FIELD AND LABORATORY EVIDENCE OF MULTIPLE HOST CONTACTS
DURING BLOOD FEEDING BY THREE SPECIES OF *CULEX* MOSQUITOES,
AND A MODEL OF THE TRADEOFF BETWEEN BLOOD-FEEDING ASSOCIATED
MORTALITY AND INCREASED FITNESS FROM MULTIPLE FEEDING

A Thesis

by

Robert A. Anderson

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Submitted to the Faculty

of

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In Partial Fulfilment of the

Requirements for the Degree

of

Doctor of Philosophy

in

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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ROBERT A. ANDERSON

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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General 0515
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Early Childhood 0518
Elementary 0524
Educational Psychology 0525
Finance 0277
Guidance and Counseling 0519
Health 0680
Higher 0745
History of 0520
Home Economics 0278
Industrial 0521
Language and Literature 0279
Mathematics 0280
Music 0522
Philosophy of 0998

Physical 0523
Reading 0535
Religious 0527
Sciences 0714
Secondary 0533
Social Sciences 0534
Sociology of 0340
Special 0529
Teacher Training 0530
Technology 0710
Tests and Measurements 0288
Vocational 0747

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LINGUISTICS**
Language
    General 0679
    Ancient 0289
    Linguistics 0290
    Modern 0291
    Rhetoric and Composition 0681
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    General 0401
    Classical 0294
    Comparative 0295
    Medieval 0297
    Modern 0298
    African 0316
    American 0591
    Asian 0305
    Canadian (English) 0352
    Canadian (French) 0355
    Caribbean 0360
    English 0593
    Germanic 0311
    Latin American 0312
    Middle Eastern 0315
    Romance 0313
    Slavic and East European 0314

**PHILOSOPHY, RELIGION AND
THEOLOGY**
Philosophy 0422
Religion
    General 0318
    Biblical Studies 0321
    Clergy 0319
    History of 0320
    Philosophy of 0322
Theology 0469

SOCIAL SCIENCES
American Studies 0323
Anthropology
    Archaeology 0324
    Cultural 0326
    Physical 0327
Business Administration
    General 0310
    Accounting 0272
    Banking 0770
    Management 0454
    Marketing 0338
Canadian Studies 0385
Economics
    General 0501
    Agricultural 0503
    Commerce-Business 0505
    Finance 0508
    History 0509
    Labor 0510
    Theory 0511
Folklore 0358
Geography 0366
Gerontology 0351
History
    General 0578
    Ancient 0579

Medieval 0581
Modern 0582
Church 0330
Black 0328
African 0331
Asia, Australia and Oceania 0332
Canadian 0334
European 0335
Latin American 0336
Middle Eastern 0333
United States 0337
History of Science 0585
Law 0398
Political Science
    General 0615
    International Law and
        Relations 0616
    Public Administration 0617
Recreation 0814
Social Work 0452
Sociology
    General 0626
    Criminology and Penology ... 0627
    Demography 0938
    Ethnic and Racial Studies 0631
    Individual and Family
        Studies 0628
    Industrial and Labor
        Relations 0629
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    Social Structure and
        Development 0700
    Theory and Methods 0344
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    General 0473
    Agronomy 0285
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        Nutrition 0475
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        Technology 0359
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    Soil Science 0481
    Wood Technology 0746
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    General 0306
    Anatomy 0287
    Animal Physiology 0433
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    Botany 0309
    Cell 0379
    Ecology 0329
    Entomology 0353
    Genetics 0369
    Limnology 0793
    Microbiology 0410
    Molecular 0307
    Neuroscience 0317
    Oceanography 0416
    Plant Physiology 0817
    Veterinary Science 0778
    Zoology 0472
Biophysics
    General 0786
    Medical 0760

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Geochemistry 0996

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Paleozoology 0985
Palynology 0427
Physical Geography 0368
Physical Oceanography 0415

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    General 0566
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    Education 0350
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    Human Development 0758
    Immunology 0982
    Medicine and Surgery 0564
    Mental Health 0347
    Nursing 0569
    Nutrition 0570
    Obstetrics and Gynecology .. 0380
    Occupational Health and
        Safety 0354
    Oncology 0992
    Ophthalmology 0381
    Pathology 0571
    Pharmacology 0419
    Pharmacy 0572
    Public Health 0573
    Radiology 0574
    Recreation 0575
    Rehabilitaion and Therapy ... 0382

Speech Pathology 0460
Toxicology 0383
Home Economics 0386

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    Agricultural 0749
    Analytical 0486
    Biochemistry 0487
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    Nuclear 0738
    Organic 0490
    Pharmaceutical 0491
    Physical 0494
    Polymer 0495
    Radiation 0754
Mathematics 0405
Physics
    General 0605
    Acoustics 0986
    Astronomy and
        Astrophysics 0606
    Atmospheric Science 0608
    Atomic 0748
    Condensed Matter 0611
    Electricity and Magnetism 0607
    Elementary Particles and
        High Energy 0798
    Fluid and Plasma 0759
    Molecular 0609
    Nuclear 0610
    Optics 0752
    Radiation 0756
Statistics 0463

Applied Sciences
Applied Mechanics 0346
Computer Science 0984

Engineering
    General 0537
    Aerospace 0538
    Agricultural 0539
    Automotive 0540
    Biomedical 0541
    Chemical 0542
    Civil 0543
    Electronics and Electrical 0544
    Environmental 0775
    Industrial 0546
    Marine and Ocean 0547
    Materials Science 0794
    Mechanical 0548
    Metallurgy 0743
    Mining 0551
    Nuclear 0552
    Packaging 0549
    Petroleum 0765
    Sanitary and Municipal 0554
    System Science 0790
Geotechnology 0428
Operations Research 0796
Plastics Technology 0795
Textile Technology 0994

PSYCHOLOGY
General 0621
Behavioral 0384
Clinical 0622
Cognitive 0633
Developmental 0620
Experimental 0623
Industrial 0624
Personality 0625
Physiological 0989
Psychobiology 0349
Psychometrics 0632
Social 0451

I dedicate this thesis to my parents, Howard and Bobbie.
They started me down the long road to a doctorate and they
are still around to see its completion. I owe them more
than I can ever possibly pay back.

ABSTRACT

FIELD AND LABORATORY EVIDENCE OF MULTIPLE HOST CONTACTS
DURING BLOOD FEEDING BY THREE SPECIES OF *CULEX* MOSQUITOES,
AND A MODEL OF THE TRADEOFF BETWEEN BLOOD-FEEDING ASSOCIATED
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By

Robert A. Anderson
Major Advisor: Dr. Reinhart A. Brust

The field and laboratory research to support this
thesis was carried out from June, 1991 to August, 1994.
Field studies were conducted in Manitoba, Canada, and
Florida, United States to evaluate the frequency of multiple
host contacts and factors that affect this behaviour by
Culex tarsalis Coquillett, *Culex restuans* Theobald, and
Culex nigripalpus Theobald, primary and secondary vectors of
several encephalitis viruses in North America.

In the summers of 1991, 1992 and 1993, blood-fed
mosquitoes were collected from box traps, each baited with a
pair of quail. One quail of each pair was injected with
rubidium and the other with cesium to permit the
determination of the source(s) of each blood meal.
Approximately 5% of all blood fed *Cx. tarsalis*, *Cx.
restuans*, and *Cx. nigripalpus* ingested blood from both quail
during overnight exposure. The frequency of multiple feeding
by these species was 0 to 18.5%, 0 to 33.3% and 0 to 17.6%,
respectively. Of the 331 mosquitoes that ingested blood
from 2 quail, 57 (17.2%) were not fully blood fed. Also,
1.0-3.5% of blood-fed mosquitoes had ingested blood before

entering the quail-baited traps.

In 48 of 70 samples collected in the field, blood feeding was skewed significantly away from an even distribution between the two quail in each cage. In 8 of 15 samples of *Cx. tarsalis* collected in 1991, the index of incomplete feeding (blood meals ≤½ full) was 2 to 8 times greater for one bird relative to the other in the same cage. In 7 of 13 samples of *Cx. nigripalpus* collected in 1992, the index of incomplete feeding was 2 to 1400 times greater for one bird relative to the other in a given cage. In 7 of 11 mixed samples of *Cx. tarsalis/Cx. restuans* collected in 1993, the index of incomplete feeding differed by 2 to 300 times. The index of interrupted blood meals was inversely related with the probability of each quail being fed upon. The probability of detecting multiple feeding was related negatively ($p < 0.0001$) with the degree to which the distribution of blood meals in each cage was skewed away from 0.5 on each bird.

Laboratory studies were conducted to test the hypothesis that the patterns of engorgement observed in the field studies were due to the behaviour of individual Japanese quail and not to differences in attraction. Quail were exposed in pairs to either *Ae. aegypti* or *Cx. nigripalpus*. Quail behaviour was recorded by videotape. Five categories of behaviour, including head shakes, foot stamps, pecks, feather fluffs and changes in body position were observed before the quail were exposed to mosquitoes. In response to mosquito attack, the frequency of each of these behaviours increased. The overall intensity of anti-mosquito activity, as measured by all categories combined, varied significantly between birds in each pair and was negatively correlated with the proportion of blood-fed

mosquitoes in each cage that had fed on the corresponding bird.

Field derived estimates of feeding success and feeding associated mortality were used as the basis for a stochastic simulation model to examine potential tradeoffs in cost and benefit from multiple feeding by *Cx. nigripalpus*. A total of 1617 *Cx. nigripalpus* were collected during the field experiment, of which 706 (44%) were blood-fed. Of the fed mosquitoes, 571 (81%) were fully fed (92% of which were single meals), and 134 were partial. Of the partially-fed females, 64 (9% of total blood feds) had taken ¼ meals, 36 (5% of total blood feds) had taken ½ meals, and 34 (5% of total blood feds) had taken trace meals. Approximately 7% of all blood meals contained blood from 2 hosts. The refeeding rate for interrupted mosquitoes was 26%. Quail hosts killed or ate approximately 24% of *Cx. nigripalpus* in field and laboratory experiments. Within the parameters evaluated for the simulation model, multiple feeding was favoured as a behavioural strategy under conditions of low, feeding-associated mortality, but this pattern was modified by the probability of feeding success.

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Among arthropods, mosquitoes are probably the most
important vectors of disease-causing pathogens (Lehane
1991). From the standpoint of disease epidemiology and
mosquito biology, blood feeding success is critical because:
(1) during feeding, female mosquitoes may acquire and/or
transmit pathogens among vertebrate hosts, and (2) blood is
of paramount importance to the mosquito as a resource that
is used directly for producing offspring. I define one
aspect of feeding success to mean that a mosquito obtains
the minimum volume of blood necessary to initiate oogenesis.
The degree of success, relative to other individuals is a
function of the amount of blood obtained. Fecundity
increases as a function of blood meal volume (Woke et al.
1956, Jalil 1974, Downe and Archer 1975, Edman and Lynn
1975, Briegel 1985, 1990). Blood meal volume may, in turn,
be affected by the interplay of mosquito feeding speed
(Gillett 1967) and the likelihood that the vertebrate host
will react to being bitten and interrupt the mosquito prior
to satiation (Edman and Kale 1971). Feeding persistence,
defined here as the tendency of mosquitoes to attack again
if blood feeding is interrupted, is one type of behaviour
that may lead to multiple feeding and increase the volume of
blood obtained for females that are interrupted before
satiation. I define multiple feeding to mean that a
mosquito has imbibed blood from at least two hosts (Edman
and Downe 1964). Differential fitness associated with blood
meal volume as a function of persistent feeding by
mosquitoes may be considered as an evolutionary context
within which to consider feeding behaviour. In other words,
one might expect that mosquitoes that display persistent

behaviour would be selected for relative to those females that are not persistent because persistence ultimately would result in more progeny. However, the relationship between blood volume and fecundity may be affected by possible costs to mosquitoes such as increased energy consumption or death from continued host contact (Edman and Scott 1987).

