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DIVERSITY AND SPECIFICITY IN CESTODES OF THE GENUS *MONIEZIA*: GENETIC EVIDENCE

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Abstract--BA C. T., WANG X. Q., RENAUD F., EUZET L., MARCHAND B. and DE MEEÛS T. 1993. Diversity and specificity in cestodes of the genus *Moniezia:* genetic evidence. *International Journal for Parasitology* **23:** 853-857. Electrophoretic study of two species of *Moniezia cestodes, M. expansa and M. benedeni,* sampled in African (Senegal) domesticated ruminants, revealed a complex of species and a degree of specificity more pronounced than that previously described. The status of the different species is validated by the probable occurrence of within species cross-mating. A European origin is suggested for *M. expansa* due to identical isoenzyme patterns in cestodes from France, whereas some atypical individuals may be derived from wild African ruminants.

INDEX KEY WORDS: Cestodes; *Moniezia;* cattle; sheep; isoenzymes electrophoresis; species diversity; specificity.

INTRODUCTION

Moniezia expansa and M. benedeni are two cestode species found in the small intestine of numerous herbivorous mammals (Schmidt, 1986). They are morphologically characterized with their interproglottidal glands (Spasskii, 1951). Numerous additional species have been described by different authors, none of which is valid (Spasskii, 1951; Troncy, Itard &Morel, 1981).

In this paper, we present a population genetic study, based on isoenzyme electrophoresis, of African (Senegal) *M. expansa* and *M. benedeni,* sampled from sheep, goats and cattle and on European (France) *M. expansa* sampled from sheep. This enabled us to test the genetic homogeneity within the two cestode species, the degree of specificity and the relevance of the interproglottidal gland criterion in species separation. This study revealed a broader diversity of species and a narrower range of host specificity than suspected. The results also suggested the European origin of, at least, *M. expansa.* The recent acquisition of African *Moniezia* from wild herbivores, morphologically indistinguishable from *M. expansa or M. benedeni* is suspected. The study raises doubts concerning the interproglottidal glands as a valid specific criterion.

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MATERIALS AND METHODS

Collection of worms. The parasites studied belong to the genus *Moniezia* (Anoplocephalidae, Cyclophyllidea). Cestodes were collected from the small intestine of cattle (30 animals), sheep (80 animals) and goats (38 animals) from the Dakar (Senegal) slaughterhouse during the summer of 1992). The exact origin of each host is unknown and, thus, the area of sampling is represented by the northern part of Senegal. For *M. expansa,* prevalences were 25 and I I % for sheep and goats, respectively (cattle not infected). For *M. benedeni,* prevalences were 4 and 17% for sheep and cattle, respectively (goats not infected). Parasites were kept alive in physiological solution [0.9% (w/v) NaCt]. After being identified under a binocular microscope, *M. expansa and M. benedeni were* stored in liquid nitrogen. The two parasites species were distinguished on the basis of the interproglottidal glands which are punctiform in *M. expansa* and linear in *M. benedeni* (Spasskii, 1951). Parasites were transported to Montpellier (France) on dry ice. Because the hosts originally came from Europe, other individuals of *M. expansa* were collected from sheep (6 animals) in the slaughterhouse of Pezenas (Languedoc, France) and were compared with African parasites.

