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A hybridization model for *Rhipicephalus appendiculatus* and *R. zambeziensis* (Acarina: Ixodidae) using glucose-6-phosphate-isomerase isoenzymes

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ABSTRACT

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In the Eastern Province of Zambia, *Rhipicephalus appendiculatus* (Neumann) and *R. zambeziensis* (Walker) are sympatric. Intermediate forms as well as typical specimens are found. No morphological criteria could detect cross-breeding between these species in the field.

Hybrids between *R. appendiculatus* and *R. zambeziensis* were produced and glucose-phosphate-isomerase (EC 5.3.1.9.; GPI) isoenzymes resolved by agarose electrophoresis. Phenotyping hybrids in the F1 and F2 generations was explained by the autosomal transmission of two loci of GPI-genes. Identification of some hybrid phenotypes offers the possibility of showing presently undetected hybridization in the field. A genetic model is proposed to explain the patterns.

INTRODUCTION

Rhipicephalus appendiculatus (Neumann, 1901), the vector of East Coast Fever (*Theileria parva parva*) occurs in southern Africa. *Rhipicephalus zambeziensis*, recently described by Walker et al. (1981), is in some areas sympatric with *R. appendiculatus*, mostly between 500 m and 700 m above sea level. *Rhipicephalus zambeziensis* was shown experimentally to transmit *T. parva parva* in cattle from nymph to adult (Lawrence et al., 1983). In areas where only one of the two species occurs, it can be identified by enzyme electrophoresis; a different pattern for glucose-phosphate-isomerase (EC 5.3.1.9.) isoenzymes on polyacrylamide gels has been described (Wouters et al., 1987). It seems that, under laboratory conditions, hybridization of the two species can occur (Zivkovic et al., 1986) yielding a fertile or a sterile hybrid.

In the Eastern Province of Zambia, there are indications that hybrids be-

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tween *R. appendiculatus* and *R. zambeziensis* occur in the dry season (May–June) and in the beginning of the rainy season (November–December). The epidemiological implications of such a subpopulation, adapted to the dryer and colder environments, could be very large. Young animals born at the end of the rainy season will die of the sickness because of a fatal reinfestation of the tick. If hybrids do not exist, there is a long period of low tick-infestation, and so animals could become seroconverted without sickness.

However, using taxonomic criteria, we cannot find definite hybrids in the field. Nevertheless, in some collections in the Eastern Province of Zambia, intermediate forms are suspected as a result of hybridization between *R. appendiculatus* and *R. zambeziensis*. Such hybridization in the field could have considerable biological and epidemiological implications. In this study we try to find a method to identify such hybrids in field-collected specimens.

MATERIALS AND METHODS

Rhipicephalus appendiculatus females collected from the Eastern Province of Zambia were reared in the laboratory after morphological identification. Larvae and nymphae originating from one female were reared in a group. Moulting and oviposition happened at 27°C (RH 85%). Larvae and nymphae were fed separately rabbits at 20°C. Resulting virgin adults 6–9 months old were used in this study. Enzyme electrophoresis of these unfed adults was done to ascertain the species identification.

Rhipicephalus zambeziensis used here were laboratory strains originating from cattle in West Nicholson, Zimbabwe (1976) and Killkenny, South Africa, received from Walker in 1986. These laboratory strains were maintained in the laboratory in the same way as *R. appendiculatus*.

Hybridization trials were undertaken by cross-breeding experiments. At each time equal numbers of adults (5–10) of *R. appendiculatus* (♂) and *R. zambeziensis* (♀) were simultaneously applied to a rabbit ear. The reciprocal cross was done on a separate rabbit. Engorged females were collected in glass tubes at 27°C in order to oviposit. After one month, the resultant larvae were put on a new rabbit and, after moulting, the resulting nymphae were fed. Some F1 adult hybrids were used for enzyme electrophoresis and some crossed to obtain F2 adults. Finally, one back-cross was made by mating *R. appendiculatus* (♂) adults with F1 hybrid females. The offspring obtained in all these cross-breeding experiments were investigated for their GPI isoenzymes. The zymograms of their GPI patterns were compared, and a genetic model for the isoenzymes is presented in this paper.

