Onderstepoort J. vet Res., 56, 235–238 (1989)

HYBRIDIZATION MODEL FOR *RHIPICEPHALUS APPENDICULATUS* AND *R. ZAMBEZIENSIS* BY GLUCOSE-P-ISOMERASE ISOENZYMES

G. WOUTERS, Institute of Tropical Medicine, Nationalestraat 155, B - 2000 Antwerp, Belgium

ABSTRACT

WOUTERS, G., 1989. Hybridization model for *Rhipicephalus appendiculatus* and *R. zambeziensis* by glucose-P-isomerase isoenzymes. *Onderstepoort Journal of Veterinary Research*, 56, 235–238 (1989)

Hybrids between *Rhipicephalus appendiculatus* and *R. zambeziensis* were reared and glucose-phosphate-isomerase isoenzymes were resolved by agarose electrophoresis. By phenotyping hybrids in F_1 and F_2 generations autosomal transmission of 2 GPI genes was demonstrated. Identification of a hybrid phenotype provides a method for identifying hybrids in field collections.

INTRODUCTION

Rhipicephalus appendiculatus, the vector of East Coast fever *(Theileria parva parva)* occurs in southern Africa. *R. zambeziensis,* which was recently described by Walker, Norwal & Corwin, 1981, is sympatric with *R. appendiculatus* in some areas, mostly between 500 m and 700 m altitude. *R. zambeziensis* was shown to transmit *Theileria parva parva* in cattle experimentally from nymph to adult (Lawrence, Norval & Uilenberg, 1983). In areas where only one of these 2 species occurs it can be identified by enzyme-electrophoresis; a different pattern for each species for glucosephosphate-isomerase (GPI) (EC.5.3.1.9) isoenzymes on polyacrylamide gels was described by Wouters, Brandt & Berkvens (1987). Under laboratory conditions both fertile and sterile hybrids of these 2 species have been reared (Zivkovic, Pegram, Jongejan & Mwase, 1986). Using morphological criteria alone it

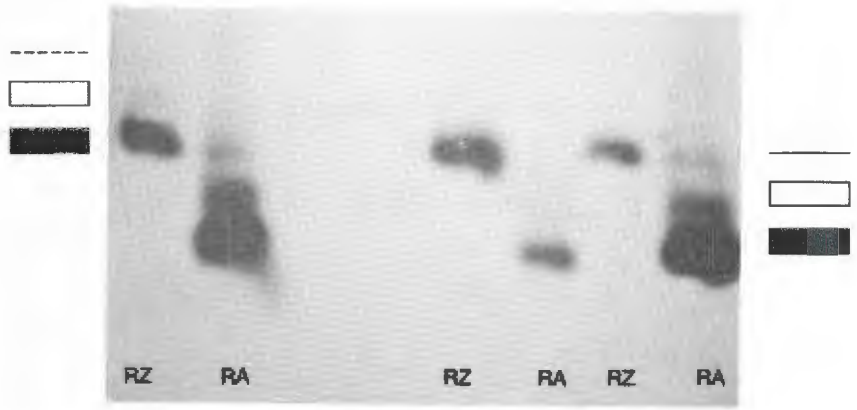

FIG. 1 GPI-phenotypes of the two species *R. appendiculatus* (RA) and *R. zambeziensis* (RZ)

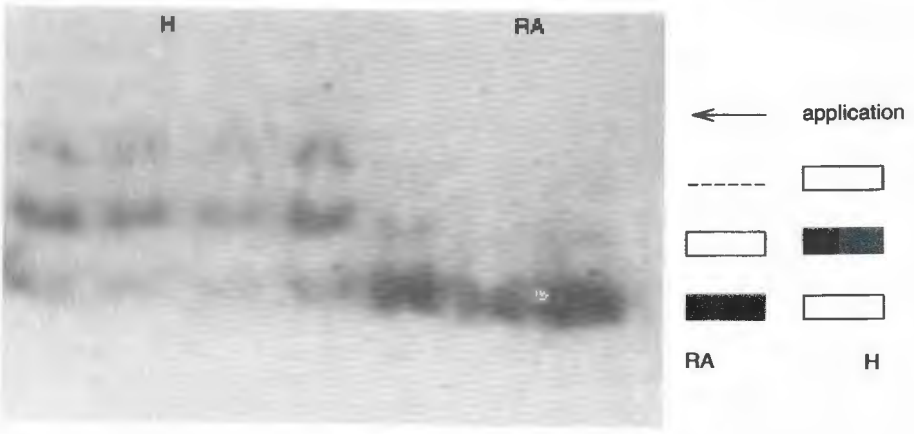

FIG. 2 Hybrid GPI-phenotype (H) between *R. appendiculatus* and *R. zambeziensis* compared to the phenotype RA.

