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A TAXONOMIC AND GENETICAL STUDY OF THE *ANOPHELES COUSTANI* GROUP OF MOSQUITOES IN SOUTHERN AFRICA

by Maureen Coetzee

A Dissertation Submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, for the Degree of Master of Science

Johannesburg, March 1982

ABSTRACT

The Anopheles coustani (Diptera:Culicidae) group of mosquitoes in southern Africa originally comprised a single species taxon A.coustani with tenebrosus and ziemanni as "varieties" thereof. Thirteen years ago (1968) the varieties were given full specific rank on morphological evidence. While the adults were easily distinguished, previous workers had not been able to find any significant differences between the immature stages. In 1972 it was suggested, without evidence, that it was possible that the three forms are in fact morphs of one polymorphic population. Using both genetical and morphological techniques, the taxonomic and evolutionary status of these similar forms was reinvestigated in this present study.

Examination of the polytene chromosomes of the fourth instar larvae provided evidence for a total of five species, and preliminary starch-gel electrophoresis studies confirmed these findings. The morphology of the adults and immatures was investigated and good discriminating characters were found in the pupae of all five species.

These studies have shown that the hypothesis that a single polymorphic species is being dealt with is incorrect, and also revealed further sibling species in two of the taxa.

As the members of the A.coustani group are highly anthropophilic, these results are potentially pertinant to studies of residual malaria transmission in Africa. They also demonstrate the utility of new approaches for the adequate study of a difficult complex of species in Anopheles.

DECLARATION BY CANDIDATE

I declare that this dissertation is my own work and that it has not been submitted before for any degree or examination in any other University. Throughout this study I have been an employee of the Department of Health.

Maureen Coetzee

This work is dedicated to my husband Dolf who has been so patient and long-suffering.

PREFACE

This study was stimulated by the need to study the morphology of the immature stages of the three species of the sub-genus Anopheles found in this country. It was only later that the genetics of the group was also considered. Current genetical studies of anophelines are proving to be almost as useful and enlightening in the study of evolutionary concepts as those carried out on members of the genus Drosophila. This study contributes in a small way to our sum of knowledge of this important genus.

I am deeply indebted to Dr. Botha de Meillon, who first awakened an interest in me for mosquitoes, and who has subsequently kept the flame alive, encouraging and supporting me whenever I looked like flagging. But for him, this dissertation would never have been written.

I am most grateful to Prof. Hugh Paterson who has made all things possible. My time spent under his supervision was most imformative and very enjoyable. It was a privelege to come under his influence and so be exposed to all the newest ideas in this field of applied entomology.

Many thanks are due to Mr. Chris Green and Mr. Rich Hunt of the Department of Medical Entomology, South African

Institute for Medical Research, Johannesburg, who put up with me for one year and helped to further my education. I appreciate their generosity in the free use of their equipment, the material they supplied me with, and for discussions regarding the ideas and techniques employed in this study. Also from the above Institute, I thank Dr. J. Ledger, Head of the Entomology department, for his interest and for granting permission to use the facilities, Mrs. J. Segerman for her help with pinned specimens and Mr. G. Newlands, Ms. C. Martindale, Mr. I. Davidson and Mr. S. Henning for their interest in my work.

For the collection of wild material I have been almost totally dependant on the co-operation of other people. In this respect I thank Dr. C. Hansford, Director of the National Institute for Tropical Diseases, Tzaneen, and the following members of his staff, in alphabetical order: Mr. H. Cross, Mr. J. Cross, Ms. D. Durand, Mr. C. du Toit, Mr. D. Eckhardt, Mr. E. Jansen, Mr. K. Newbetry, Mr. A. Quan, Mr. W. Sekgobela, Mr. J. Shipalane, Mr. D. Theron and Mr. G. van Eeden. Also Dr. P. Taylor of Blair Research Institute, Salisbury, Zimbabwe and Mr. J. le Grange of the Department of Health, Namibia.

For helpful discussions I am indebted to Mr. C. Green, Mr. R. Hunt, Mr. K. Newberry and Ms. D. Durand. I thank Dr. D. Lambert for ideas on the presentation of this work.

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Identification

The need for the accurate identification of mosquitoes was appreciated at an early stage when observations showed that in any particular area malaria was transmitted by only one or two species of anophelines (Watson, 1921). This has led to a vast amount of literature being published on mosquito identification. In 1938, Edwards published a catalogue of mosquitoes of the world in which he recognised 1400 species. The most recent catalogue published (Knight & Stone, 1977) recognises 2960 species, more than double that of Edwards'. While there were morphological differences, classical taxonomists could describe new species. Problems arose however, when taxonomic species were found to comprise of cryptic genetical species. These are commonly referred to as sibling species.

Paterson (1975) states "... that medical entomological studies must be based on sound identification of genetical species (my emphasis) and gives an example of the resultant chaos if this is not adhered to, by quoting the

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Anopheles gambiae complex in relation to malaria control. Firstly, from the time of Ross' visit to West Africa until the discovery of the complex (Paterson, 1962, Davidson, 1962), malaria entomologists in Africa studied these important insects as if they were all members of a single species. As a consequence, it became necessary to collect once more all the biological data, but this time for each individual species. Secondly, the refusal by some authors to recognise the complex as late as 1974 served merely to confuse the picture. As the different members of the gambiae complex have different behavioural and physiological characters and consequently different vector capacities, it is obviously most important to identify correctly which member of the complex one is dealing with in malaria control programmes.

Before starting on a study of species, it is necessary to state which concept of species one is adopting. In this case, because speciation is a genetical event, "species" is defined in genetical terms (as opposed to the taxonomic species concept which is concerned only with classification). According to the Recognition Concept (Paterson, 1978, 1980), species are populations of individual organisms which share a common specific-mate recognition system (SMRS) and habitat preference. The SMRS comprises a co-adapted signal-response reaction chain whose function is to ensure fertilization. The SMRS may take the form of visual, auditory, chemical or

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