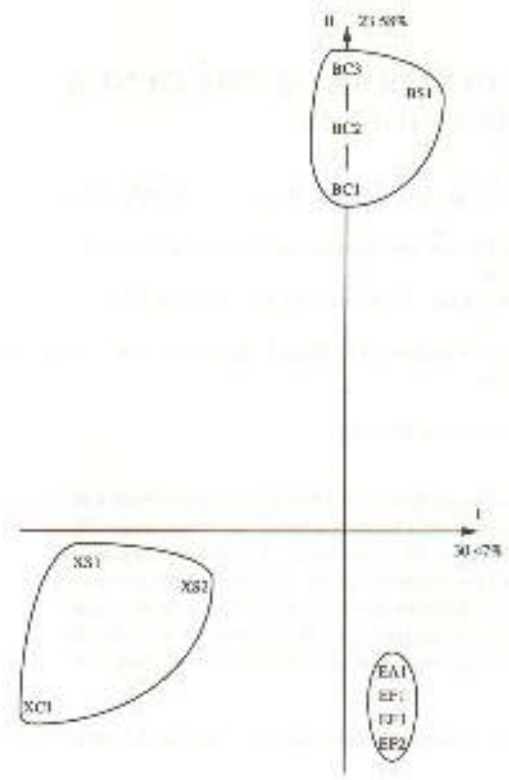

FIG. 1. Genetic variability of different cestodes studied using a factorial correspondence analysis for the first two axes (I and II). EA and EF are *M. expansa* from Senegal and France, respectively (sampled in sheep or goat). BC and BS are M. *benedeni* from cattle and sheep, respectively. XS and XC are atypical *M. expansa* and *M. benedeni* from sheep and cattle, respectively. The percentages of genetic variability explained by each axes are represented in the graph.

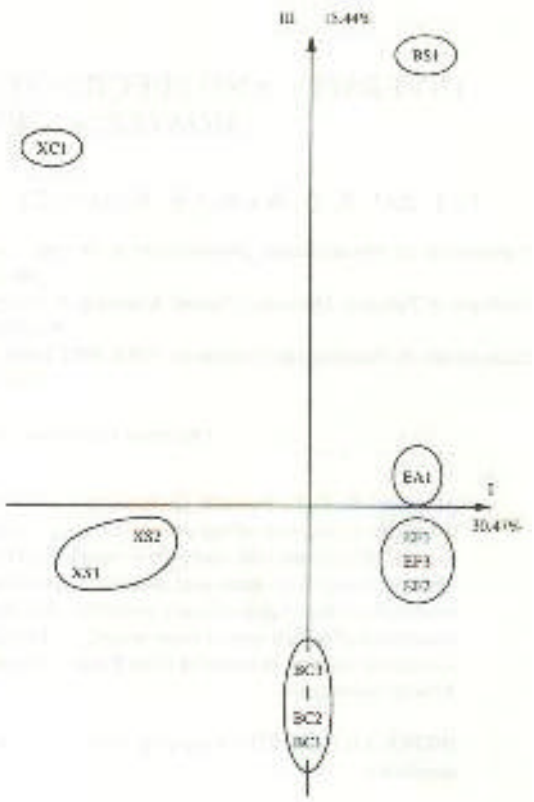

FIG. 2. Genetic variability of different cestodes studied using a factorial correspondence analysis for axes I and 111. EA and EF are *M. expansa* from Senegal and France, respectively (sampled in sheep or goat). BC and BS are *M. benedeni* from cattle and sheep, respectively. XS and XC are atypical *M. expansa* and *M. benedeni* from sheep and cattle, respectively. The percentages of genetic variability explained by each axes are represented in the graph.

Preparation of worms. At Montpellier, the worms were thawed, one portion was fixed in alcoholic Bouin 's fluid, stained with acetic carmine, mounted between slides in Canada balsam (Martoja & Martoja, 1967), and observed under a photonic microscope. This enabled a precise identification of the cestodes. Another portion of each parasite, corresponding to a volume of 0.5 ml was homogenized in an Eppendorf tube filled with an equal volume of distilled water, centrifuged at 10,000 g for I min, and the homogenate used as the enzyme source.

Electrophoresis. Electrophoresis was performed on 10% starch gel as described by Renaud & Gabrion (1988). The enzyme systems studied were as follows: glucose phosphate isomerase (GPI, EC 5..3.19), malate dehydrogenase (MDH, EC 1.1.1.37), mannose phosphate isomerase (MPI, EC 5.3.1.8), phosphoglucomutase (PGM, EC 2.7.5.1), peptidase c (PEP-C, EC 3.4.1 1) and hexokinase (HK, EC 2.7. 1. 1). For each parasite species, the number of worms analysed is given in Table. 1.

