43

ADVANCES IN PARASITOLOGY

Edited by

J.R. BAKER R. MULLER D. ROLLINSON

Advances in PARASITOLOGY

VOLUME 43

Advances in PARASITOLOGY

Edited by

J.R. BAKER

Royal Society of Tropical Medicine and Hygiene, London, England

R. MULLER

International Institute of Parasitology, St Albans, England

and

D. ROLLINSON

The Natural History Museum, London, England

VOLUME 43

ACADEMIC PRESS

San Diego London Boston
New York Sydney Tokyo Toronto

ACADEMIC PRESS
a division of Harcourt Brace & Company
525 B Street, Suite 1900, San Diego,
California 92101-4495, USA
http://www.apnet.com

ACADEMIC PRESS
24-28 Oval Road
LONDON NW1 7DX
http://www.hbuk.co.uk/ap/

A catalogue record for this book is available from the British Library

ISBN 0-12-031743-5

Typeset by Mathematical Composition Setters Ltd, Salisbury, Wiltshire
Printed in Great Britain by MPG, Bodmin, Cornwall

99 00 01 02 03 04 MP 9 8 7 6 5 4 3 2 1

CONTRIBUTORS TO VOLUME 43

P.J. BRINDLEY, *Molecular Parasitology Unit, and Australian Centre for International & Tropical Health & Nutrition, Queensland Institute of Medical Research, Post Office, Royal Brisbane Hospital, Queensland 4029, Australia*

J.P. DALTON, *School of Biological Sciences, Dublin City University, Dublin 9, Republic of Ireland*

W. GIBSON, *School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG, UK*

P. GÖTZ, *Institute of Zoology, Free University of Berlin, Königin-Luise-Str. 1-3, 14195 Berlin, Germany*

A. HEMPHILL, *Institute of Parasitology, Faculties of Veterinary Medicine and Medicine, University of Bern, Langass-Strasse 122, CH-3012 Bern, Switzerland*

D. KNOX, *Moredun Research Institute, 408 Gilmerton Road, Edinburgh, EH17 7JH, UK*

P. ROSENTHAL, *Department of Medicine, Box 0811, San Francisco General Hospital, University of California, San Francisco, CA 94143-0811, USA*

J. STEVENS, *Department of Biological Sciences, University of Exeter, Exeter, EX4 14PS, UK*

J. TORT, *School of Biological Sciences, Dublin City University, Dublin 9, Republic of Ireland*

A. VILCINSKAS, *Institute of Zoology, Free University of Berlin, Königin-Luise-Str. 1-3, 14195 Berlin, Germany*

K.H. WOLFE, *Department of Genetics, University of Dublin, Trinity College, Dublin 2, Republic of Ireland*

This Page Intentionally Left Blank

PREFACE

This volume opens with an account by Wendy Gibson and Jamie Stevens, of the University of Bristol (UK), of what is known about sexual reproduction in the family Trypanosomatidae. The occurrence of sex in this group of parasitic flagellates is a relatively new discovery. When the late Cecil Hoare was writing his monograph on the trypanosomes of mammals about 25 years ago, he stated that 'All the available evidence indicates that the reproduction of trypanosomes throughout their life cycle ... is asexual' (Hoare, C.A. (1972): *The Trypanosomes of Mammals*. Oxford and Edinburgh: Blackwell Scientific Publications, pp. 48–49), although from very early in the history of 'trypanosomatology' various accounts of alleged sexual processes were reported in the literature. Hoare was rightly sceptical of these early accounts, referring to the authors' 'obsession with sexuality', 'fantastic views' and 'highly fanciful' descriptions. However, Gibson and Stevens discuss the careful work, in which the senior author played a significant part, beginning with the crossing experiments reported by Leo Jenni and his co-workers in 1986, which eventually led to general acceptance of the occurrence of genetic exchange — at least in the species *Trypanosoma brucei* and very probably in other species and genera as well, by a process which bore no relationship to the 'fanciful' processes described by the earlier authors. It remains true, however, that no one has yet succeeded in visualizing the process involved, so its mechanism remains unclear.