In nature, mosquitoes have been observed to be eaten by their hosts (Corbet and Downe 1966, Edman et al. 1984), but quantitative information on the probability of death is limited to laboratory studies in which the number of mosquitoes that survived exposure to hosts was recorded (Day and Edman 1984, Edman et al. 1972, Kale et al. 1972, Webber and Edman 1972). Mosquitoes may trade off the increased fecundity from additional blood meals for lower risk of death associated with not attempting a second meal, but this hypothesis has not been addressed in any studies that I could locate. Such a trade off may have been important in the evolution of feeding persistence. Roitberg and Friend (1992) suggested an analogous tradeoff between sugar and blood feeding by mosquitoes.

Multiple feeding is a phenomenon for which the causes are reasonably well documented (Davies 1990), but one for which quantitative data under different conditions are lacking, especially for *Culex* vectors of encephalitis viruses in North America. Interactions between these species and their avian hosts are worthy of study with respect to costs to mosquitoes associated with blood feeding because many birds are at least facultatively insectivorous (Morse 1971). As such, these hosts may pose extra risk to attacking mosquitoes because the mosquitoes can end up as prey.

The contribution that multiple feeding makes to

variation in disease transmission dynamics needs to be established. Based on a study in Thailand, Scott et al. (1993) speculated that multiple-host contacts within a single gonotrophic cycle by *Aedes aegypti* (L.) may be important in the rapid and focal transmission of dengue virus among individuals in the same or adjacent households. In this example, the authors suggested that one explanation for multiple feeding by *Ae. aegypti* is that these mosquitoes seldom feed on sugar and that frequent, small blood meals may provide metabolic resources in the place of plant nectar (Edman et al. 1992). Multiple feeding by mosquitoes and the behavioural factors that determine it associated with mosquitoes and their hosts are important topics for study.

OBJECTIVES OF THE RESEARCH

The aims of this thesis are as follows:

1) To determine the frequency of multiple feeding by three species of *Culex* mosquitoes when two hosts of the same species are available in close proximity to each other and to examine the implications of multiple feeding for arbovirus transmission.

2) To relate variation in probability of obtaining blood, blood-meal volume and multiple feeding to the defensive behaviour of avian hosts.

3) To consider how a tradeoff between increased fecundity due to multiple feeding and potentially increased mortality due to extra host contacts may have influenced feeding persistence as a behavioural strategy.

THESIS ORGANIZATION

This thesis is organized into four research chapters as they have been prepared and submitted for publication. Differences between the manuscripts submitted for publication and the chapters herein are a result of compilation into one document. The tables and figures are numbered consecutively from the beginning of the thesis without regard to the chapters. One aggregate list of references is given at the end. Each chapter that corresponds to a publication is organized into title, abstract, introduction, materials and methods, results, and discussion. Figures pertinent to each chapter are located at the end of each chapter. The question of interest and the objectives of the research are presented in the overall introduction preceding the research chapters. Pertinent literature is considered in the literature review. Chapter 1 is a paper on the subject of the frequency and amount of variation in multiple feeding by *Cx. tarsalis* Coquillett, *Cx. restuans* Theobald, and *Cx. nigripalpus* Theobald, with a discussion of the theoretical impact of multiple feeding on arbovirus transmission. Chapter 2 is a paper in which variation in interrupted and multiple feeding is related to differences in the degree to which individual quail differ in their tendency to be fed on by mosquitoes. Chapter 3 is a paper in which I present laboratory evidence to validate the assumption that the blood feeding patterns observed in Chapter 2 were due, at least in part, to variation in behaviour among individual quail. In Chapter 4, I present a field-derived estimate of the probability of feeding associated mortality pertinent to the experimental design used in Chapters 1, 2, and 3. I also present data on

feeding success of field-caught mosquitoes and use the data on mortality and feeding success as the basis of a simulation model with which I examine potential tradeoffs in cost and benefit from multiple feeding. A general discussion and summary, respectively, are given following the research chapters.

LITERATURE REVIEW

Females of most species of mosquito are obligate
haematovores that must seek blood one or more times as
adults to acquire sufficient protein for egg development.
As a result of this direct link to reproduction, blood
feeding should be a behavioural priority for mosquitoes.
Blood feeding is also crucial to the role of mosquitoes as
vectors of disease agents because such pathogens are
acquired from and transmitted to vertebrates during blood
feeding (Lehane 1991). Thus, blood feeding is at the center
of a tripartite ecological relationship that involves the
mosquito, the vertebrate as a blood source, and the
transmitted parasite, which relies on the interaction
between the other two for continued existence.

In North America, several important diseases are caused
by viruses transmitted by mosquitoes. Two such noteworthy
viruses are western equine encephalomyelitis virus (WEEV)
and Saint Louis encephalitis virus (SLEV). *Cx. tarsalis* is
the primary enzootic and epizootic/epidemic vector of WEEV
and SLEV in western North America (Reisen and Monath 1988,
Reeves 1971). *Cx. nigripalpus* is the primary vector of SLEV
in Florida (Edman and Taylor 1968). *Cx. restuans* may also
be an important enzootic vector of WEEV and SLEV (Reiter
1988). WEEV and SLEV are transmitted enzootically and
epizootically among birds, mostly passeriformes (Reisen and
Monath 1988). Clinical disease and inapparent infections in
human and other non-avian hosts result from tangential
transmission outside of the normal disease cycle during
years when mosquito numbers and virus infection frequency
are high (Reeves 1971, Reisen and Monath 1988). Humans are
considered dead-end hosts and do not contribute to the

maintenance or amplification of these zoonoses, but viral infections in people can result in significant morbidity and mortality (Reisen and Monath 1988).

In the wild, *Cx. tarsalis* and *Cx. nigripalpus* feed primarily on birds, but they also feed on mammals (Edman 1974, Tempelis et al. 1965). There is a shift from obtaining blood meals predominantly from birds in the spring to a more balanced ratio of avian and mammalian blood meals later in the summer (Edman and Taylor 1968, Tempelis et al. 1965). In essence, *Cx. tarsalis* and *Cx. nigripalpus* are opportunistic blood feeders and observed host utilization patterns most likely reflect availability of vertebrate hosts, rather than an innate preference for one type over another (Edman and Downe 1964, Edman and Spielman 1988). *Cx. tarsalis* and *Cx. nigripalpus* feed predominantly on birds early in the season and transmit WEEV or SLEV among them. As a result, both species of mosquito amplify viruses in bird populations (enzootic/epizootic transmission). However, they are also epidemic vectors to non-avian hosts because of feeding opportunism. This is in contrast to *Cx. restuans*, which seldom feeds on non-avian hosts (Nasci and Edman 1981) and, thus, acts as a vector only within and among avian populations.

Blood feeding success is as important to pathogen transmission as are host utilization patterns. However, coincidence of mosquitoes and potential hosts in time and space may not guarantee successful feeding, and thus successful transmission, because host defensive behaviour may limit mosquito engorgement (Edman and Spielman 1988). Blood feeding success may be considered as a composite measure of the probability of obtaining any amount of blood weighted by the volume of blood obtained. To become

infected and eventually infectious (after completion of the extrinsic incubation period of the pathogen), female mosquitoes must imbibe sufficient blood from an infective vertebrate host and then transmit the pathogen to a susceptible vertebrate host during the next blood-feeding attempt (Scott 1988).

Factors that increase the number of feeding attempts by a female mosquito potentially increase its vectorial capacity because each extra feeding attempt also represents an additional opportunity for transfer of pathogens from mosquito to vertebrate or vice versa. Interruption of blood feeding prior to satiation is one such factor because the probability of continued host seeking and/or refeeding is high for individuals with a small volume of blood obtained during the first meal (Edman et al. 1975). There is variation in the proportions of host-seeking *Cx. tarsalis*, *Cx. nigripalpus* and *Cx. restuans* that obtain blood and seldom do all fed females obtain a full meal (Blackmore and Dow 1958, Dow et al. 1957, Edman and Downe 1964, Magnarelli 1977). Transmission of pathogens varies with the square of the biting rate such that small increases in the rate of contact between vector and vertebrate may disproportionately increase transmission (Dye 1992). Most models of pathogen transmission are based on the assumption that each mosquito makes only one host contact per gonotrophic cycle (de Moor and Steffens 1970, Macdonald 1952, Scott et al. 1983) despite evidence that many mosquito species take blood more than once between successive ovipositions (Anderson et al. 1990, Boreham 1975, Edman and Downe 1964, Klowden and Lea 1979c, Nasci and Edman 1981, Rempel et al. 1946, Washino and Tempelis 1983, Zoltowski et al. 1978). Models of disease transmission should include a parameter to reflect the

probability of multiple feeding.

Although, many species of mosquitoes take multiple meals (Xue and Edman 1991), few experimental studies have been undertaken specifically to examine multiple feeding, especially by vector species of *Culex*. Furthermore, the link between multiple feeding and reproductive success and disease transmission has seldom been addressed. Each host contact by an infected mosquito that results in transfer of saliva to vertebrate tissue represents potential transmission of a pathogen. For example, *Cx.* pipiens pipiens (L.) infected with St. Louis encephalitis transmitted this virus more than once in a single gonotrophic cycle as a result of multiple feeding (Mitchell et al. 1979). One of the primary limitations to generating information on the significance of multiple blood feeding by mosquitoes has been an inability of researchers to easily identify multiple blood meals accurately in wild-caught mosquitoes (Washino and Tempelis 1983). Multiple blood meals taken from non-related hosts can be identified by serological methods, but meals from two or more individuals of the same species can not be easily distinguished (Tempelis 1989, Washino and Tempelis 1983). Multiple feeding on hosts of the same species may be important from an epidemiological point of view, especially in those disease systems that involve a narrow range of vertebrate hosts (Holden et al. 1973). Several important new techniques are available for the study of multiple feeding on closely related hosts.

Romoser et al. (1989) developed a histological process that allows visualization of the components of a multiple blood meal based on the deposition of a peritrophic membrane, the peritrophic plug, a zone of digestion around

each blood meal and color differences among blood-meal components. The formation of these structures occurs over varying amounts of time, depending on the species of mosquito (Romoser et al. 1989, Scott et al. 1993, Wekesa et al. 1995). To be useful, the peritrophic membrane and zone of digestion must form during the interval between separate feedings. Histological preparation of individual blood meals is labour intensive. On its own, this technique does not distinguish between separate meals taken from one host versus separate meals taken from different hosts. This limitation may be overcome if the histological technique is combined with serological identification of the components of the blood meal, but the processing of individual blood meals becomes more labor intensive than with histology alone. No field data based on this technique have been published for *Cx. tarsalis*, *Cx. nigripalpus* or *Cx. restuans*.

