GENETICS OF RESISTANCE TO DISEASE IN AN ECOLOGICAL CONTEXT

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SUMMARY

In developing countries vector transmitted diseases like trypanosomosis, theileriosis, and malaria create serious health problems in man and livestock. Control strategies for the vectors and the parasites have not yet lead to adequate results. Genetics may have a role to play in the vector-parasite relationship. So far, most genetic and immunological studies have concentrated on mosquito vector competence. Approximately 2 to 3 quantitative trait loci are responsible for this trait in different species of mosquito, and with different parasites. However, mosquito vector competence is not the most important factor in transmission, where biting frequency and mosquito longevity play a significant role. Even when these traits are genetically determined, the integration with existing control measures like impregnated bed-nets and with other mosquito transmitted diseases like dengue fever and filariasis have to be resolved.

Mosquito genetics can be a useful model to study genetics in tick-borne diseases and trypanosomosis. A most useful lesson is, that through epidemiology important traits of tranmission be determined first. The successful introduction of genetically selected populations, or possibly transgenic vectors requires even more research regarding the biology, the environment, the ethics and the societal concerns. As a model for research selection lines and/or transgenics have proven to be valuable tools.

Vector transmitted diseases have a tendency to occur both in humans and livestock in the same environment. Integrated control methods therefore should be aimed at both humans and livestock to improve their health simultaneously.

INTRODUCTION

In Africa infectious and parasitic diseases of livestock and wildlife are responsible for severe losses through epidemics and through reduced production and performance. Arthropods play an important role in major disease problems in the developing world like trypanosomiasis, tick-borne diseases and malaria in humans. The parasites transmitted by vectors like tsetse flies, ticks and mosquitoes cause frequent and serious human and livestock diseases such as sleeping disease in humans, nagana in cattle, surra in camels, babesiosis, anaplasmosis, heartwater, theileriosis and malaria. The diseases can have an acute and/or chronic form and frequently lead to mortality.

Strategies aimed for vector eradication have been developed in the past, but most have not succeeded. The new concept for vector control is control of the disease to an acceptable level. Control can be aimed at resistance at animal/human level, the vector and the parasite

itself. Unfortunately there appear to be few integrated strategies, because researchers and their host institutions tend to support a single perspective instead of a holistic approach.

Control of these diseases requires an integrated approach, bringing together parasitology, entomology and ecology, economics, organisation of veterinary and health services, social and cultural structures and environmental issues.

In this paper I will concentrate on the genetics of the vector-parasite relationship. Genetic tools are now available to study the resistance to parasites at the genome and gene level in livestock and in the vectors. For trypanosomiasis recent reports about genetic resistance in livestock are available. However, there are also new developments in vector control, albeit far away, that may complement the strategy for genetic resistance at the animal level. Finally, also molecular genetic analysis of the parasite itself is leading to new approaches for controlling the disease. The research results in malaria vector genetics outnumber by far the publications in tsetse and tick genetics. Therefore malaria will be given priority in this publication.

Advances in mosquito genetics for control of malaria. Malaria is the most serious disease in the world. The mosquito hosts numerous diseases like malaria, lymphatic filariasis, yellow and dengue fever. Control of the mosquito host through insecticide application has been tried on a large scale, but has led to pesticide resistant vector populations. The same is happening with chemotherapy leading to resistant parasites. Vaccine research has not yet produced the solution. Careful use of prophylactics, combined with impregnated bed-nets and repellents is the only way for personal anti-malaria protection at the moment.

Mosquitoes differ widely in their susceptibility or refractoriness to a given species of malaria parasite. The genetic basis of the host-parasite relationship has been known since 1929 (Kilama and Craig 1969). Selection of mosquito populations for susceptibility or refractoriness and crossing to an F2 or backcross population revealed that a limited number of genes, behaving according to Mendelian laws determine vector competence. For example, Cooper *et al* (1994), detected high refractoriness to *Plasmodium falciparum* in an *Anopheles farauti* strain. *A. farauti* is native to Australia and can therefore be used in vaccine trials in Australia.