Andrew Hemphill, of the University of Bern (Switzerland), reviews the protozoan parasite, *Neospora caninum*. This genus was erected as recently as 1988 to describe an apicomplexan parasite, antigenically distinct from *Toxoplasma*, causing neuromuscular disease in dogs. The same parasite was then found to be responsible for abortion in cattle, and it has now been reported from other herbivores in many parts of the world. The life cycle has very recently been elucidated. The author describes new sensitive and specific molecular tools for the detection of the parasite which should help to determine its importance as a cause of disease in animals, and possibly in humans.

The volume continues with two linked reviews of the proteolytic enzymes of parasites. First, the proteases of almost all the medically important protozoan parasites of humans are discussed by Philip Rosenthal of the University of California, San Francisco (USA), who finds that almost all

express multiple proteases. Cysteine proteases, which act either intracellularly or extracellularly in lysosome-like organelles, are present in all, and the importance of these and of other proteases in the life cycle of the parasites suggests that they may present a powerful new target for antiparasitic chemotherapy.

We are also extremely fortunate to have a chapter dedicated to the proteinases of parasitic helminths written by key workers in this field: Jose Tort, Kenneth Wolfe and John Dalton from Dublin, Republic of Ireland, Paul Brindley from Brisbane, Australia and Dave Knox from Edinburgh, Scotland.

The proteinases of helminths, and their associated genes, have been under close scrutiny in recent years because of their recognized importance in many aspects of the parasitic way of life. Proteinases are involved in tissue penetration, digestion and evasion of host immune responses. In this review, particular attention is given to digenean trematodes of medical and economic importance, cestodes and nematodes parasitizing animals and plants. Interesting points emerge from the wealth of literature reviewed, and of the four major groups of peptidases — serine, aspartic, cysteine and metalloproteinases — it is the papain superfamily of cysteine proteinases which has been most characterized from parasitic worms. The final section of the review is devoted to an in-depth phylogenetic analysis of this group of proteinases, which includes a comparison with similar proteinases from protozoan parasites.

The volume ends with an account by Andreas Vilcinskas and Peter Götz, from the Free University of Berlin (Germany), of parasitic fungi which infect insects. The authors examine the ways in which these two groups of disparate organisms interact at the molecular level, concentrating on fungal molecules involved in virulence and on insect molecules involved in the humoral immune response. Study of this rather unusual model system has shed new light on mechanisms of fungal pathogenesis, and the authors hope that their studies may be extended to the molecular interactions between insects which act as vectors and the parasites which they transmit to humans and other animals.

J.R. Baker
R. Muller
D. Rollinson

CONTENTS

Proteases of Protozoan Parasites

P.J. Rosenthal

Proteinases and Associated Genes of Parasitic Helminths

J. Tort, P.J. Brindley, D. Knox, K.H. Wolfe and J.P. Dalton

Parasitic Fungi and their Interactions with the Insect Immune System

A. Vilcinskas and P. Götz

This Page Intentionally Left Blank

Genetic Exchange in the Trypanosomatidae

Wendy Gibson[1] and Jamie Stevens[2]

[1]*School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG, UK, and*
[2]*Department of Biological Sciences, University of Exeter, Exeter, EX4 14PS, UK*

ADVANCES IN PARASITOLOGY VOL 43
ISBN 0-12-031743-5

ABSTRACT

The only trypanosomatid so far proved to undergo genetic exchange is *Trypanosoma brucei*, for which hybrid production after co-transmission of different parental strains through the tsetse fly vector has been demonstrated experimentally. Analogous mating experiments have been attempted with other *Trypanosoma* and *Leishmania* species, so far without success. However, natural *Leishmania* hybrids, with a combination of the molecular characters of two sympatric species, have been described amongst both New and Old World isolates. Typical homozygotic and heterozygotic banding patterns for isoenzyme and deoxyribonucleic acid markers have also been demonstrated amongst naturally-occurring *T. cruzi* isolates.