Another technique, based on marking host blood with either rubidium or cesium, permits the identification of multiple meals taken from at least two hosts of the same or different species and is applicable in both field and laboratory situations (Anderson et al. 1990). Large numbers of blood meals can be processed easily in this manner. If the host-blood marking technique of Anderson et al. (1990) is used in association with the technique of Romoser et al. (1989), it may be possible to estimate more accurately the frequency of multiple feeding in nature than is currently available with conventional serological methods. Neither technique can be used to identify probing attempts that do not result in uptake of blood. Such information would be useful in estimating the contribution to transmission rates of unfed, but infective mosquitoes.

Information from studies of multiple feeding behaviour could be used to examine the potential effect of multiple feeding on pathogen transmission by incorporating the increase in feeding rate due to multiple feeding into a mathematical model. Historically, malaria has attracted the most interest from a mathematical point of view, based on early attempts to calculate a threshold density of anopheline mosquitoes, below which, malaria transmission would decline to zero (Dye 1992, Macdonald 1952). This model of vectorial capacity, which incorporates entomological components, is generally given as follows (Reisen 1989): $C = ma^2P^n/-\log_e P$.

Where,

C = vectorial capacity or number of new infections arising per day from one infected host,

m = number of mosquitoes per host,

a = average number of bites per day on a relevant host per mosquito (a squared term as mosquitoes must bite twice to acquire and then transmit a pathogen),

P = probability of daily survival of mosquitoes, and

n = extrinsic incubation period of the pathogen in days.

This model was originally developed for a one-vertebrate host system, i.e. malaria, and it was assumed that all mosquitoes that bite an infected human become infected (Dye 1992). It was also assumed that all mosquito bites subsequent to the meal during which the mosquito was infected and after the duration of the extrinsic incubation period were infective for the vertebrate. This is the simplest form of the vectorial capacity equation and it has been expanded by some to account for immunity in the vertebrate population and refractoriness in the vector population (Dye 1992). Theoretically, these additional

parameters make the model more representative of malaria transmission, but more difficult to estimate because the errors associated with each parameter must be multiplied to arrive at a confidence limit for vectorial capacity (Dye 1992).

The basic vectorial capacity model described above has been adopted to consolidate the enormous amount of bionomic data on the enzootic transmission of WEEV among birds by *Cx. tarsalis* in California (Smith 1987), but it is also applicable to other vectors such as *Cx. nigripalpus* and *Cx. restuans*. A parameter, 'V' (proportion of mosquitoes that become infectious after biting an infective host), has been added by Reisen (1989) to account for the varying efficiency with which different populations of mosquitoes acquire and transmit disease (Hardy et al. 1983). This model is as follows: $C = ma^2VP^n/-\log_e P$.

The manner in which the vectorial capacity model parameters are specified indicate those likely to be of most significance to disease transmission. Biting rate appears as a squared parameter and thus has a significant influence on transmission. Transmission requires vector/host contact at least twice. The first contact must involve ingestion of some blood for pathogen infection in the vector to take place, but blood ingestion during the second attempt is not critical because transmission occurs during the salivation, i.e. usually before blood uptake occurs (Edman and Spielman 1988). The squared form of the biting rate parameter 'a' means that small changes in the number of host contacts, especially by infected vectors, may result in a disproportionate increase in transmission (Smith 1987). Data on the importance of multiple feeding to disease transmission may contribute to an understanding of why diseases such as WEE and SLE persist despite low isolation frequencies in nature and the tenuous nature of insect life

cycles that support them (Edman and Spielman 1988). This is
the context in which interrupted and multiple feeding is
most significant. Models such as those described above can
be used to estimate a population threshold for stable or
epidemic transmission for different values of the biting
rate (Reeves 1971). Specific host and mosquito-associated
factors that affect the magnitude of the biting rate are
discussed below.

It is reasonable to predict that a female mosquito
would not break off feeding unless forced to do so or until
neural feedback from abdominal stretch receptors indicates
that the midgut is full (Klowden and Lea 1979b). In fact,
virtually all mosquitoes do feed to repletion when allowed
to feed on restrained hosts that are incapable of defending
themselves (Edman et al. 1985, Klowden 1988, Reeves 1971).
Partially fed mosquitoes are frequently observed in nature
(Magnarelli 1977), thus some factor acts to reduce access to
host blood (Klowden and Lea 1979c). Behaviour of both
mosquitoes and their hosts is one major determinant of blood
feeding success (Edman and Scott 1987). In reality, one
behavioural category often depends on the other, especially
as blood feeding is an interactive process (Scott et al.
1988).

The initiation of blood feeding by individuals within a
cohort may not be synchronous so that the proportion host-
seeking increases with time. Newly emerged females seldom
seek hosts until two to three days after emergence or until
after mating has occurred (Edman and Spielman 1988). Not
all female mosquitoes are responsive in synchrony to cues
that indicate the presence of a host (Bowen 1991). Gravid
mosquitoes are usually refractory to host cues because of a
humoral inhibitor associated with the presence of eggs in
the ovary (Klowden and Lea 1979a), but this inhibition may
decline as the eggs are held longer (Klowden 1988) or may

not strongly inhibit blood feeding by some species (Klowden and Briegel 1994). Distention-induced inhibition of feeding activity is the most important mechanism that affects the potential for interrupted and multiple feeding (Klowden 1988). The degree of distention is dependent on volume of blood ingested and decreases with time after feeding as the blood is digested and the volume reduced (Klowden 1988, Edman et al. 1975).

The nutritional status of female mosquitoes may determine blood feeding success because sugar-deprived mosquitoes tend to give up more quickly when disturbed by the host (Walker and Edman 1985b). Sugar feeding also modifies the response of partially fed female mosquitoes to host-associated cues (Klowden 1988). Individuals denied sugar, but allowed to imbibe less than a full blood meal are more likely to continue to feed than are individuals with the same amount of blood and supplemental sugar (Klowden 1988). Host seeking (Klowden et al. 1988) and biting persistence (Nasci 1991) may also be reduced by low nutritional resources during larval development.

Pathogen infection, a critical requirement for disease transmission, can also directly affect the ability of a female mosquito to feed (Rossignol 1988). This is primarily a result of parasite-induced pathology in the salivary glands that inhibits secretion of apyrase (Rossignol 1988), an enzyme that inhibits hemostasis and increases feeding efficiency (Ribeiro 1987, 1988). Most of these data have been collected for species of *Anopheles* infected with malaria, but La Crosse virus-infected *Ae. triseriatus* (Say) also probe more frequently and are less successful at blood feeding than are uninfected individuals (Grimstad et al. 1980).

Adult age also affects host seeking and blood feeding (Klowden 1988). Parous females (those that have undergone

at least one cycle of blood feeding and oviposition) recover
more quickly from distention-induced neural and ovary-
derived hormonal feeding inhibition, and show less
inhibition than do nulliparous females of the same cohort
(Klowden 1988).

Interactions among individual mosquitoes may enhance
blood feeding success, (Ahmadi and McClelland 1985, Edman et
al. 1985). The authors speculated that this was likely
mediated by a non-specific odour produced by successfully
engorging mosquitoes that attracts other individuals to the
feeding site; however, the data of Ahmadi and McClelland
(1985) and Edman et al. (1985) are enigmatic because an
increase in vector density usually results in a decrease in
feeding success because of changes in host behaviour (Edman
and Scott 1987).

Blood feeding by mosquitoes causes annoyance and can be
detrimental to the health of their vertebrate hosts. As a
result, vertebrates often defend themselves from attack. It
follows that a consideration of host biology, and
specifically, defensive behaviour, is important in the
context of the feeding success and vectorial capacity of
mosquitoes. The host has the greatest chance of affecting
the feeding success of the foraging mosquito once physical
contact has been made (Scott 1988). Host species, body
size, age, individual variation in tolerance of mosquito
attack, and health are five factors that have been
identified as important determinants of interactions between
mosquitoes and the animals on which they feed because
defensive behaviour may vary with each of these factors
(Edman et al. 1985, Edman and Scott 1987, Scott 1988).
Physical characteristics such as hair or feather density and
peripheral vascularization may also affect blood-feeding
success (Edman and Spielman 1988, Walker and Edman 1985a).
For example, the probability of a given body region of

anesthetized chipmunks (*Tamius striatus* (L.)) and gray
squirrels (*Sciurus carolinensis* Gmelin) being selected as a
feeding site by *Ae. triseriatus* was inversely related to the
length and density of hair on the hosts. Walker and Edman
(1985a) speculated that labellar tapping by the mosquitoes
functioned to discriminate among areas with different
arteriole and venule densities.

Host species is an important determinant of mosquito
blood feeding success because of variable defensive
behaviour of different animals (Dow et al. 1957, Edman and
Kale 1971, Edman et al. 1974, Kale et al. 1972, Walker and
Edman 1986). Small passerine birds tended to prevent a
larger proportion of mosquitoes from engorging than did
larger birds, although this relationship was not absolute
(Edman et al. 1974). The feeding success of *Cx. nigripalpus*
on various ciconiiform species appeared to be related to the
intensity of host anti-mosquito behaviour which was related
to the species of ciconiiform (Edman and Kale 1971, Kale et
al. 1972). The black-crowned night heron, the great blue
heron, and the green heron did not exhibit intense anti-
mosquito behaviour, probably because they are sit-and-wait
foragers which places a premium on minimizing body
movements. On the other hand, five other ciconiiform
species that are active foragers prevented most mosquitoes
from engorging (Edman and Kale 1971). Differences in
foraging behaviour of gray squirrels and chipmunks were also
related to their tendency to display anti-mosquito defensive
behaviour, and thus influence mosquito feeding success
(Walker and Edman 1986), although observational techniques
may have affected the results obtained, at least for
chipmunks (Cully et al. 1991).

There are numerous observations that mosquito feeding
success tends to increase with increasing size of the
vertebrate host (Edman and Scott 1987, Port and Boreham

1980, Sota et al. 1991), perhaps because of larger surface area on which to feed or because some areas of the body such as the belly and behind the ears are almost inaccessible to grooming movements (Edman and Spielman 1988). Host size tends to be confounded by age which can also be an important determinant of mosquito feeding success (Scott et al. 1988, Sota et al. 1991).

The development of behaviour in many animals is an ontogenetic process (Krebs and Davies 1991) and anti-mosquito defensive behaviour is no exception (Blackmore and Dow 1958, Kale et al. 1972, Scott et al. 1988). There is general agreement that young animals are less able to defend against mosquito attack than are adults (Edman and Scott 1987, Scott et al. 1990), but there are exceptions; for example some precocial birds (Reeves 1971). Age-related changes in the ability of vertebrates to limit mosquito feeding success have significant implications for pathogen transmission. Young animals tend to be more susceptible to arboviral infection and to produce higher viremias that are more likely to infect mosquitoes (Scott 1988).

Individual animals of the same age and species vary in their ability or tendency to repel attacking mosquitoes (Dow et al. 1957, Edman and Scott 1987, Kale et al. 1972, Reeves 1971, Scott et al. 1988). This is an important concept in models of pathogen transmission, because non-random feeding success by mosquitoes can significantly alter the probability of acquiring and transmitting a pathogen, especially if disease mediates a reduction in host-defensive behaviour by making the vertebrate ill (Day et al. 1983, Dye 1992).

The host-associated factors that behaviourally mediate blood-feeding success of mosquitoes may be modified by mosquito density or biting pressure (Edman and Scott 1987, Edman and Spielman 1988, Edman et al. 1972, Edman et al.