Preparation of the ticks was done individually as described in a previous study (Wouters et al., 1987). To recap briefly, ticks were homogenated in an enzyme stabiliser and, after ultracentrifugation, 3 µl of the cytosol fraction were applied to an agarose-gel (0.8%) (Sigma A-6013). The gel buffer was a

dilution (1:20) with distilled water 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, wells were cut in the gel for sample application. Agarose-gel electrophoresis was carried out at room temperature and 275V 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. Blood samples of the rabbit were run simultaneously to eliminate any endogenous rabbit enzyme.

RESULTS

Species identification of *R. appendiculatus* and *R. zambeziensis* was confirmed on the agarose-gels when compared to previous studies.

No difference was seen in the patterns between males and females of both species.

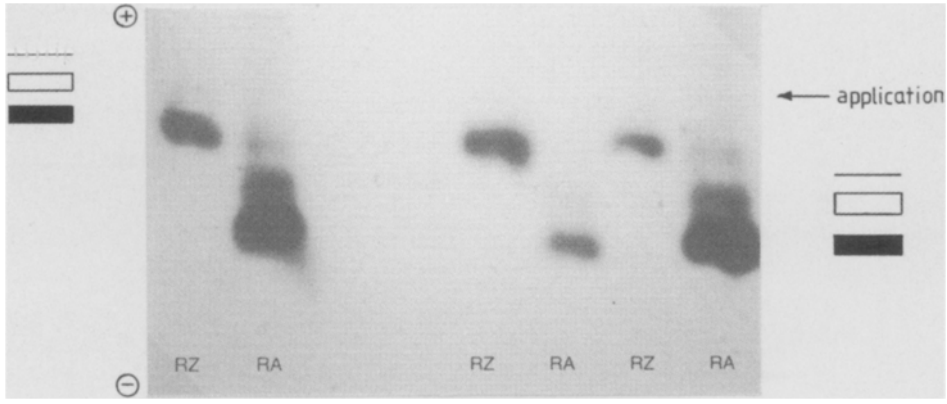


Fig. 1. GPI phenotypes of the two species *R. appendiculatus* (RA) and *R. zambeziensis* (RZ).

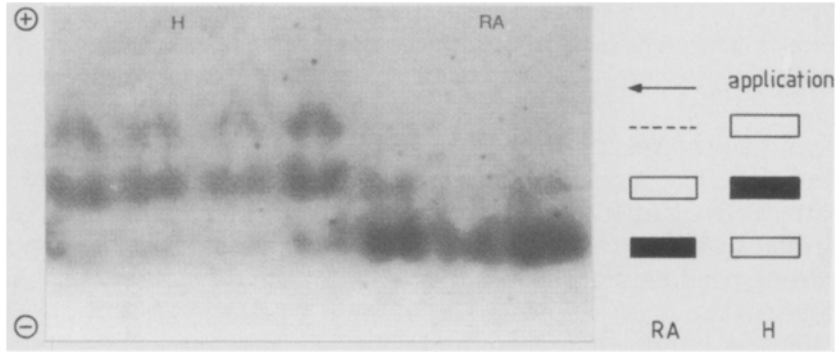
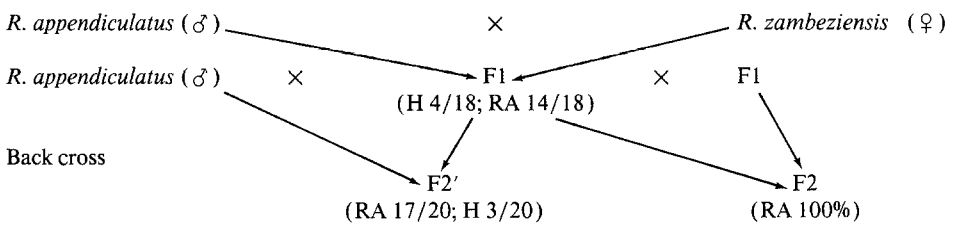


Fig. 2. Hybrid GPI phenotype (H) between *R. appendiculatus* and *R. zambeziensis* compared to the phenotype RA.