The mechanism of genetic exchange in *T. brucei* remains unclear, although it appears to be a true sexual process involving meiosis. However, no haploid stage has been observed, and intermediates in the process are still a matter for conjecture. The frequency of sex in trypanosomes in nature is also a matter for speculation and controversy, with conflicting results arising from population genetics analysis.

Experimental findings for *T. brucei* are discussed in the first section of this review, together with laboratory evidence of genetic exchange in other species. The second section covers population genetics analysis of the large body of data from field isolates of *Leishmania* and *Trypanosoma* species. The final discussion attempts to put the evidence from experimental and population genetics into its biological context.

1. INTRODUCTION

Trypanosomatids are unicellular, obligate parasites found in a wide variety of invertebrates and vertebrates in nature. Some of these flagellates have monogenetic life cycles, but the notorious members of the family are the digenetic species responsible for the vector-borne diseases leishmaniasis and trypanosomiasis, which affect humans and domestic livestock in the warmer regions of the world.

Genetic exchange in trypanosomatids has been unequivocally demonstrated in only one species, *Trypanosoma brucei*, and these laboratory findings will be discussed in the first section of this review, together with experimental evidence of genetic exchange in other species. The second section covers population genetics analysis of the large body of data from field isolates of *Leishmania* and *Trypanosoma* species. Our final discussion

attempts to put the evidence from experimental and population genetics into its biological context.

2. LABORATORY EXPERIMENTS ON GENETIC EXCHANGE

2.1. Experimental Crosses of *Trypanosoma brucei*

2.1.1. *Experimental Design*

The first successful laboratory cross was carried out by Jenni *et al.* (1986), who co-transmitted two clones of *Trypanosoma brucei* ssp. through tsetse flies (*Glossina* spp.) and demonstrated hybrid progeny among the metacyclic forms from the salivary glands. The two parent clones were distinguishable by isoenzymes and restriction fragment length polymorphisms (RFLPs), and the cloned hybrid progeny had inherited a mixture of markers from both parents. Further crosses followed this general scheme (Figure 1). Briefly, equal numbers of the two parental trypanosome clones were mixed and fed to groups of teneral tsetse flies. Not all flies became infected after the infected feed, and only some infected flies produced hybrids. Thus large numbers of flies and trypanosome populations needed to be screened to identify those containing hybrids. Mating is non-obligatory, so the metacyclic population from the salivary glands may contain a mixture of parents and hybrids. Before the use of selectable markers, hybrid-containing populations were identified by the presence of either heterodimeric isoenzyme bands unique to hybrids (Jenni *et al.*, 1986) or non-parental karyotypes after pulsed field gel electrophoresis (PFGE; Gibson, 1989).

The development of methods for the stable transformation of trypanosomes with exogenous deoxyribonucleic acid (DNA) (Lee and Van der Ploeg, 1990; Ten Asbroek *et al.*, 1990; Eid and Sollner-Webb, 1991), led to a second approach using selectable markers. In the cross described by Gibson and Whittington (1993), each of the parental clones was transformed with a different construct designed to integrate a gene for drug resistance into the tubulin locus by homologous recombination. In this way, parental clones resistant to the antibiotics hygromycin or G418 were created. After co-transmission through the fly, hybrid progeny were selected by resistance to both drugs. This strategy has obvious advantages over the previous 'finding a needle in a haystack' approach. However, since only one of the allelic tubulin arrays carried a drug resistance marker, and assuming Mendelian inheritance, only a quarter of the hybrid progeny would be expected to be doubly resistant and distinguishable from the parents by selection.

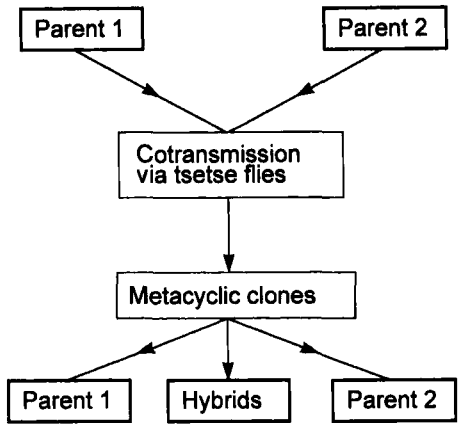

Figure 1 General scheme of an experimental cross of *Trypanosoma brucei*. Genetic exchange is non-obligatory and so both hybrids and parents are found among the metacyclics.