1985). In these studies the probability that a vertebrate would protect itself from mosquito attack increased with the number of feeding mosquitoes. This was attributed to increased irritation and perhaps represents an evolutionary solution to serious blood loss (Edman and Spielman 1988).

Partial blood meals often result from host attempts to limit access to blood. Mosquitoes with small blood meals may be at a reproductive disadvantage relative to other individuals with more complete meals. In this case, one would expect that partially fed mosquitoes would attempt to refeed. This sort of behaviour would increase the average rate of host contact for a population of mosquitoes relative to one in which mosquitoes feed once only each gonotrophic cycle, without regard to blood-meal volume. The contact rate of mosquito vectors and their vertebrate hosts is a significant determinant of vectorial capacity (Dye 1992). However, the extent of multiple feeding by *Culex* vectors of encephalitis viruses and the contribution of multiple feeding to virus transmission has not been explicitly worked out. Furthermore, the possible effect of feeding-associated mortality on feeding persistence has not been studied for *Culex* vectors of encephalitis. Consequently, the relationship between host behaviour/host-induced mortality and partial/multiple feeding by mosquitoes deserves more attention with regard to reproductive success of these blood feeders and pathogen transmission.

CHAPTER 1

Field Evidence for Multiple Host Contacts During Blood
Feeding by *Culex tarsalis*, *Culex restuans* and *Culex
nigripalpus* (Diptera: Culicidae)

ABSTRACT

Field studies were conducted in Manitoba, Canada, and
Florida, United States, to evaluate the frequency of
multiple host contacts by *Cx. tarsalis*, *Cx. restuans*, and
Cx. nigripalpus, primary and secondary vectors of several
encephalitis viruses in North America. Blood-fed mosquitoes
were collected from box traps, each baited with a pair of
quail (Japanese quail in Manitoba, northern bob-white in
Florida). One quail of each pair was injected with rubidium
and the other with cesium to permit the determination of the
source(s) of each blood meal. Approximately 5% of all
blood-fed *Cx. tarsalis*, *Cx. restuans*, and *Cx. nigripalpus*
ingested blood from both quail during overnight exposure.
The frequency of multiple feeding by these species ranged
from 0 to 18.5%, 0 to 33.3% and 0 to 17.6%, respectively.
Of the 331 mosquitoes that ingested blood from two quail, 57
(17.2%) were not fully blood fed. Also, 1.0-3.5% of blood-
fed mosquitoes had ingested blood before entering the quail-
baited traps. As a result of the fact that virus
transmission increases with the square of the biting rate,
any increase in the number of host contacts as a result of
multiple feeding, however modest, may increase
disproportionately the rate at which virus is transmitted.

INTRODUCTION

Some mosquitoes attracted to bait hosts have ingested blood recently (Mitchell and Millian 1981, Trpis and Hausermann 1986). It has been shown repeatedly in blood-meal identification studies that many North American *Culex* vectors of encephalitis viruses ingest blood from more than one type of animal in a single gonotrophic cycle (Cupp and Stokes 1976, Edman and Downe 1964). In the first major study to document multiple feeding by many species of mosquitoes (Edman and Downe 1964), the authors speculated that mosquitoes may have been induced to take multiple meals because one of the meals (presumably the first) was taken from a less acceptable host, and, consequently, the mosquitoes switched to another animal. In later studies, the importance of host-defensive behaviour as a factor that induced mosquitoes to interrupt feeding was documented (Edman and Kale 1971, Edman and Scott 1987). However, there are no published studies that address the question of whether *Culex* species take multiple meals when two or more individuals of the same species of bird are available in close proximity to each other.

I use the term, multiple feeding, to describe the situation in which a mosquito ingests some blood from at least two hosts during a single gonotrophic cycle. This is distinct from the situation in which a mosquito is interrupted during blood uptake, but returns to the same host to complete the blood meal. Two meals are involved in each situation, but multiple feeding involves two separate hosts. Multiple feeding during a single gonotrophic cycle may occur for either of two distinct reasons. In one case, two or more hosts may be bitten if mosquitoes are prevented from acquiring sufficient blood from one host to induce neural and hormonal mechanisms which inhibit further blood

feeding (Klowden 1988). Interruption of blood uptake before satiation is associated most commonly with defensive behaviour by the hosts (Davies 1990, Edman and Scott 1987). Alternatively, species that require several blood meals for oogenesis or for metabolic reserves (gonotrophically discordant) may continue to seek hosts (perhaps daily) between one oviposition event and the next. This is the case for some *Anopheles* (Klowden and Briegel 1994) and *Aedes aegypti* (Scott et al. 1993, Trpis and Hausermann 1986). Multiple feeding of gonotrophically concordant species (those that require only one blood meal per reproductive cycle) is likely a result only of the first mechanism, whereas either or both mechanisms may affect the feeding frequency of discordant species.

The objective of my study was to determine whether *Cx. tarsalis*, *Cx. restuans*, and *Cx. nigripalpus* take multiple meals when more than one individual of the same species of host is available. Such information would provide the basis for conclusions as to whether multiple feeding on conspecific hosts is a possibility that has been overlooked in previous blood-feeding studies based on serological methodology.

MATERIALS AND METHODS

Following the preliminary work of Kimsey and Kimsey (1984), in which rubidium was used as a host-blood marker, Anderson et al. (1990) developed a blood-marking technique in which rubidium was injected into one of two chickens and cesium was injected into the other. Pairs of birds marked in this way were made available to host-seeking mosquitoes and the blood meals assayed for the presence of both rubidium and cesium. This technique permitted the identification of mosquitoes that had obtained blood from

one or both birds in the pair, although interrupted meals resumed on the same host were not detectable.

The technique of Anderson et al. (1990) was used to study blood feeding by wild mosquitoes at Delta Marsh (in Manitoba, Canada) during 1991 and at Winnipeg during 1993. Delta Marsh is a large freshwater marsh (>20,000 ha) at the south end of Lake Manitoba. The Winnipeg site is located on the University of Manitoba campus along the Red River. Both sites were observed to harbor passerine birds and mosquitoes during the summer.

Plywood box traps (30 cm by 30 cm by 30 cm) (Fig. 1) with baffled, slotted entrances (narrowing from 30 cm by 8 cm to 30 cm by 2 cm) on the underside and a surgical stocking sleeve on one side were used to capture mosquitoes attracted to the quail. The baffled entrances were constructed of fine mesh (1 mm by 1 mm) to permit downward movement of host odours while restricting the ability of the mosquitoes to escape once in the cage. Traps were suspended ~1 m above the ground on the edge of wooded areas at each location. Traps were baited with pairs of numbered Japanese quail, *Coturnix japonica* Temminck & Schlegel (Saskatchewan wild type, Quail Genetic Stock Centre, University of British Columbia). Quails were 8-12 wks old and weighed, on average, 120 g. Overall, 102 pairs of quail were used in 1991, and 40 pairs were used in 1993. Japanese quails were used to attract mosquitoes because they are small, easy to handle, and readily available. An avian host was considered necessary because *Cx. tarsalis* and *Cx. restuans* feed on birds in nature (Washino and Tempelis 1983) and I was interested in an animal model that would approximate the natural hosts of both species of *Culex*. A preliminary attempt to trap and use wild, yellowheaded blackbirds (*Xanthocephalus xanthocephalus* (Bonaparte)) was not successful because the blackbirds could not be adapted to

handling and too few were trapped to be of use.

Quails were placed in cylindrical, wire cages (25 cm long by 15 cm diameter, mesh size 1.3 cm by 1.3 cm) (Fig. 1), which were inserted through the stocking sleeve and placed between the baffles in the box traps (Fig. 1). These small cages prevented the quails from eating mosquitoes that rested on the inside of the box traps; however, the quails had sufficient room to turn around, stretch, and groom themselves. One member of each pair of quails was injected with rubidium (500 mg/kg) and the other with cesium (750 mg/kg) according to the methods of Anderson et al. (1990) to document multiple feeding.

Box traps were placed in their field locations ~30 min before sunset and were collected within 30 min of sunrise. At collection, the no-return entrances were sealed with foam rubber plugs and the quail cages were removed through a sleeve of surgical stocking. Box traps were placed in a freezer at -20C to kill the mosquitoes. New quails were used each night.

The same method was used to collect blood-fed *Cx. nigripalpus* in Florida in August of 1992, except that northern bob-white, *Colinus virginianus* (L.), were used as bait animals to attract *Cx. nigripalpus* and to measure multiple feeding by this species. Japanese quails were not available at the Florida research site. The average weight of the northern bob-white was 95 g. Twenty-six pairs of bob-white were used during this part of the study. All mosquitoes were collected in the hardwood hammock that surrounds the Florida Medical Entomology Laboratory at Vero Beach. Box traps were of similar design to those used in Manitoba, except that they were made from clear acrylic (plastic) rather than plywood. Exposure times also were from before sunset to after sunrise, but the number of hours exposure was not equivalent because of significant

latitudinal differences between central Florida and
Manitoba. For example, sunset in Manitoba was approximately
2100 h and sunrise was approximately 0500 h, whereas sunset
was approximately 1900 h and sunrise approximately 0600 h in
Florida.

Mosquitoes collected from all quail-baited traps were
identified and blood-fed individuals were retained for
rubidium and cesium analysis by atomic emission flame
spectrophotometry (Anderson et al. 1990). Prior to analysis
for rubidium and cesium, blood meals were assigned to one of
four size classes: trace, one-quarter full, one-half full,
and full according to the criteria of Edman et al. (1975) to
provide information on the extent of multiple feeding by
partially and fully fed mosquitoes. The mosquitoes
collected in one box trap during a given sunset-to-sunrise
collection period were defined as a sample. Thus, each
sample provided a replicate measure of the frequency
(expressed as per cent of blood-fed mosquitoes) of multiple
host contacts. Only blood-fed mosquitoes positive for
either rubidium or cesium were included in the number of
blood-fed mosquitoes per sample. The few blood-fed
mosquitoes in some of the samples that were negative for
both rubidium and cesium presumably obtained blood from
other sources and were excluded from the blood-fed
categories described above.

The frequency of multiple feeding per sample was
calculated as the number of blood-fed mosquitoes with both
rubidium and cesium, divided by the total, marked, blood-fed
individuals of that species. Initially, all blood-fed *Cx.
tarsalis* collected in 1991 were lumped together to calculate
the overall frequency of multiple feeding by this species.
The same approach was used for *Cx. nigripalpus* collected in
1992 and for *Cx. restuans* and *Cx. tarsalis* collected in
1993. Estimates of the range in frequency of multiple

feeding were based on samples in which at least 17
mosquitoes blood fed on the quail. According to the
binomial expansion, 17 is the minimum number of blood-fed
mosquitoes per sample for which an increase of one multiple
blood meal does not result in rejection of the null
hypothesis that the true frequency of multiple feeding is
5%. In other words, samples smaller than 17 were considered
unreliable. The overall frequency of multiple feeding by
Cx. tarsalis, *Cx. restuans* and *Cx. nigripalpus* was close to
5% for each species based on combining the data within each
species collection. The mean, standard error and confidence
limits of the frequency of multiple feeding by species and
year of collection were calculated directly from the
quotients of number of two-host meals divided by the number
of quail-fed mosquitoes multiplied by 100. The means of the
percentages of multiple feeding for each species collection
were compared by analysis of variance. Confidence limits of
the percentage of multiple feeding that resulted in partial
blood meals were calculated from the binomial expansion
(Sokal and Rohlf 1981).