Received 29 June 1989—Editor

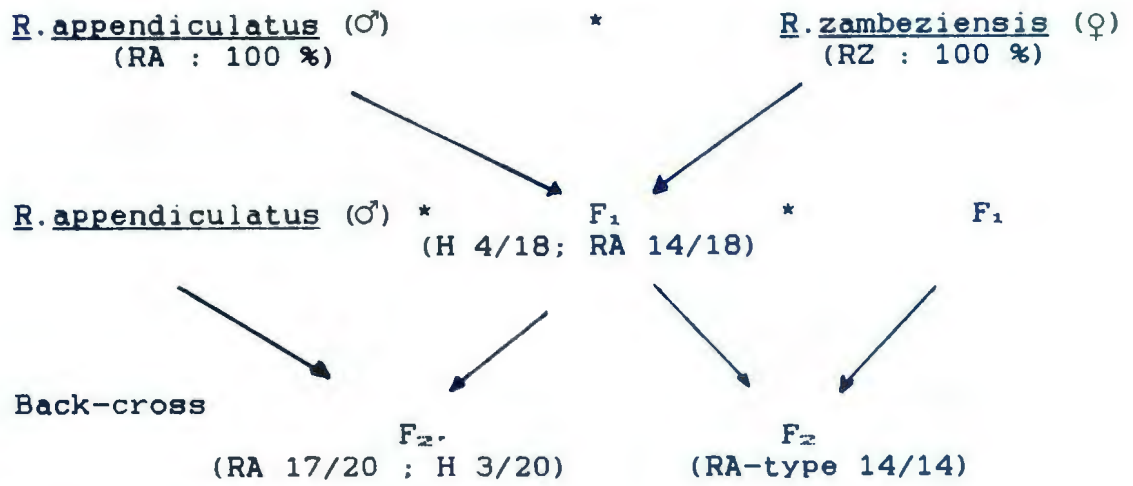

FIG. 3 Pedigree of cross-breeding experiments

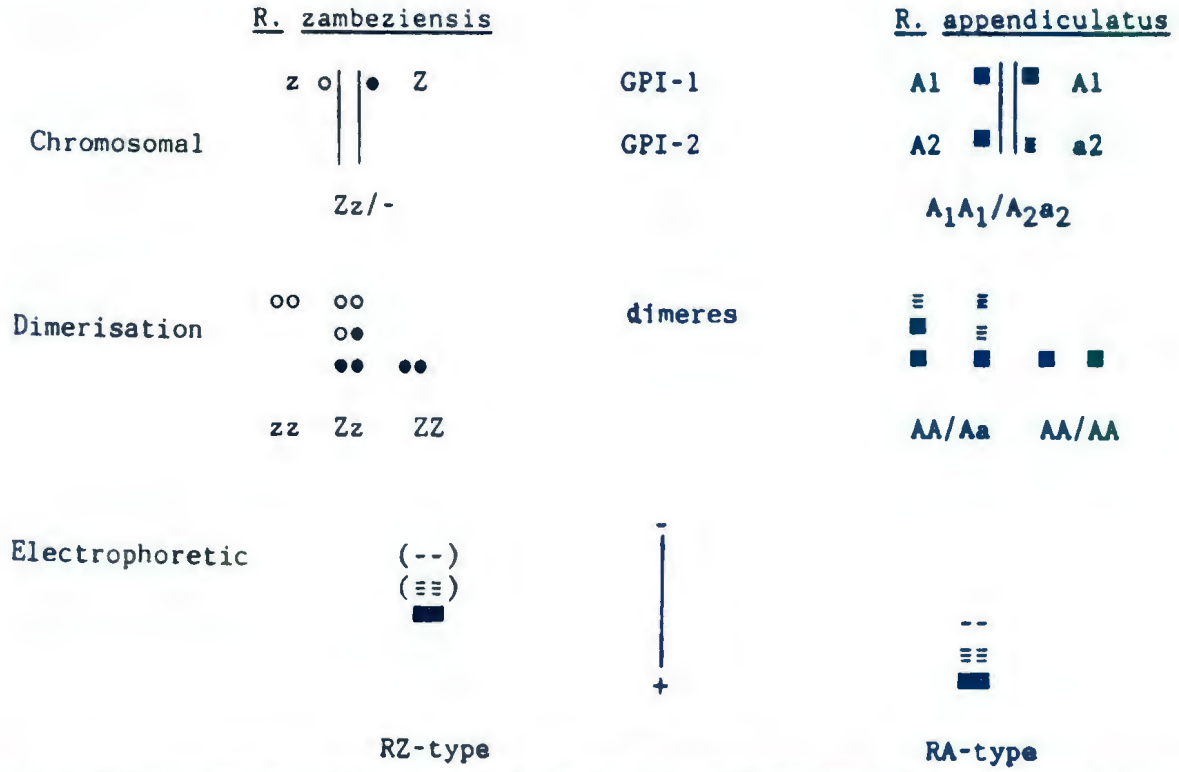

FIG. 4 Molecular explanation of the GPI zymograms of *R. appendiculatus* and *R. zambeziensis* (above: chromosomal presentation, middle: dimerisation; below: electrophoresis pattern)

has been impossible to identify hybrids in field collections with certainty. Nevertheless, in some collections from the Eastern Province of Zambia morphologically intermediate forms have been seen that are suspected to be hybrids between *R. appendiculatus* and *R. zambeziensis*. Such hybridization in the field could have considerable biological and epidemiological implications. In this study, an attempt has been made to find a method of identifying hybrids in field-collected specimens.

MATERIALS AND METHODS

R. appendiculatus females collected in the Eastern Province of Zambia were morphologically identified and then reared in the laboratory. Moulting and oviposition took place at 27 °C, R.H. 85 %. Larvae and nymphae were fed on rabbits at 20 °C. The resulting adults were used in this study. Enzyme-electrophoresis of these adults was done to confirm the species identification.

The *R.zambeziensis* ticks used came from 2 laboratory strains, one originating from ticks collected from cattle in West Nicholson, Zambabwe, in 1976 and the Kilkenny strain from South Africa, received from Dr J. B. Walker in 1986. These strains were maintained in the laboratory in the same way as *R. appendiculatus*.

A number of cross-breeding experiments were performed with these ticks. Each time equal numbers of adults (5–10) of *R. appendiculatus* males and *R. zambeziensis* females were simultaneously placed on a rabbit's ear. The reciprocal cross was done on a