Little success with generation of hybrids *in vitro* has been reported, despite the use of selectable markers (Gibson and Whittington, 1993). Schweizer *et al.* (1991) observed a heterodimeric isoenzyme band after long-term co-cultivation of procyclics of each homozygotic type, but did not succeed in isolating the putative hybrid trypanosomes. Later work (see Section 2.1.3) has indicated that genetic exchange probably takes place in the salivary glands of the fly, and thus the failure to reproduce the process *in vitro* probably results from the difficulty of growing these life-cycle stages outside the fly.

2.1.2. *Mating Compatibility*

A number of different crosses followed the pioneering experiment of Jenni and colleagues (1986), using all combinations of subspecies except Group 1 *T. b. gambiense* (as defined by Gibson, 1986); this is the classical avirulent type of *T. b. gambiense*, which is very difficult to transmit through tsetse flies in the laboratory. Most of the parental isolates used in these crosses originated from the two sides of Africa, an experimental design chosen to maximize allelic differences between the parents. However, Schweizer *et al.* (1994) crossed two parental isolates from East Africa (Tanzania and Uganda) and Degen *et al.* (1995) used two sympatric *T. b. brucei* isolates from Uganda.

From Table 1, which lists the laboratory crosses carried out to date, it appears that there is no barrier to genetic exchange between different

Table 1 Published experimental crosses of *T. brucei* spp.

	Parents[a]			References
A	*T. b. brucei* STIB 247	×	*T. b. gambiense* Group 2[b] STIB 386	Jenni *et al.*, 1986; Paindavoine *et al.*, 1986a; Wells *et al.*, 1987; Sternberg *et al.*, 1988, 1989
B	*T. b. brucei* STIB 247	×	*T. b. brucei* TREU 927/4	Turner *et al.*, 1990
C	*T. b. brucei* TREU 927/4	×	*T. b. gambiense* Group 2[b] STIB 386	Turner *et al.*, 1990
D	*T. b. brucei* STIB 247	×	*T. b. brucei* STIB 777	Schweizer *et al.*, 1994
E	*T. b. brucei* TSW 196	×	*T. b. rhodesiense* 058	Gibson, 1989; Gibson & Garside, 1990; Gibson *et al.*, 1992
F	*T. b. brucei* TSW 196	×	*T. b. brucei* J10	Gibson & Garside, 1991
G	*T. b. brucei* KP2N	×	*T. b. rhodesiense* 058H	Gibson & Whittington, 1993; Gibson & Bailey, 1994; Gibson *et al.*, 1997a
H	*T. b. rhodesiense* 058H	×	*T. brucei* spp. P20[c]	Gibson *et al.*, 1995
I	*T. b. brucei* STIB 826	×	*T. b. brucei* STIB 829	Degen *et al.*, 1995
J	*T. b. rhodesiense* 058H	×	*T. b. gambiense* Group 2[b] TH2	Gibson *et al.*, 1997a
K	*T. b. brucei* KP2N	×	*T. b. gambiense* Group 2[b] TH2	Gibson *et al.*, 1997a

[a] Trypanosome origins: STIB 247, hartebeest, Tanzania, 1971; STIB 386, human, Côte d'Ivoire, 1978; TREU 927/4, tsetse, Kenya, 1970; STIB 777, tsetse, Uganda, 1971; TSW 196, pig, Côte d'Ivoire, 1978; 058, 058H, human, Zambia, 1974; J10, hyena, Zambia, 1973; KP2N, tsetse, Côte d'Ivoire, 1982; STIB 826, 829, tsetse, Uganda, 1990.

[b] Group 2 virulent *T. b. gambiense* as defined by Gibson (1986).

[c] F1 hybrid from KP2N × 058H cross (Gibson and Bailey, 1994).