Data analysis. Data were analysed by a factorial correspondence analysis (Fca) (Benzécri & Coll, 1973; Lebart, Morineau & Warwick, 1984) adapted for allozyme charac-ters (She, Autem, Kotulas, Pasteur & Bonhomme, 1987). Each individual is characterized for each existing allele by the values 2, 1 or 0 whether it has 2 (homozygotes), 1 (heterozygote) or 0 copies of the considered allele. Individuals were then analysed as active variables using Fca. This method of analysis characterises each sample simultaneously according to all the genetic variables (alleles) and shows the contribution of each allele to the overall variability of the samples.

RESULTS

The different genotypes found in *M. expansa* and *M. benedeni* respectively are shown in Table 1. Six entities can be distinguished. First, *M. expansa* appears very different from *M. benedeni. M. expansa* from France are very similar to those sampled in Africa (little

TABLE 1--GENOTYPE OBSERVED IN *Moniezia expansa*, FOUND IN SHEEP OR GOATS, AND IN *Moniezia benedeni* FOUND IN CATTLE OR SHEEP

	N	*Mpi*	*Mdh*	*Pgm*	*Pep-c*	*Pgi*	*Hk*
EAI	4	2/2	2/2	2/2	5/5	2/2	3/3
EA2	9*	2/2	2/2	2/2	4/5	2/2	3/3
EA3	42*	2/2	2/2	2/2	4/4	2/2	3/3
EFI	1	1/2	2/2	2/2	4/4	2/2	3/3
EF2	1	1/1	2/2	2/2	3/6	2/2	3/3
EF3	6	1/1	2/2	2/2	4/4	2/2	3/3
XS1	2	3/3	3/3	1/1	7/7	1/1	2/2
XS2	1	2/3	2/3	1/1	7/7	1/2	2/2
BCI	2	3/3	1/1	2/2	1/1	2/2	1/1
BC2	2	3/3	1/1	2/3	1/1	2/2	1/1
BC3	4	3/3	1/1	3/3	1/1	2/2	1/1
BSI	2	2/2	1/1	3/3	2/2	2/2	4/4
XC1	1	4/4	3/3	2/2	8/8	1/1	2/2

First letter: E = *expansa*, *B* = *benedeni* and X = unknown. Second letter: A = Africa, F = France, S = sheep and C = cattle. N = Number of individuals.

Alleles numbered according to their anodal mobility.

*One cestode from goat.

TABLE 2--F_{IT} VALUES FOR THE POLYMORPHIC LOCI OBSERVED IN EACH SPECIES

	Loci	N	F_{it}
EA	*Pep-c*	55	0.395
EF	*Mpi, Pep-c*	8	0.18
XS	*Mpi, Mdh,, Pgi*	3	- 0.20
BC	*Pgm*	8	0.53

EA and EF: African and European *M. expansa; XS*: atypical *M. expansa* from sheep; BC: *M. benedeni* from cattle; N: Number of individuals.

geographical variation). All *M. expansa* were found in sheep or goats, never in cattle. Only two cestodes, displaying a *M. expansa* genotype, were found in goats. Two species were found in the group *"M. benedeni"*, one specific to cattle and the other specific to sheep (three diagnostic loci: *Mpi, Pep-c, Hk*, Table 1). Two other groups of *Moniezia* were distinguished (XS1, XS2 on one hand and XC1 on the other hand, Table 1). Three individuals (two XS1, one XS2, Table 1), collected from sheep and identified morph-ologically as *M. expansa*, appeared to be different from this species at three diagnostic loci *(Pgm, Pep-c, Hk*, Table 1). One individual (XCI, Table 1), collected from cattle and identified as *M. benedeni*, could not be included in any of the other species found in this group (five and six diagnostic loci in comparison with *M. benedeni* from cattle and sheep, respectively, Table 1). Even with the small number of loci examined, if the "per cent fixed difference" system of Richardson, Baverstock & Adams (1986) is used, the five different genetic entities revealed differed at 33 % of loci at least, a level that confirms their specific status.