The potential impact on arbovirus transmission of an
increase in the biting rate of mosquitoes due to multiple
feeding was evaluated with an epidemiological model
previously used by Smith (1987) to calculate the vectorial
capacity of *Cx. tarsalis* for Western Equine
Encephalomyelitis virus. I calculated the change in virus
transmission for the range in estimates of multiple feeding
from my study relative to transmission if one assumed no
multiple feeding.

RESULTS

In Manitoba in 1991, 13,857 female mosquitoes were
collected with pairs of marked quail as bait. Of these

mosquitoes, 5,218 were *Cx. tarsalis* and 3,102 (59%) had
ingested blood from at least one quail. In Manitoba in
1993, 4,141 female mosquitoes were collected, of which 2,027
were *Cx. restuans* and 1,764 were *Cx. tarsalis*. Overall,
1,409 (70%) of the *Cx. restuans* and 1,207 (68%) of the *Cx.
tarsalis* ingested blood from at least one quail. In Florida
in 1992, 2,110 female mosquitoes were collected, of which
2,041 (97%) were *Cx. nigripalpus*; 857 (42%) had ingested
blood from at least one quail. Very few (<1% of all
mosquitoes collected in the box traps) were gravid or
teneral (newly emerged) females, or males.

Overall, 331 of 6,575 (5.03%) fed *Culex* took blood from
two quails. The percentage of multiple host contacts by
species were 5.09% of 3,102 fed *Cx. tarsalis* collected in
1991, 4.14% of 1,207 fed *Cx. tarsalis* collected in 1993,
5.39% of 1,409 fed *Cx. restuans* collected in 1993, and 5.48%
of 857 fed *Cx. nigripalpus* collected in 1992. The range in
frequency of multiple blood meals is given for samples with
at least 17 marked, blood-fed mosquitoes in Table 1. The
frequency of multiple blood feeding did not differ
significantly among the species studied.

The number and frequency of mosquitoes that took blood
from both quail, but for which the blood meals were graded
as partial are given by species in Table 2. Multiple host
contacts that resulted in partial meals by *Cx. nigripalpus*
occurred at a significantly greater frequency than for *Cx.
tarsalis* collected in 1991.

In addition to the direct evidence of multiple feeding
by double-marked mosquitoes described above, two sources of
indirect evidence for other mosquitoes with high potential
for refeeding are provided. First, mosquitoes with a half
blood meal or less (partial meals) and both rubidium and
cesium were individuals that had taken blood from two hosts
and were considered likely to refeed again. Edman et al.

(1975) found that *Cx. nigripalpus* with half a blood meal or less were more likely to refeed than were females with greater than a half blood meal. Second, mosquitoes in the box traps that contained fresh blood, but negative for both markers were assumed to be host-seeking, although already partially engorged. The observed number and proportion of blood-fed mosquitoes that had ingested blood before attraction to the quail (unmarked with either rubidium or cesium) are given in Table 3. These estimates do not include previously fed mosquitoes that obtained blood from either quail once they entered the cages. The frequency of unmarked blood meals was greatest for *Cx. nigripalpus* and the 1993 *Cx. tarsalis* collection. More than 85% of unmarked blood meals were partial according to the grading scheme of Edman et al. (1975) (Table 3).

In my study, multiple feeding that involved at least two hosts was found potentially to increase vectorial capacity from 10% to 69% relative to the situation in which no multiple feeding occurred (Figure 2).

DISCUSSION

Evidence of multiple feeding by mosquitoes of many species has been demonstrated in independent studies (Cupp and Stokes 1976, Edman and Downe 1964). These studies were based on serological techniques in which multiple meals could be detected, providing that the components of the multiple meals had been taken from different species of host. Multiple feeding on individuals of the same species of host however, has been demonstrated for only a few anopheline and culicine species feeding on humans with distinct ABO blood groups or haptoglobins (Boreham et al. 1978, Boreham and Lenahan 1979, Burkot et al. 1988). Birds are important hosts for *Cx. tarsalis*, *Cx. restuans*, and *Cx.*

nigripalpus (Washino and Tempelis 1983). Often, a few
species of passerine birds are most important for virus
amplification (Holden et al. 1973). Consequently, multiple
feeding on conspecific birds may be of importance in the
enzootic transmission of virus.

Furthermore, many avian species often aggregate in
colonial nesting areas, at roosts, and at feeding sites
(Weatherhead 1981, 1983), such that many potential hosts of
the same species may be simultaneously available to hungry
mosquitoes. In studies of behavioural interactions between
host-seeking mosquitoes and avian hosts, it has been shown
that birds may interrupt blood feeding such that mosquitoes
potentially may contact more than one host of the same
species in the course of obtaining a full blood meal (Kale
et al. 1972, Webber and Edman 1972).

In my study, *Cx. tarsalis*, *Cx. restuans*, and *Cx.
nigripalpus* took multiple meals from conspecific avian
hosts. Although the overall frequencies were close to 5%,
the maximum observed frequencies ranged from 13.6% for *Cx.
tarsalis* to 33.3% for *Cx. restuans*. Observed proportions of
multiple feeding by mosquitoes on each pair of birds varied
inversely with the relative difference in the probability of
each bird being fed on (Chapter 2). The host-blood marking
technique used in my study is capable only of detecting
multiple meals that contain blood from both hosts. It is
possible that some meals are interrupted and then resumed on
the same individual. This probability increases with the
degree of difference between hosts in terms of the
probability of being fed on (Burkot et al. 1988). However,
from the standpoint of vectorial capacity, mosquitoes that
are interrupted and then resume feeding on the same host
probably do not increase the rate of virus transmission
unless a vertebrate is more likely to be infected as a
result of multiple bites by the same mosquito or unless the

mosquito is more likely to be infected by the greater amount
of blood obtained from two or more meals.

In comparison to the proportions of blood-fed *Culex*
with two-host meals from my study, data from other studies
are as follows. Edman (1974) recorded <1% multiple feeding
by *Cx. nigripalpus* from Florida. Edman and Downe (1964)
recorded overall percentages of multiple meals by 13 species
of mosquitoes in five genera, including *Cx. tarsalis*, (21.5%
multiple), *Cx. salinarius* Coquillett (36.7% multiple), and
Cx. pipiens L.(20% multiple). Cupp and Stokes (1976) noted
that 13% of 328 *Cx.* salinarius took multiple meals. Anderson
et al. (1990) observed that 19% of *Cx. quinquefasciatus* Say
ingested blood from two chickens in the laboratory.
Additionally, multiple feeding by *Culex* mosquitoes on
conspecific hosts is not restricted to ornithophagic
species. For example, Boreham et al. (1978) found that from
7.5% to 19.8% of *Cx. quinquefasciatus* Say collected in
Kisumu, Kenya, imbibed blood from two or more human hosts.
In all of the studies, except that of Anderson et al. (1990)
and Boreham et al. (1978), the separate hosts represented in
the multiple meals were of different species.

The box trap used in my study was designed to retain
mosquitoes during and after blood feeding on the quail, and
this may have resulted in unnaturally high multiple feeding
by keeping the mosquitoes in close proximity to hosts.
Also, my use of quail as model avian hosts may not have
simulated accurately the response of mosquitoes to passerine
birds. However, with one exception (Edman 1974), the
estimates of the frequency of multiple feeding on
conspecific hosts by the species in my study accorded well
with estimates from other studies of multiple feeding by
Culex mosquitoes on natural hosts (Cupp and Stokes 1976,
Edman and Downe 1964).

Despite the potential bias presented by the trap

design, I believe that the frequencies of multiple feeding observed in my study likely represent an underestimate of the frequency of host contacts that involve secretion of saliva. I measured only host contact based on blood uptake. Mosquitoes may salivate into a host without ingesting blood (Ribeiro 1987). Furthermore, many mosquitoes (up to 32% in my study, Table 2) that had made at least two host contacts and ingested detectable amounts of blood were likely to blood feed again because the total amount of blood obtained from two hosts probably was still not sufficient to inhibit further blood feeding (Edman et al. 1975). Additionally, I observed that from 1.0 to 3.5% of blood-fed mosquitoes attracted to the quail had first ingested blood from other sources (Table 3).

My data provide a basis for challenging the assumption of one host contact per mosquito per gonotrophic cycle for the purposes of modeling vectorial capacity (Smith 1987). Multiple host contacts may increase the number of opportunities for individual mosquitoes both to acquire and transmit pathogens. Contact between mosquitoes and vertebrate hosts appears twice in the vectorial capacity model as multiplied terms. Effectively, disease transmission increases as the square of the increase in frequency with which mosquito vectors feed on amplifying vertebrate hosts (Dye 1992). For example, 5% multiple feeding may result in more than a 10% increase in transmission (Fig.2). Vectorial capacity may be underestimated if it is assumed that each mosquito bites only one host each gonotrophic cycle. My contention that transmission may increase as a result of multiple feeding rests on two assumptions. First, I assumed that small meals taken during multiple feeding by uninfected mosquitoes produce infective vectors. Second, I assumed that a single mosquito is capable of delivering virus to more than one

host during serial probing. This assumption is supported by studies where, once infected with Western Equine Encephalomyelitis Virus or Saint Louis Encephalitis Virus, *Cx. tarsalis* are infected for life (Hardy 1987, Henderson et al. 1979, Mitchell et al. 1980) and thus may transmit virus each time they salivate into a susceptible host. According to the data I have presented on multiple feeding, transmission may be enhanced substantially (from 10 to 70%) in situations that favour multiple feeding. Because I was able to demonstrate multiple feeding by three species of North American *Culex* in two geographically distinct locations, I conclude that this behaviour may be possible for many other vector species. More work should be done to document the dynamics of interrupted and multiple blood feeding on similar hosts.

Table 1. Frequency of multiple host contacts by *Cx. tarsalis* and *Cx. restuans* on Japanese quail (Manitoba) and *Cx. nigripalpus* on northern bob-white (Florida).

	% Frequency			
	MEAN[1]±SE	LCL-UCL	Min.-Max	n

Cx. tarsalis				
1991	5.5±0.88	3.7-7.3	0-15.2	25
1993	5.0±1.34	2.2-7.8	0-18.5	20
Cx. restuans	5.5±2.00	1.4-9.6	0-33.3	18
Cx. nigripalpus	6.2±1.02	4.1-8.4	0-17.6	20

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[1]There are no significant differences among the mean percentages by ANOVA. LCL, lower confidence limit; UCL, upper confidence limit; confidence interval = 95%. n, number of samples in which ≥17 mosquitoes blood-fed on ≥1 quail.

Table 2. Number and frequency of multiple blood meals that were partial (those for which the total volume was ≤½).

Species	Partial/Multiple		LCL-UCL
	n	%[1]	95%CI
Cx. tarsalis			
1991	21/158	13.3a	9.0-19.6
1993	8/50	16.0ab	8.6-29.1
Cx. restuans	13/76	17.1ab	10.4-27.5
Cx. nigripalpus	15/47	31.9b	20.9-47.1

[1]Percentages followed by the same letter are not significantly different by the test for equality of percentages (Sokal and Rohlf 1981).

Table 3. Number of blood-fed mosquitoes that acquired blood
(unmarked with rubidium or cesium) before entering traps
baited with quail.