Mosquito genetics has advanced like plant, animal and human genetics with the application of molecular biology. Molecular markers such as restriction fragment length polymorphisms (RFLP) and microsatellite sequences or simple sequence repeats (SSR) markers have been developed. Mosquitoes contain small amounts of genomic DNA. However, by regenerating Southern blots up to 24 rounds, many RFLP loci can be examined for each mosquito. An RFLP marker linkage map has been developed for the yellow fever mosquito *Aedes aegypti*. The map covers 153 cM, consisting of 81 RFLP loci. The RFLP markers are partially integrated into the classical genetic linkage map based on mutant and allozyme markers (Severson 1994 and Severson *et al* 1993). A physical map of *A. aegypti*, based on labelled recombinant cosmids, is being developed by Brown *et al* (1995).

Microsatellites are attrative as less than 10 ng of genomic DNA template is required for a PCR reaction. Up to 200 loci per individual can be characterised (Severson 1994). SSR marker genetic linkage maps are being produced for the principal human malaria vector, *Anopheles gambiae*. The RFLP markers are probaly most useful to study the genome structure of different mosquito species, because cDNA sequences are usually conservative. Therefore the same markers may be used for other closely related species.

Mosquitoes exhibit cellular and humoral immune responses to parasites such as melanotic encapsulation of invading organisms. Phenoloxidase plays an important role in this process. Understanding of this mechanism will be improved since a cDNA for prophenoloxidase has been cloned in *An. gambiae*. Other components of the immune system include lectins and opsonins for hemocytes. Defensin proteins show antibacterial properties in *Ae. Aegypti* and *An. gambiae*. Model insect systems, like *Drosophila melanogaster*, may also help to understand the genetic mechanism of mosquito immune response.

Utilising mosquitoes with different suspectibility to *P. falciparum* has been shown in *Anophelis stephensi;* the early initiation of haemoglobin degradation and higher amino peptidase activity were responsible for limited parasite development in the mosquito midgut. This was shown in the refractory strain with reverse results in the susceptible strain (Feldmann *et al.* 1990). Instead of using the parasite itself negatively charged sephadex beads provoke the same reaction in *An. gambiae* (Paskewitz and Richle 1994). The bead assay can be used to investigate refractoriness through genetic mapping and to study the biochemistry of recognition and melanization.

Mapping of quantitative trait loci (QTL) is progressing steadily in mosquitoes. In *Ae. Aegypti* susceptibility to the filarial worm parasite *Brugia malayi* has been studied. Two QTLs were detected, one locus on chromosome 1, showing a recessive effect on susceptibility and a second locus on chromosome 2 with an additive effect (Severson *et al.* 1994). Significant epistasis was detected between the two loci, and the genetic background of the mosquito strains influences these effects too. Another study by Severson *et al.* (1995) showed that two QTLs were involved in oocyst development of *P. gallinaceum* in the mosquito midgut. One QTL accounted for 49 and 65% respectively of the phenotypic variance in two populations, the other QTL for 10 and 14% of the variance. There was also evidence, that the QTL locus on chromosome 2 is involved in susceptibility to *Brugia malayi* and to yellow fever virus. This could either be explained by a single locus with epistatic effects or a tightly linked cluster of genes. See also Beerntsen *et al.* (1995). Zheng *et al.* (1997) studied the encapsulation reaction in *An. Gambiae* to *P. cynomolgi B.* and genetic mapping showed one major and two minor QTLs. One of these QTLs maps in the general area where the prophenoloxidase gene is located. Positional cloning is needed to verify the influence on vector competence directly. The mapping of QTLs and therefore also positional cloning has focused on vector competence (C.F. Curtis 1994). It appears that few loci, 2 to 3, are responsible for refractoriness and the mechanisms involved are melanisation of oocytes and fast haemoglobin detection. Selection pressure for refractoriness doesn't happen naturally, because mosquito infection rates seldom exceed 10%. To introduce refractoriness genes into mosquito populations would require

highly creative and technical solutions and may be challenging (Curtis 1994). Another phenomenon, that may have a genetic basis, is the biting behaviour of mosquitoes. *Anopheles* mosquitoes include zoophilic and anthropophilic species. Recombinant strains could be produced by backcrossing or genetic engineering. Still the question remains how these populations would replace the anthropophilic wild populations.

Spielman (1994) argues that reduced vector competence may have little to do with the epidemiology of malaria. Vector capacity, vector survival and human-biting frequency are major determinants in transmission. Besides mosquitoes transmit other diseases than malaria, although some genes appear to affect all in the same direction (Severson 1995). Further problems reviewed by Spielman (1994) are the use of bednets in locations were refractory populations are released and the increase in density of hematophagous mosquitoes leading to protests by the local residents. Genetic determination of QTLs for vector survival and human-biting frequency has received less attention. In combination with the present options of bednets and window screens in local communities this might be the best approach for genetic research of a long term nature.

