the difficulties to culture these ciliates axenically (Williams and Coleman 1992). The only possibility to overcome these problems seems to be a molecular analysis of the various ciliate communities and a comparison of the ciliates identified solely by their 18S rDNA with a collection of “type” ciliates validated at the morphological and molecular level.

Here we report the results of a molecular assessment of the diversity of “gut” ciliates from a broad range of hosts, i.e. from the ruminants cattle, sheep, goats, European red deer (*Cervus elaphus*), to hindgut-fermenters such as horse, zebra and Indian and African elephant. We will show that the intestinal ciliates constitute a monophyletic cluster, and that the very diverse ciliates from ruminants most likely are not host-specific, but shared by the various ruminant hosts. In contrast, we will show that the ciliates from the hindgut fermenters are more diverse than anticipated from morphological studies but rather host-specific in their distribution.

Material and Methods

Sample collection and DNA extraction

Morphologically identified ciliates were obtained from monofaunated sheep (Jouany and Senaud 1979) and in vitro cultures. Alternatively, individual ciliates were isolated from the rumen fluid of sheep or cattle, respectively, with the aid of a micromanipulator and subsequently determined. For the analysis of the ciliate communities in the rumen, samples of rumen fluid from sheep were obtained from the Kielanowski Institute of Animal Physiology and Nutrition at Jablonna (Poland), and the University of Utrecht (The Netherlands), respectively. The samples of rumen fluid from cows were from the University of Utrecht, and the Agricultural University of Wageningen (The Netherlands), respectively. The rumen fluid of a goat was obtained from the central animal laboratory of the Radboud University Nijmegen (The Netherlands), and rumen fluid of a red deer from INRA, Clermont Ferrand/Theix (France). The faeces of an Indian elephant were from Burger’s Zoo, Arnhem (The Netherlands), faeces from horses were collected in Nijmegen (The Netherlands) and Kleve (Germany). Zebra and African elephant faeces were collected in the Serengeti National Park (Tanzania).