Species	Total[1]/Blood-fed[2]	%[3]	Partial/Total[1]	%[4]
Cx. tarsalis				
1991	39/3141	1.2a	38/39	97
1993	25/1232	2.0ab	24/25	96
Cx. restuans				
	15/1424	1.0a	13/15	87
Cx. nigripalpus				
	31/ 888	3.5b	27/31	87

Numbers followed by the same letters are not
significantly different by the test for equality of
percentages (Sokal and Rohlf 1981).

[1]Total blood-fed mosquitoes in which neither rubidium
nor cesium was detected: defined as unmarked.

[2]Marked + unmarked, blood-fed mosquitoes in quail-
baited traps.

[3]Unmarked blood meals as a percentage of all blood-fed
mosquitoes.

[4]Percentage of unmarked, partial blood meals, according
to the criteria of Edman et al. (1975). No percentages in
this column are significantly different by the test for
equality of percentages (Sokal and Rohlf 1981).

Figure 1. Box trap used to collect blood-fed mosquitoes attracted to marked quails. (A) Trap design. (B) Wire cage for quail. The position of the wire cage within the trap is indicated by an arrow.
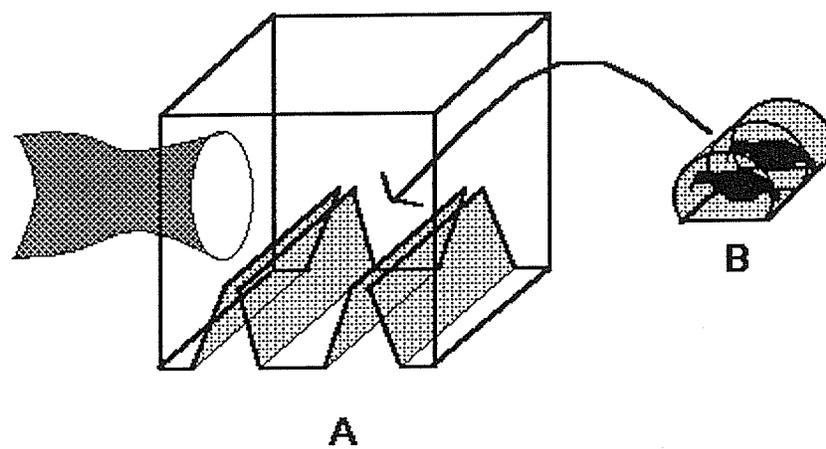
A

B

Figure 2. Relationship between increase in reproductive rate
(R) of an arbovirus during amplification in an avian
population and increase in vectorial capacity caused by
multiple feeding of mosquitoes. The relationship is
calculated for the range in multiple feeding (0-30%)
observed in my study, and according to the formula for
vectorial capacity adapted for *Cx. tarsalis* and WE
transmission by Smith (1987). Increase in transmission is
calculated relative to R=1 for stable transmission. From
Smith's model (1987), two parameters associated with
mosquito-host contact, "M" (average number of mosquitoes per
host per day) and "B" (average number of blood meals per
mosquito per day), have been increased by the proportion
representative of multiple feeding such that the calculation
yields a squared relationship between the increase in host
contacts caused by multiple feeding and the increase in R.

$$Y = 2.0X + X^2$$
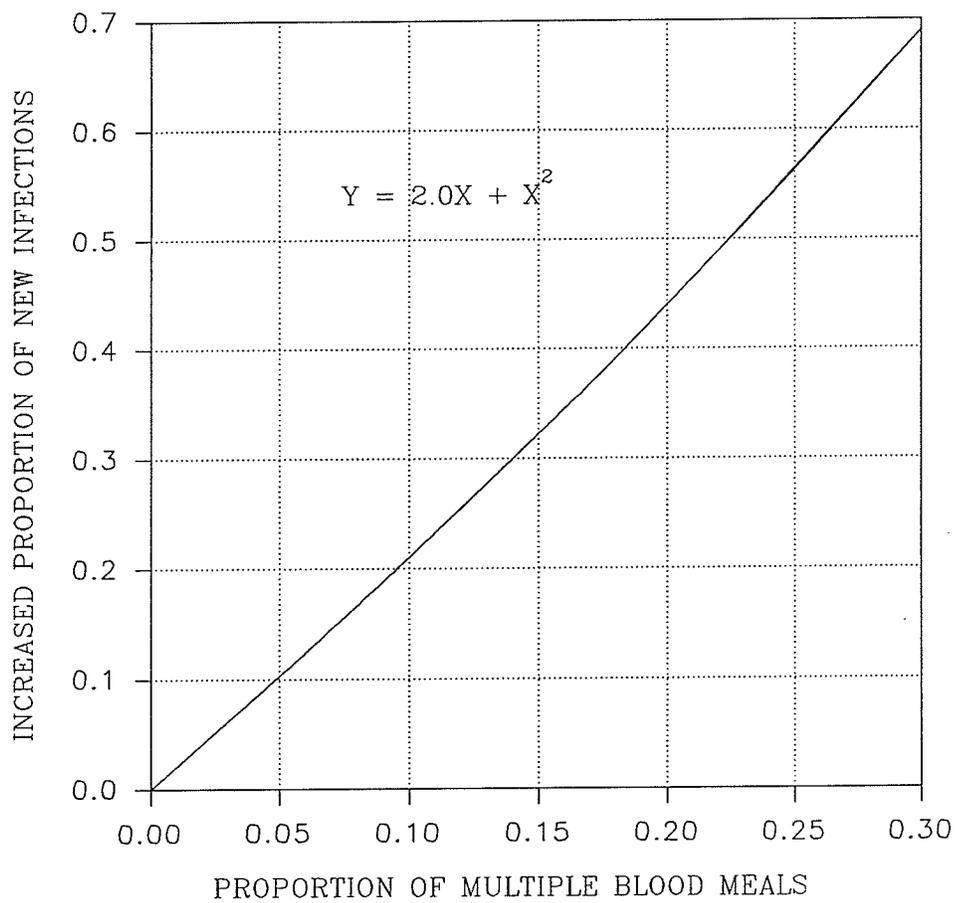
CHAPTER 2

Interrupted Blood Feeding by *Culex* (Diptera: Culicidae) in Relation to Individual Host Tolerance to Mosquito Attack.

ABSTRACT

Field studies were conducted at Delta Marsh and Winnipeg, Manitoba, and Vero Beach, Florida, to examine variation among individual quail hosts in terms of the success of mosquitoes that fed on them. *Cx. tarsalis*, *Cx. restuans* and *Cx. nigripalpus* accounted for most of the blood-fed mosquitoes collected in box traps, each baited with two quail. *Cx. tarsalis* was the predominant blood-fed species collected at Delta Marsh in 1991, *Cx. nigripalpus* was the predominant species collected at Vero Beach in 1992 and both *Cx. tarsalis* and *Cx. restuans* were collected in approximately equal numbers in mixed collections at Winnipeg in 1993. In 48 of 70 samples with adequate numbers of blood-fed mosquitoes collected over the three years, blood feeding was skewed significantly from an even distribution between the two quail in each trap. In 5 of 13 samples of *Cx. tarsalis* collected in 1991, an index of incomplete feeding (proportion of blood meals $\leq$ half full) was from 2 to 8 times greater on one bird relative to the other. In 7 of 13 samples of *Cx. nigripalpus* collected in 1992, an index of incomplete feeding was from 2 to 1400 times greater on one bird relative to the other. This index was from 2 to 300 times greater for one bird relative to the other in 7 of 11 mixed *Cx. restuans*/*Cx. tarsalis* samples collected in 1993. The index of interrupted feeding was related inversely with the probability that the quail would be fed on for both rubidium-marked quails (p=0.0057, r^2=0.19, slope=-0.41) and the cesium-marked quails (p=0.0038,

r^2=0.20, slope=-0.30). The proportion of detectable multiple meals in a given trap night was related negatively (p<0.0001, r^2=0.26, slope=-0.161) with the degree to which the distribution of blood meals in each trap night was skewed away from 0.5 on each bird.

INTRODUCTION

The frequency with which mosquitoes contact vertebrate hosts is an important aspect in the epidemiology of vector-borne pathogen transmission (Dye 1992), particularly for the *Culex* vectors of encephalitis viruses in North America (Day and Edman 1988, Smith 1987). In at least two published models of pathogen transmission that involve *Culex tarsalis* Coquillett, an important North American arbovirus vector, it is assumed that there is only one host contact per mosquito per gonotrophic cycle (Scott et al. 1983, Smith 1987). Based on laboratory studies with *Aedes aegypti* (L.), Klowden (1988) described some of the physiological mechanisms, including abdominal stretch receptors and hormone-mediated, oocyte-induced inhibition, that under ideal conditions would limit a gonotrophically concordant species to one blood meal per reproductive cycle. However, he also indicated that under natural conditions, these mechanisms do not always work. Several *Culex* species may feed more than once each cycle and, in so doing, contact more than one host (Chapter 1, Anderson et al. 1990, Boreham et al. 1978, Edman and Downe 1964). Edman et al. (1972) demonstrated that avian hosts may interrupt blood feeding *Cx. nigripalpus* before the mosquitoes acquired sufficient blood to inhibit further feeding (Edman et al. 1975). This provided evidence that the rate with which mosquitoes are interrupted by their hosts is an important determinant of the frequency of mosquito-host contact.

Burkot et al. (1988) used an extended form of a model proposed by Boreham and Garrett-Jones (1973) to calculate the expected frequency of interrupted blood meals, including cryptic meals (by their definition, those in which serial feeding attempts involve individuals of the same host species). Boreham and Garrett-Jones (1973) used serology to

identify the proportions of patent, one-host and multiple-host blood meals taken by *Anopheles sacharovi* Favre. They used these proportions to calculate the expected frequency of cryptic, multiple blood meals. Burkot et al. (1988) pointed out that Boreham and Garrett-Jones (1973) implicitly assumed that the probability of interruption on each of the hosts was equal and that if this assumption is true, it is possible to estimate the overall probability of interruption from the proportions of detectable, multiple meals. In this usage, interrupted meals included those in which feeding was broken off and not resumed, those in which feeding was broken off, but resumed on the same host (cryptic multiple meals of Burkot et al. 1988) and those in which feeding was broken off and then resumed on the other host (patent multiple meals). The formula of Burkot et al. (1988) is $I_H = I_N =$ proportion of patent, mixed blood meals/$2Q(1-Q)$ where I_H and I_N are equal to the probability of the human host and the non-human host, respectively, interrupting a feeding mosquito, and Q is the probability of the human host being fed on. The model is based on two hosts (one human and one non-human) being available.

Although the method described above might be adapted to calculate the probability of interruption for *Culex* species from published, feeding studies, Edman et al. (1972) showed a correlative link between the probability of partial (assumed by the authors to have been interrupted by host-defensive behaviour) blood meals taken by *Cx. nigripalpus* and the intensity of anti-mosquito, host-defensive behaviour. Furthermore, there is evidence that feeding success (proportion of mosquitoes with some blood) of *Cx. nigripalpus* on different individual hosts of the same or different species is correlated with the intensity of anti-mosquito behaviour exhibited by each individual (Edman et al. 1972, Kale et al. 1972). In the context of these

studies, it seemed unsafe to use the mathematical method of
Burkot et al. (1988) to calculate the frequency of
interrupted feeding of *Culex* mosquitoes without independent
verification of the key assumption of their model.