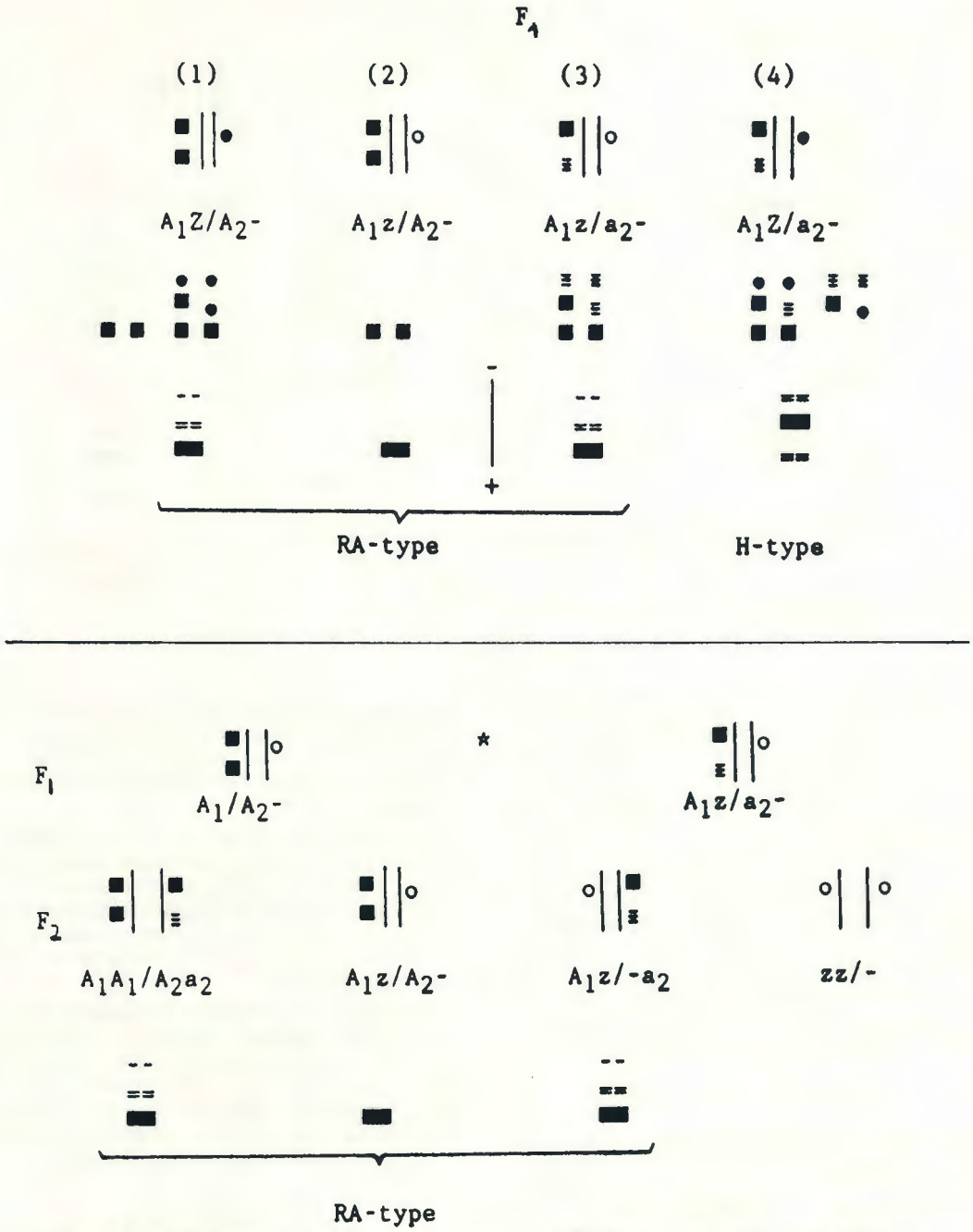

FIG. 5 Segregation in the F_1 and F_2 generations of the *R. appendoculatus* * *R. zambeziensis* cross with a molecular explanation of zymograms.

separate rabbit. Engorged females were placed in glass tubes at 27 °C. After a month the larvae were put on a new rabbit, and after feeding and moulting the resulting nymphae were also fed. Some of the F_1 adult hybrids were used for enzyme-electrophoresis and some were crossed to obtain F_2 adults. A back-cross was also made by mating *R. appendiculatus* males with F_1 hybrid females. The offspring of all these cross-breeding experiments were investigated for their GPI-isoenzymes. The zymograms of their GPI-patterns were compared and a heredity model for the isoenzymes is presented.

Preparation of the ticks was done individually as described by Wouters *et al.