TABLE 1

Pedigree of cross-breeding experiments



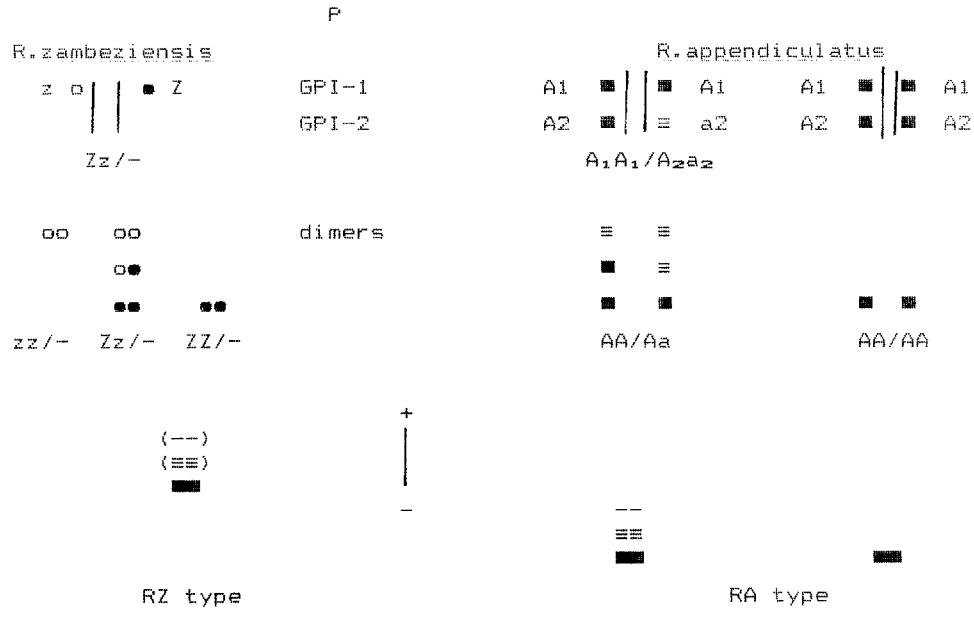


Fig. 3. Explanation of the GPI zymograms of *R. appendiculatus* (♂) and *R. zambeziensis* (♀) (above: chromosomal presentation; middle; dimerisation; below; electrophoresis pattern).

Only the cross between *R. appendiculatus* (♂) and *R. zambeziensis* (♀) gave viable larvae. The parent *R. appendiculatus* and *R. zambeziensis* show a distinct pattern, RA and RZ respectively, with three equidistant bands, of decreasing intensity to the cathode (Fig. 1). A more unusual RA pattern with only one strong band exists. The strongest band of the RZ pattern had the same mobility as the most cathodal band of the RA pattern. Four of the 18 F1 adults show an intermediate pattern (H, Fig 2). These three GPI phenotypes (RA, RZ, H) are the only patterns found in the F1, F2 and back-cross. In generation F1 and in our F2 (parents are F1 ticks with unknown pheno-