The objective of my field study was to examine
variation in the frequency with which individual bird hosts
of the same species are fed on by three species of *Culex*
when the hosts are of the same age, sex, size and health
status and when two hosts are in close proximity to each
other. Another objective was to use the physical criterion
of blood-meal size (specifically, meals ≤ half of a full
meal) to calculate an index of the frequency of interrupted
blood meals on individual hosts and to assess the validity
of assuming that the probability of interruption is equal
among hosts. Additionally, I analyzed variation in the
frequency of multiple feeding by *Culex* (Chapter 1) in
relation to variation in the degree to which mosquitoes were
able to feed on individual hosts.

<p style="text-align:center">MATERIALS AND METHODS</p>

Details of collection sites, methods for estimating the
frequency of multiple feeding and species of hosts and
mosquitoes studied were given in Chapter 1. I extended
analysis of the frequencies of multiple feeding reported in
that chapter. Methods and data described in the current
chapter pertain to blood-meal size and the blood-feeding
frequency on individual hosts.

Pairs of quail were exposed to field populations of
mosquitoes. Quail did not vary significantly in weight and
all were in good health at the time of use. Quail paired
together were of the same age as they had been reared
together from a common cohort of eggs. Males were paired
with males and females were paired with females, although

individual quail were selected randomly. Each quail was used once only. One quail in each pair was injected with rubidium chloride and the other was injected with cesium chloride as blood markers according to the method of Anderson et al. (1990).

All mosquitoes were identified and fed specimens retained. The size of each blood meal was scored as partial ($\leq\frac{1}{2}$) or full ($>\frac{1}{2}$) according to the criteria of Edman et al. (1975). Blood meals were analyzed for the presence of rubidium and cesium by atomic emission flame spectrophotometry (Anderson et al. 1990). A blood meal was defined as simple if it was positive for only one marker, multiple if positive for both markers. Mosquitoes with a simple, partial meal were assumed to have been interrupted once by the correspondingly marked host. Full, simple meals were assumed to represent uninterrupted feeding to satiation by the mosquitoes. Multiple meals were interrupted, but then resumed on the second host, although I could not determine the order in which the respective quail were attacked, or if in fact more than two feeding attempts were required to complete a blood meal.

The proportion of blood meals from a given quail in a trap night was calculated as the number of meals with the corresponding mark divided by the total meals on both quail (number of simple meals from both birds + two times the number of mosquitoes with multiple meals). For the purposes of this calculation, mosquitoes with one marker were assumed to have taken one meal, while those positive for both markers (multiple meals) were considered to have taken two meals, thus the factor of two in the denominator above. The proportions of blood meals taken by *Culex* mosquitoes from each host in each cage were plotted by year of collection to illustrate the frequency with which blood feeding was distributed among individual quail and to show that a

similar pattern could be demonstrated for different species of mosquitoes collected in two geographical locations and with two species of quail. For each sample size (represented by the number of blood meals per trap night), I calculated the binomial probability of obtaining the proportion representing the actual distribution of blood meals between the two quail, assuming the true value to be 0.5. The observed proportion was considered significantly different from 0.5 if the binomial probability was <0.05 (2-tailed). Trap nights with <17 blood-fed mosquitoes were not included in this analysis because sample sizes smaller than this were considered unreliable (Chapter 1).

An index of the likelihood that a given quail would interrupt feeding mosquitoes was calculated. A weighted estimate was calculated that accounted for partial meals (those that met the physical criteria of having been interrupted according to the definition of Edman et al. 1975) and multiple meals (because multiple meals must have been interrupted between hosts). This index was calculated by an iterative process as follows. For the multiple meals, and in the absence of physical criteria for deciding which quail had been fed on first, and thus had interrupted the mosquito, a probability of 0.5 was initially assigned to each of the quail. Thus, the first estimate of the probability of interruption for each quail in a pair was calculated by dividing the number of partial, simple meals + one half of the multiple meals by the sum of the number of partial, simple meals + 0.5 of multiple meals + the number of full, simple meals. Before the next iteration, these estimates, designated p1 and p2 for quail 1 and quail 2 respectively, were then used to calculate a new relative weighting of the proportion of multiple meals that had been interrupted by each of the two quail. The weighting for p1 was calculated as p1 divided by the sum of p1 + p2. The

weighting for p2 was calculated as 1-(weight of p1). The
estimate of the probability of interruption for each quail
was recalculated as in the first step except that the weight
was changed from 0.5 to the value calculated above. Each
time new values of p1 and p2 were calculated, they were used
to calculate a new weight for the proportion of multiple
meals interrupted on each of the quail. The process was
repeated until the difference between successively
calculated values of the index (for each quail) was less
than 0.00001.

The indices of interruption for both quail in each pair
were compared as a ratio of the greater-to-lesser value for
each trap night to show the relative magnitude of the
difference between individual quail. The frequency
distribution of this ratio was used to calculate the
probability with which differences in the rate of
interruption would occur for a given pair of quail. Only
trap nights with ≥10 engorged mosquitoes from each quail
were used for this analysis because, for small sample sizes,
one individual can account for a large change in a
proportion.

With regard to the probability that each bird would be
fed on, for a two-host treatment, my null hypothesis was
that 0.5 of the blood meals would come from each bird.
Therefore, if the proportion of blood meals from a given
bird deviated from 0.5, I expected to see a corresponding
decrease in the proportion of multiple meals because a
larger proportion of interrupted meals would be expected to
be completed on the bird on which they had been commenced
(Burkot et al. 1988). Accordingly, I used regression
analysis to examine the relationship between the probability
of detecting multiple meals and the deviation from an even
distribution of blood meals on each bird by trap night.

RESULTS

Seventy box trap collections (trap nights) had $\geq$ 17
blood fed *Culex* (Chapter 1). Twenty-five of these trap
nights occurred in 1991 at the Delta Marsh site, at which
Cx. tarsalis was the dominant species (>80% of all
mosquitoes), both in terms of total number of mosquitoes and
number of blood-fed mosquitoes. Twenty trap nights occurred
in 1992 at Vero Beach, where *Cx. nigripalpus* comprised >95%
of the total. Twenty-five trap nights occurred in 1993 at
Winnipeg, where *Cx. tarsalis* and *Cx. restuans* were
represented in approximately equal proportions and often
were collected together in the same traps. The blood-
feeding frequency of *Cx. tarsalis* (68% of 1,764) collected
in 1993 did not differ significantly (p>0.05) from that of
Cx. restuans (70% of 2,027) from the same collections.
Combined, these two species accounted for >90% of all
mosquitoes collected in 1993. As the primary purpose of
this study was to examine variation among individual hosts
with respect to its effect on mosquito blood feeding
success, and as there were no significant differences in
the feeding frequency of *Cx. restuans* and *Cx. tarsalis*
collected in 1993, I presented data from that year for both
species combined.

For *Cx. tarsalis* collected in 1991, the proportion of
blood meals taken from one of the two quail in each box trap
was 0.047 to 0.954 (Fig.3A). In 18 of 25 trap nights, the
distribution of blood meals deviated significantly from 0.5
(Fig.3A). Not all of the apparently skewed proportions
deviated significantly from 0.5, because confidence limits
of proportions increase with decreasing numbers of
individuals collected (Fig.3A).

For *Cx. nigripalpus* collected in 1992, the proportion
of blood meals taken from one of the two quail in each trap

was 0.154 to 0.778 (Fig.3B). The distribution of blood
meals deviated significantly from 0.5 in 6 of 20 trap nights
(Fig.3B).

For the *Cx. tarsalis/Cx. restuans* combined collections
from 1993, the proportion of blood meals taken from one of
the two quail in each trap was 0 to 1.0 (Fig.3C). The
distribution of blood meals deviated significantly from 0.5
on each bird in 23 of 25 trap nights (Fig.3C). The catch of
fed *Cx. tarsalis* in 1991 and *Cx. tarsalis/Cx. restuans* in
1993 was higher than for *Cx. nigripalpus* collected in 1992
(Fig.3).

A total of 40 trap nights from the three years of
collection contained ≥10 blood fed mosquitoes marked with
rubidium and ≥10 marked with cesium. Fifteen of these were
trap nights with *Cx. tarsalis* collected in 1991. The index
of interrupted blood meals was 0.0 to 0.57 for the rubidium-
marked quail and 0.0 to 0.65 for the cesium-marked quail
(Fig.4A). In 5 of 13 cases, the index of interrupted blood
meals was from 2 to 8 times greater for one bird relative to
the other in a given pair (Fig.4A). In two of the trap
nights, a ratio could not be calculated because the
denominator was zero.

Fourteen trap nights with *Cx. nigripalpus* collected in
1992 contained ≥10 blood fed mosquitoes marked with rubidium
and ≥10 marked with cesium. The index of interrupted blood
meals was 0.0001 to 0.65 for the rubidium-marked quail and
0.0 to 0.61 for the cesium-marked quail (Fig.4B). In 7 of
13 cases, the index of interrupted blood meals was from 2 to
>10 times greater for one bird relative to the other in a
given pair (Fig.4B).

For 11 trap nights with ≥10 rubidium-marked and ≥10
cesium-marked *Cx. tarsalis* and/or *Cx. restuans* collected in
1993, the index of interrupted blood meals was 0.0006 to 1.0
for the rubidium-marked quail and 0.12 to 0.62 for the

cesium-marked quail (Fig.4C). In 6 of 11 cases, the index
of interrupted blood meals was from 2 to ≥10 times greater
for one bird relative to the other in a given pair (Fig.4C).

The index of interruption was found to underestimate
the actual probability of interruption when the iterative
calculation was used in a simulation of the behaviour of the
index.

The index of interruption for each quail was related
negatively with the probability that the quail would be fed
on for both rubidium-marked quails ($p=0.0057$, $r^2=0.19$,
slope$=-0.41$) and the cesium-marked quails ($p=0.0038$,
$r^2=0.20$, slope$=-0.30$). The proportion of detectable
multiple meals was correlated inversely with the degree of
deviation from equal blood feeding success on each quail
($p<0.0001$, $r^2=0.26$, slope$=-0.161$) (Fig.6).

DISCUSSION

Individual quail of the same species varied
significantly in the probability of being fed on by
mosquitoes. This pattern was repeatable across at least two
species of quail and in two different geographical
locations. In the most extreme cases in my study, some
quail were not fed on at all, while others accounted for all
of the blood meals in a given trap. It is possible that the
observed patterns of blood feeding were due to differential
attractiveness of individual birds; however, I controlled
for age, size, gender and health by ensuring that quail
within pairs were equivalent with respect to these
characteristics. These are four of the five host
characteristics identified by Edman and Scott (1987) as
important in determining attractiveness and feeding success
of mosquitoes. The fifth characteristic was individual
tolerance to mosquito attack. If there was no difference in

tolerance to mosquito attack between the birds in a trap, I would have expected equal proportions of blood meals from each bird in each cage. Although my experimental design did not rule out subtle differences in attractiveness (not associated with the criteria identified by Edman and Scott 1987) within pairs of quail, most likely, the observed variation in the probability of individual quail being fed on was a result of variation in the intensity of anti-mosquito defensive behaviour, as I observed in a separate laboratory study (Chapter 3) and as observed by Kale et al. (1972) for several species of ciconiiform birds. Rossignol and Rossignol (1988) showed with a simulation model, that a bias in relative feeding success of mosquitoes toward one of two hosts is possible in the absence of a difference in attraction if feeding is easier on one host relative to the other.