* (1987). Briefly, ticks were homogenized in an enzyme-stabilizer and after ultracentrifugation, 3 $\mu\ell$ of the cytosol fraction was applied to an agarose-gel (0,8 %) (Sigma A-6013). The gel buffer is a 1:20 dilution of the bridge-buffer (Tris 0,9 M; Boric acid 0,5 M; EDTA 0,02 M), pH 8,6. After 20 min at 4 °C slices were cut in the gel for application. Agarose-gel electrophoresis was carried out at 275 V for 60 min. After electrophoresis the gel was stained for glucose-phosphate-isomerase (Wouters *et al.*, 1987) and incubated at 38 °C for 30 min.

RESULTS

Only the cross between *R. appendiculatus* males and *R. zambeziensis* females produced viable larvae. The parent *R. appendiculatus* and *R. zambeziensis* each showed a distinct pattern, RA and RZ respectively, with 3 equidistant bands, with a decreasing intensity towards the cathode (Fig. 1). The strongest band of the RZ-pattern had the same mobility as the

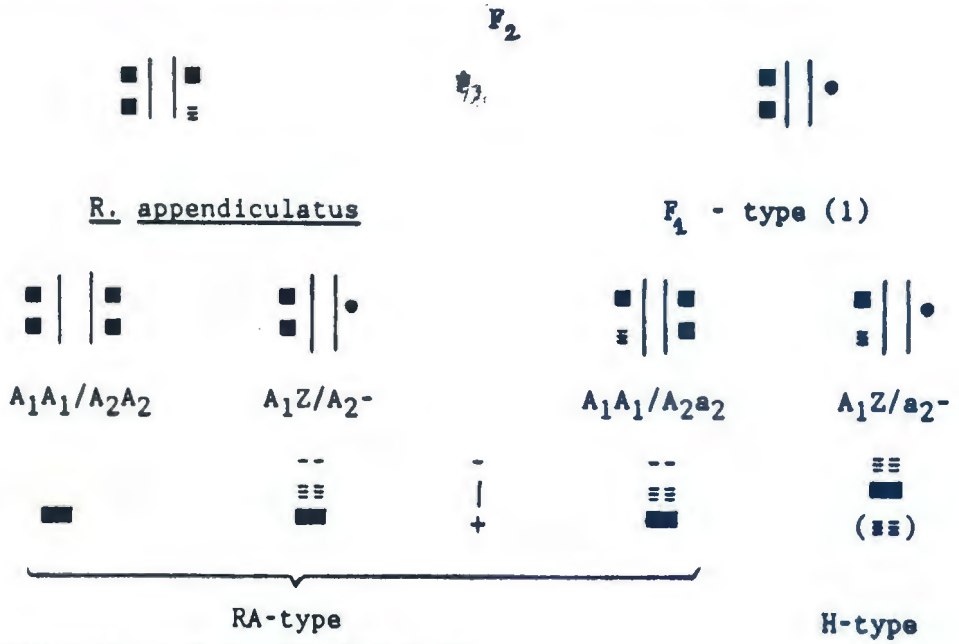

FIG. 6 Back-cross between *R. appendiculatus* (♂) and an F-type (1)