Tick genetics. There appear to be few researchers who have studied the genetics of susceptibility to *Theileria* species or other parasites in ticks. Under laboratory conditions it has been shown, that variation in susceptibility to infection with *Theileria parva* exists. The ticks originated from different locations, but these differences between tick stocks could have an environmental and/or genetic basis. A.S. Young *et al.* (1995) studied two tick (*Rhipicephalus appendiculatus)* populations representing different stocks. They used the number of salivary gland acini infected per tick as the parameter to measure susceptibility. The range of the number of infected acini by *T. parva* in individual ticks ranged from 0 (found in approximately 10% of ticks) to over 700. Estimates of heritability of the susceptibility to infection with *T. parva* at day 16 after exposure to infection were .24 and .26 respectively. The heritabilities were even higher when they were measured at day 17 than day 16. The genetic extremes after selection can be exploited for studies of the immunology and the biology of *T. parva* in the tick. Field challenge can be mimicked by using the lines of ticks with low susceptibility. Major gene effects could be detected by application of molecular genetics in future.

Tsetse genetics. Vector-parasite relationships have been studied between different species of tsetse, between species of the parasite and in different combinations. Screening of natural populations of *Glossina p. palpalis* has been carried out by Maudlin (1980). Based on a larger body of data it may be concluded that *Trypanosoma vivax* easily infects tsetse, while other trypanosome species have more difficulty. Within *Glossina* species variability has been established and the difference between sexes has long ago been observed. Males are being more readily infected than females. In a well organised study of inheritance of susceptibility to *Trypanosoma cruzi* infection in *Rhodnius prolixus* the parameter was the scoring of trypanosomes in the faeces of the bugs. The heritability was low, i.e. 5.5%. The trait was sex linked or sex limited (Maudlin 1976). In a study of susceptibility to *T. congolense* infection in *Glossina morsitans,* maternal effects did determine the infection rates (Maudlin 1982). Many reseachers have asked the question why infection rates are often very low. Maudlin (1985)

recites 10% for *Glossina morsitans*, 7% were *T. vivax* infections, 2.6% were *T. congolense* and only .3% *T. brucei*. In this paper again the extra chromosal nature of factors inherited from their mothers are presented. Large differences between families in susceptibility can be selected. Susceptible families of *Glossina m. morsitans* had a midgut infection rate of 76.9% and a mature infection rate of 47.9%. In the refractory families these figures were respectively 11.1 and 6.3% (Maudlin and Dukes 1985). These two lines were then challenged with different stocks of *T. congolense*, *T. brucei* and *T. b. gambiense*. *T. congolense* and *T. b. brucei* showed similar responses in the lines. However, *T. b. gambiense* deviated from this pattern, showing similar midgut infection rates but much lower mature infection rates. The trypanosome genotype appears to have a major effect on susceptibility (Maudlin *et al.* 1986). The effects of X-linked genes have been associated with the establishment of a mature infection. Female flies showed consistently smaller proportions in mature infections. Longer maturation times are associated with lower levels of transmission (Milligan *et al.* 1995).

Genetic variability for vector competence has been established in tsetse. However, the outcome is dependent on the specific tsetse trypanosome interactions. Major maternal and sex-linked effects have been established. Likelihood of transmission depends on the fly susceptibility to infection and might be characteristic of fly populations in defined locations. Linkage of behaviour and physiology of tsetse populations with molecular genetic data could be an interesting approach for the future.

CONCLUSIONS

Vector transmitted diseases like trypanosomosis, theileriosis and malaria create serious health problems in the developing world. New approaches are needed to control these diseases, integrating existing control measures with innovative vector and parasite control methods.

Refractoriness to the parasites in the vector has a genetic basis that could be exploited. Vector competence however is only one aspect of the transmission which also includes vector longevity and biting frequency. In mosquitoes molecular and immunological knowledge about genes affecting the competence have already been identified. It would be interesting to explore the other traits genetically and model the transmission system in malaria.

In tsetse and tick genetics much basic genetic work still needs to be done. The lesson learned from malaria is to study the epidemiology, including transmission, first to identify the most appropriate traits.

Even when genetics by selection or transgenesis could play a role in vector control, many biological, environmental, ethical, and societal issues have to be resolved.

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