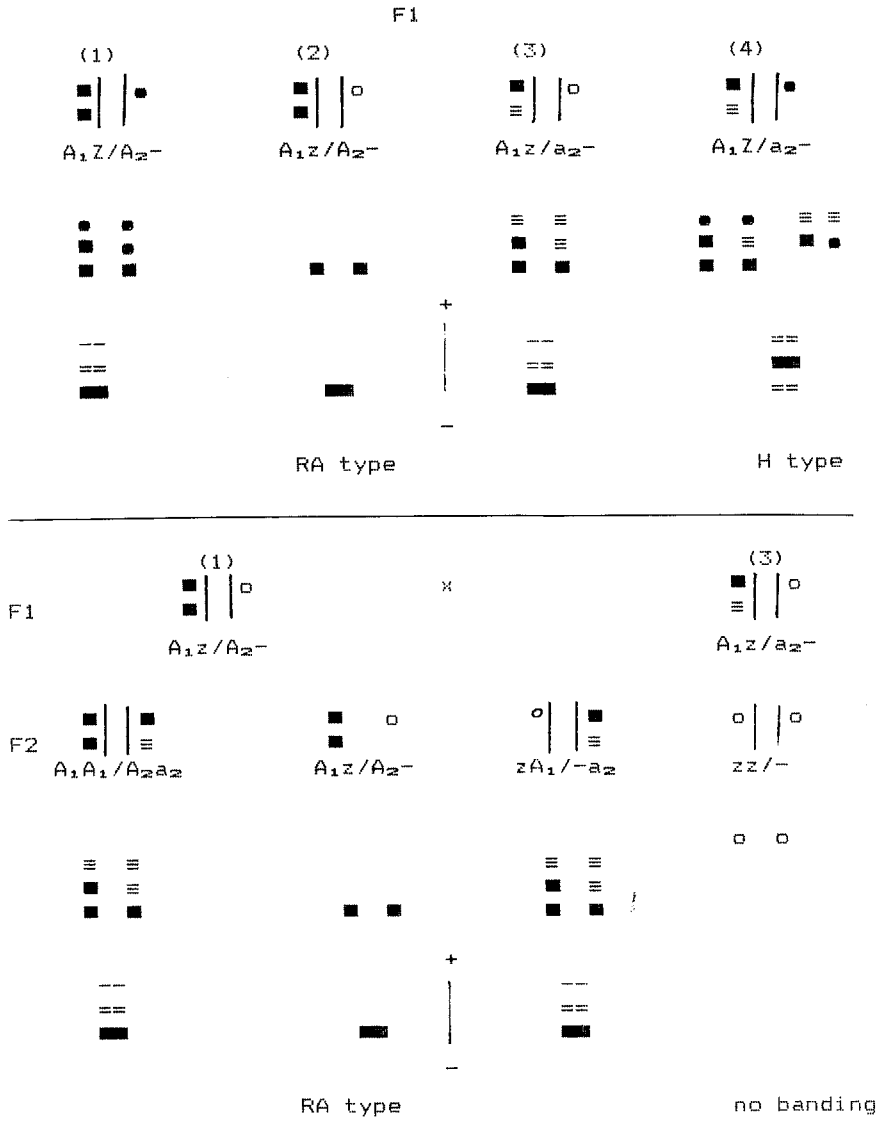


Fig. 4. Segregation in the F1 and an example (1 v 3) of an F2 generation of the *R. appendiculatus* × *R. zambeziensis* cross with a molecular explanation of the zymograms.

type) we could not find the RZ type of *R. zambeziensis*. Table 1 gives a schematic representation of the different crossing experiments and the observed frequencies of GPI phenotypes found in the progeny.

Because of the crossing results and the dimeric structure of GPI (Dixon et

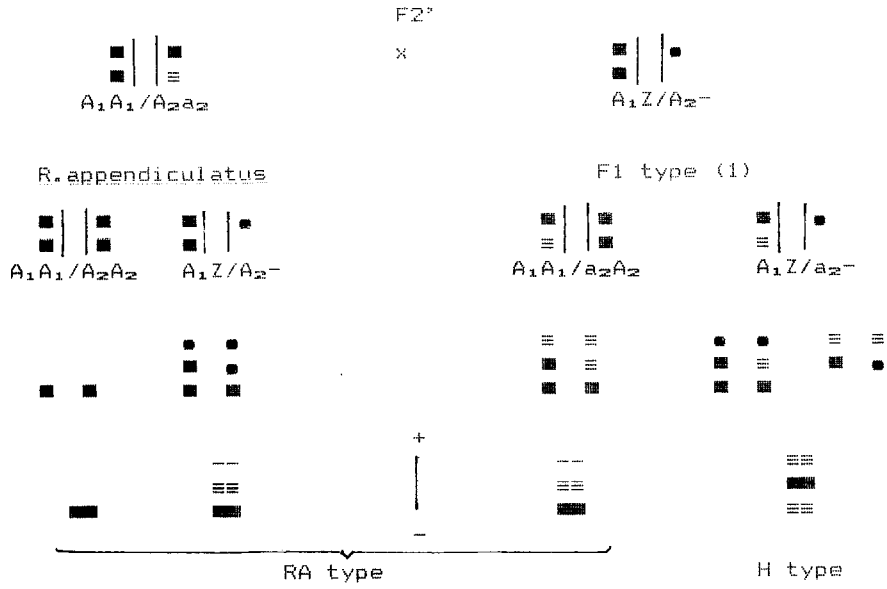


Fig. 5. Example of a back-cross between *R. appendiculatus* (♂) and an F1 type (♀) with a molecular explanation of the zymograms.

al., 1979), a genetic model is proposed in Figs. 3, 4 and 5. This assumes two different non-allelic duplicated autosomal loci GPI-1 and GPI-2 in *R. appendiculatus*. *Rhipicephalus zambeziensis* has one GPI locus with two alleles (Z and z) with a dominance relationship between them. It seems that the Z allele is more translated than the z allele.