most cathodal band of the RA-pattern. Four of the 18 F_1 adults show an intermediate pattern (H) (Fig. 2). These 3 GPI phenotypes (RA, RZ, H) are the only patterns found in the F_1, F_2 and in the back-cross. Fig. 3 gives a schematic representation of the different cross-experiments and the observed frequencies of the GPI-phenotypes found in the progeny. Because of the dimeric structure of GPI a model is given in Figs 4, 5 and 6, assuming 2 different non-allelic duplicated autosomal genes GPI-1 and GPI-2 in *R. appendiculatus*. The 2 pairs of genes (A_1A_1 and A_2C_2) each realise the RA-phenotype, the result of a dimerisation of the polypeptide chains A and a. *R. zambeziensis* has one GPI locus with 2 alleles (Z and z) with codominance between them.

DISCUSSION

Species identification of *R. appendiculatus* and *R. zambeziensis* on agarose-gels confirmed the results obtained in previous studies by Wouters *et al.* (1987). The model gives an explanation of the patterns on the zymogram in terms of genes and genotypes. It therefore has advantages over identification based on morphological features, which may be variable or difficult to see. Even with GPI-isoenzymes, though, identification of hybrids is difficult because only about one quarter of them have a typical H-pattern. Nevertheless, our model strongly suggests that if the H-type is found in the field it must result from hybridization in the past.

The intensity of the bands could be explained by a different affinity between the polypeptide chains A, a, Z and z. The dimerisation process between 2 subunits in the cell is a stochastic phenomenon depending on the concentrations and affinities between subunits. Not all these dimers differ in their migration patterns or are even formed. In the F_1 generation and in our F_2 (parents are F_1-ticks with unkown phenotype) we could not find the RZ-type of *R. zambeziensis*. Moreover hybrids, which are intermediate between the two species in appearance and may be found in areas where these 2 species are sympatric, can express the RA-phenotype. So in this area, identification by GPI-isoenzymes is unclear because of species interference or the introgression of genes.

Interbreeding between related species is very common in a microevolutionary context and could have important consequences. Lewontin & Birch (1966) has shown that the hybridization between the flies *Dacus tryoni* and *D. neohumeralis* led to a superior hybrid population able to survive at extremely high temperatures.

In the Eastern Province of Zambia there are indications that hybrids between *R. appendiculatus* and *R. zambeziensis* occur in the dry season (May–June) and in the beginning of the rainy season (November–December). Because of the epidemiological implications of adaptation of such a subpopulation it is important to be able to elucidate this.

REFERENCES

LAWRENCE, J. A., NORVAL, R. A. I. & UILENBERG, G., 1983. *Rhipicephalus zambeziensis* as a vector of bovine theileriae. *Tropical Animal Health and Production*, 15, 39–42.

LEWONTIN, R. C. & BIRCH, L. C., 1966. Hybridization as a source of variation for adaptation to new environments. *Evolution*, 20, 315–336.

WALKER, JANE, B., NORVAL, R. A. I. & CORWIN, M. D., 1981. *Rhipicephalus zambeziensis* sp. nov., A new tick from eastern and southern Africa, together with a redescription of *Rhipicephalus appendiculatus*, Neumann, 1901 (Acarina, Ixodidae). *Onderstepoort Journal of Veterinary Research*, 48, 87–104.

WOUTERS, G., BRANDT, J. & BERKVENS, D., 1987. Species characterisation of *Rhipicephalus appendiculatus* and *Rhipicephalus zambeziensis* (Ixodidae: Acarina) by enzyme-electrophoresis. *Annales de la Société Belge de Médecine Tropicale*, 67, 267–270.

ZIVKOVIC, D., PEGRAM, R. G., JONGEJAN, F. & MWASE, E. T., 1986. Biology of *Rhipicephalus appendiculatus* and *R. zambeziensis* and production of a fertile hybrid under laboratory conditions. *Experimental and Applied Acarology*, 2, 285–298.

Printed by the Government Printer, Private Bag X85, Pretoria, 0001