The Fca analysis confirmed these conclusions (Figs. 1 and 2). Figure I shows the projection on the plane defined by the two major axes (i.e. axes I and 11) which represent 54.1% (30.5 + 23.6%) of the overall variability of the samples. It discriminates three groups: the *"expansa "* group (EA, EF), the *"benedeni"* group (BC, BS) and the group containing the atypical individuals (XS, XC). Figure 2 shows the plane formed by axes I and III which represent 45.9% (30.5 + 15.4%) of the overall variability. It discriminates individuals from cattle from those in sheep in the *"benedeni"* and in the atypical groups. In this figure, the African (ES) and European (EF) *M. expansa* are separated slightly by the third axis of the Fca, but this depends only on rare diagnostic alleles in European *M. expansa (i.e. Mpi/1; Pep-c/3; Pep-c/6).*

During this study, some loci appeared polymorphic within the different species. Heterozygote deficits, measured with Wright's (1922) F_{it}, ranged from -0.2 to 0.53 in the different species (Table 2). The only negative value obtained was in a very small sample (the three atypical *Moniezia* from sheep), which, in the presence of three polymorphic loci, was likely to artificially underestimate the F_{it}. When considering

only *Pep-c* in African *M. expansa,* because of a larger sample size (Table 1), a *G* test for goodness of fit gave a significant value (8.1, $P<0.025$) when testing for panmixia. These observations suggest that heterozygote deficits are expected to be high in the species studied.

DISCUSSION

The biochemical discrimination between *M. expansa* and *M. benedeni* has already been performed by Johnson & Hoberg (1989). Moreover, as demonstrated in similar studies, parasite species diversity is often much more complex than previously postulated by morphological taxonomy. This is true for various kinds of organisms: cestodes (e.g. Renaud, Gabrion & Pasteur, 1983; Renaud & Gabrion, 1984; Baverstock, Adams & Beveridge, 1985; Renaud & Gabrion, 1988; de Chambrier, Vaucher & Renaud, 1992), trematodes (e.g. Reversat, Renaud & Maillard, 1989), nematodes (e.g. Nascetti & Bullini, 1982; Andrews, Beveridge, Adams & Baverstock, 1989; Chilton, Beveridge & Andrews, 1992), acanthocephalans (e.g. de Buron, Renaud & Euzet, 1986) or caligid copepods (Zeddam, Berrebi, Renaud, Raibaut & Gabrion, 1988).

M. expansa seems specific to sheep (never found in cattle) where it dominates (in number) the other species of the genus. It is rare in goats. This species seems to have originated in Europe where the *M. expansa* sampled displayed a similar isoenzyme pattern. The two species observed in the *M. benedeni* group proved to be host specific. One species was found only in cattle and the other only in sheep. Two additional species could be characterized. One species, in sheep, was morphologically related to *M. expansa.* Another individual, in cattle, was identified morph-ologically as *M. benedeni.* The genetic differentiation of these two atypical parasite species, in comparison with the other taxa studied and their rarity within our sample could suggest an external origin. This could represent a colonization of domestic cattle with wild animal cestodes. However, this point will remain speculative, unless additional data on cestodes from wild African ruminants are obtained.

Nevertheless the lack of morphological differentiation between these atypical species and M. *expansa and M. benedeni* raises doubts about the relevance of the interproglottidal glands for species determination within the *Moniezia genus.*

The geographical area of sampling was large (i.e. northern Senegal). This may explain the heterozygote deficits observed. Nevertheless, frequent migration of domesticated cattle could prevent such structuring. It seems that the heterozygote deficits observed are more likely to arise from selling (suspected to be frequent in cestodes; Euzet & Combes, 1980). However, cross-mating must be frequent in order to explain the heterozygosity observed.

In this paper species diversity in the *Moniezia genus* appears higher than that admitted by Spaaskii, 1951 and Troncy *et al.,* 1981. For certain organisms, in particular cestodes, selling may make species characterization more difficult (Lymbery, 1992). Here, the presence of numerous heterozygotes (outcrossing) and the high levels of genetic differentiation strongly validate the five species characterized even for those represented by few individuals.

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