DISCUSSION

The alleles Z, z and A_1, are all multiple alleles at the GPI-1 locus. These genes produce polypeptide chains, of which two assemble randomly. The dimers Az are not formed while AZ and Zz have a different mobility. The GPI-2 locus has two alleles A_2 and a_2. As illustrated in Fig. 3, the gene products of A_2 A_1 are the same (polypeptide chain A).

The differences in intensity of the bands could be explained by a different affinity between the polypeptide chains A, a, Z and z. The dimerisation process between two subunits in the cell is a stochastic phenomenon depending on concentrations and the affinity between the subunits. Not all these dimers differ in their migration pattern or even are formed. The electrophoretic mobility of the aa dimer is equal to the mobility of the ZZ dimer. Aa has an intermediate mobility and this is the same for the AZ dimers. The dimers Za

and za are not formed while Zz and zz dimers are very feeble or almost undetectable. Dimers of Az are not realized, i.e. z is recessive to A (no o chain is formed) or affinities are not appropriate.

Rhipicephalus zambeziensis lacks locus GPI-2. Each gene produces a distinct chain, thus *R. zambeziensis* ticks have one of the three genotypes (ZZ, Zz, zz). Each of these dimers has a different phenotypic electrophoretic mobility; only the ZZ dimer is clearly visible on the gels.

Rhipicephalus appendiculatus has an extra duplicated locus (GPI-2) over and above locus GPI-1. Thus, four distinct chains in theory are possible; however, in all *R. appendiculatus* the first locus (shared with z and Z) is always allele A at both genes, whilst locus GPI-2 always has one gene of A and one of a. It is assumed that the A gene in the GPI-2 locus is the same as that in the GPI-1 locus, but the a gene is a variant. All the A genes produce the same chain ▒ whilst the a gene produces chain ≡. This all occurs irrespective of the complement of genes; a is not recessive to A at locus GPI-2. Another assumption made is that the lack of a paired gene in $A_2/-$ or $a_2/-$ does not affect the expression of A_2 or a_2. The two GPI-loci (A_1A_1 and A_2A_2) produce chains A and a which dimerise at random to yield ▒ ▒, ▒ ≡ and ≡ ≡, the lower, middle and upper detectable bands for the RA phenotype respectively.

This model gives an explanation of the patterns on the zymograms in terms of loci, genes and genotypes. It therefore has advantages over identification based on morphological features, which may vary or be difficult to see. However, identification of hybrids even with GPI isoenzymes is difficult because only about one-quarter of them have the typical H pattern. Nevertheless, if the assumptions of the model are true, it strongly suggests that if the H-type is found in the field it must be a result of hybridization in the past. Moreover, these results show that hybrids between the two species, which we may find in sympatric areas of the two species as intermediate forms, can express the RA-phenotype. So in such cases identification by GPI isoenzymes is unclear. Only if the RZ pattern is obtained can we unequivocally state the tick's identity.

Interbreeding of related species is very common, and in a microevolutionary context could have important consequences. Lewontin and Birch (1966) have shown that hybridization between *Dacus tryoni* and *D. neohumeralis* in Australia exists and has led to a superior hybrid population. The extreme high temperature of the south has made natural selection and competition between the two species. *Dacus tryoni* is less sensitive to high temperature than *D. neohumeralis*, but the hybrids have a higher population growth rate and have migrated southwards. The climatological conditions have changed the species spectrum.

Further cross experiments are set up between individuals to check the assumptions above and to show, in particular, if it is possible to produce an RZ pattern in the F2. Until now, no RZ phenotype was found in other F2 crosses.

To confirm the genetic hypothesis, protein isolation and fractionation in vitro is needed to separate different polypeptide chains.

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