The pattern of deviation in the distribution of blood meals between two possible hosts was similar for the three years in which mosquitoes were collected. Confidence limits of proportions increased in width as the number of individuals counted declined. Therefore, it was more difficult to reject a null hypothesis that the proportion of blood meals from each bird was equal to 0.5 for trap nights with small numbers of blood-fed mosquitoes. This may explain why relatively fewer (6 of 20) of the 1992 trap nights deviated in a statistically significant manner from an even distribution of blood meals compared with the number of 1991 and 1993 trap nights that did so (18 of 25 and 23 of 25, respectively). Another possible explanation for the larger number of skewed proportions in the 1991 and 1993 collections compared with 1992 is that greater densities of mosquitoes may exacerbate differences among birds. The quails used in 1991 and 1993 were subject to greater average densities of attacking *Culex*. Alternatively, the smaller

amount of variation in the 1992 samples may have been due to differences between species of mosquitoes in the way in which they responded to the defensive behaviour of hosts. *Cx. nigripalpus* were collected in Florida in 1992, whereas *Cx. tarsalis* were collected in Manitoba in 1991 and mixed collections of *Cx. tarsalis* and *Cx. restuans* were collected in Manitoba in 1993. It is also possible that the strain of Japanese quail used in Manitoba was more variable in behaviour than was the strain of northern bob-white used in Florida. Quarantine regulations prevented transport of Japanese quail from Canada to the United States and northern bob-white from Florida to Canada so that I could not conduct field experiments to control for these possibly confounding variables. When *Cx. nigripalpus* were allowed to feed on Japanese quail, the proportion of blood meals from one bird in a cage could vary from near zero to one (Chapter 3). This may be cautiously interpreted as evidence that the major source of variation stemmed from the behaviour of individual quail and not the species of mosquito. Although I did not use wild birds as bait, there is variation in feeding success of mosquitoes on such hosts (Dow et al. 1957, Kale et al. 1972). I demonstrated that variation among individuals of the same species, size, age, gender and health status may be an important factor that affects the blood-feeding dynamics of wild populations of host-seeking mosquitoes.

The index of the proportion of mosquitoes with interrupted blood meals varied significantly (0 to 1.0) for individual quail in my study. The iterative calculation of this factor was adopted so that the information from multiple meals could be used to estimate the proportion of interrupted blood meals on each quail, but also because it reduced the bias in this estimate associated with the assumption that multiple meals were equally likely to have

been interrupted by each of the two quail. This assumption
was initially necessary because I had no way of telling in
which order the birds had been fed on by the mosquitoes with
two-host meals. However, if a weighting algorhythm had not
been used, this assumption would have introduced a bias
because of the weight associated with the number of multiple
meals relative to partial meals in both the numerator and
the denominator. Additionally, the assumption that multiple
meals were equally likely to have been interrupted by both
quail was hard to justify if one considered the blood meal
distribution data presented in Fig. 3. The iterative
calculation had the effect of weighting the multiple blood
meals based on the observed proportions of partial meals for
a given quail. In about half of the trap nights tested (18
of 37), the index for one quail was at least twice as large
as for the other quail. I interpreted this as evidence that
hosts are as likely as not to be dissimilar with respect to
their tendency to interrupt feeding mosquitoes. I could not
detect cryptic, multiple meals interrupted and resumed on
the same host with my marking technique so no attempt was
made to estimate the probability of this type of event.

My results have two important implications. First,
variation in the degree to which individual hosts tolerate
mosquito attack may be a important factor that determines
the average host contact rate of mosquitoes from one
location to another because those interrupted during
feeding may resume on other hosts (Chapter 1, Edman and
Downe 1964). Second, the method of Burkot et al. (1988) for
calculating the probability of blood meal interruption is
not likely to be reliable for *Culex* mosquitoes that feed on
birds because one of the key assumptions (that different
hosts interrupt mosquitoes at the same rate) is often
violated. This caution is reinforced when one considers my
data (Fig.5) and the study by Edman et al. (1972), in which

individual avian hosts varied in the degree to which they tolerated mosquito attack. In the study by Edman et al. (1972), fewer mosquitoes overall, obtained blood and more took partial blood meals from the most defensive avian hosts.

Burkot et al. (1988) calculated the overall probability of interruption (including cryptic double meals from the same host) for three species of *Anopheles* in Papua New Guinea based on the theoretical relationship between the observed proportion of patent, multiple meals and the overall proportion of meals from each host and based on the assumption that the probability of interruption was equal for each host. Scott et al. (1993) used the same equation to calculate the probability of interruption for *Ae. aegypti*. However, no data were presented in either study to support the assumption that different hosts had the same tendency to cause mosquitoes to break off blood feeding, even though the probabilities of each type of host being fed on varied considerably from location to location. In both of these studies, more than one type of host as well as more than one individual host of each type were involved with the attendant potential for variation among hosts operating at two levels. I question the reliability of theoretical estimates of the frequency of interrupted feeding that rest on the assumption that different hosts are equally likely to interfere with feeding mosquitoes. I recommend that researchers be alert for a possible correlation between blood-meal size and the proportion of mosquitoes that feed on different hosts before they assume that the probability of interruption is equal across hosts.

The iterative method used for calculating an index of interruption resulted in underestimates of the actual probability of interruption when this process was simulated, however the weak negative relationship between the index of

interruption and the probability of being fed on was similar
to that obtained by Edman et al. (1972) when they used only
the proportion of all blood meals that were partial as an
estimate of the probability of interruption. The point of
this exercise in my study was to demonstrate that there is a
possible relationship between the probability of a specific
host being fed on and its probability of interrupting a
feeding mosquito. Following from this, it would not be
appropriate to assume that different hosts interrupt
mosquitoes with the same probability, especially in cases
where the probability of feeding on each of two alternate
hosts is different. This assumption was made by Burkot et
al. (1988) for data from *Anopheles* spp. that had fed on
human and non-human hosts.

I have shown that a significant amount of variation in
the observed frequencies of multiple feeding is correlated
with relative differences in the degree to which individual
quail are fed on. Burkot et al. (1988) calculated that
patent multiple feeding is expected to be maximal when the
probability of feeding on either of two hosts is equal (i.e.
0.5). Explicitly, if both hosts have some probability of
interrupting mosquitoes, but one host does not allow any
amount of feeding (intolerant) relative to the other,
probability does not favor an interrupted meal begun on the
tolerant host being successfully resumed on the intolerant
host. It is more likely that, if a mosquito is interrupted
by the tolerant host, the meal will be successfully resumed
on the same host. Thus, two-host, multiple meals are likely
to result only for those meals initially interrupted by the
intolerant host. Alternatively, if both hosts are
relatively equal in tolerance to mosquito attack, an
interrupted meal on either host is equally likely to be
resumed on either of the two hosts. In this case, two-
host, multiple meals can occur in two ways compared with the

one way described for the previous situation. My data were consistent with this model, but the poor fit of the regression to the data left open the possibility that there were other factors that also determined the detected frequency of multiple feeding. One implication of the relationship presented in Fig.6 is that the host contact rate of mosquitoes may increase substantially in habitats in which the most available hosts are uniformly intolerant of mosquito attack.

Multiple feeding may occur by two fundamentally different mechanisms. Gonotrophically discordant species such as *Anopheles* (Klowden and Briegel 1994) may blood feed several times between egg batches to supplement nutritional reserves. Also, mosquitoes may contact more than one host because an earlier meal was interrupted before satiation (Klowden 1988), usually by host defensive behavior (Davies 1990). In the latter situation, serial feeding attempts are likely to occur within one or a few hours on the same night (Edman et al. 1975). For gonotrophically concordant species, refeeding avidity tends to decrease as the delay between serial meals is increased (Edman et al. 1975). Multiple feeding by *Cx. tarsalis*, *Cx. restuans* and *Cx. nigripalpus* was most likely a result of meals interrupted by host behaviour because these species of mosquitoes are gonotrophically concordant (Clements 1992). In either situation, even relatively low frequencies of multiple host contacts may be important because of additional opportunities for the mosquito to acquire or transmit pathogens (Smith 1987, Chapter 1).

Figure 3.   Distribution of mosquito blood feeding on each of
two quail for each trap night with ≥17 blood fed, marked
Culex mosquitoes.   Each bar represents one trap night.
Black bars represent the proportion of blood meals marked
with rubidium.   White bars represent the proportion of blood
meals marked with cesium.   Trap nights were sorted by
magnitude of the proportion value that represents the split
between the blood meals from the rubidium and cesium-
injected quail.   Bars capped by solid circles denote trap
nights in which the proportions of blood meals from each
bird were significantly different from 0.5.   The number of
blood-fed, marked mosquitoes for each trap night is given by
the height of the crosshatched bar in the bottom graph.   A)
1991 collections made with Japanese quail as bait: all
blood-fed mosquitoes shown were *Cx. tarsalis* (25 trap
nights), B) 1992 collections made with northern bob-white as
bait: all blood-fed mosquitoes shown were *Cx. nigripalpus*
(20 trap nights), C) 1993 collections made with Japanese
quail as bait: mixed *Cx. tarsalis* and *Cx. restuans* (25 trap
nights)

PROPORTION (█ RB. ☐ CS)

1.00
0.75
0.50
0.25
0.00

A.   B.   C.

BLOOD-FED MOSQUITOES PER TRAP NIGHT

600
400
200
0
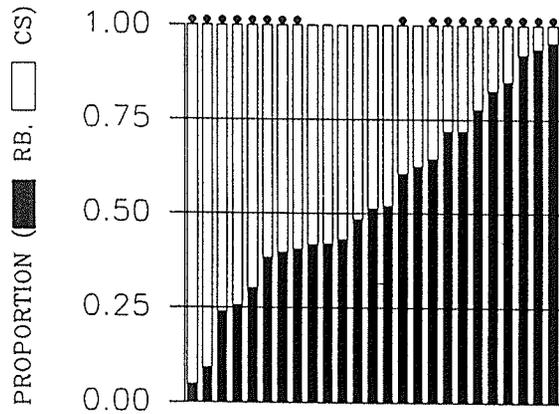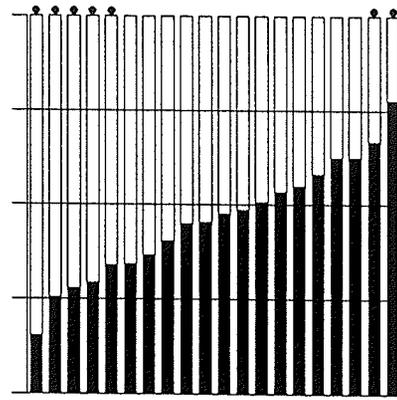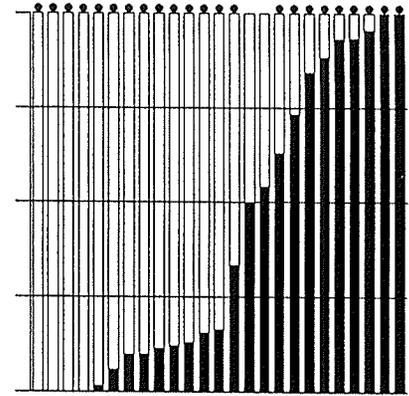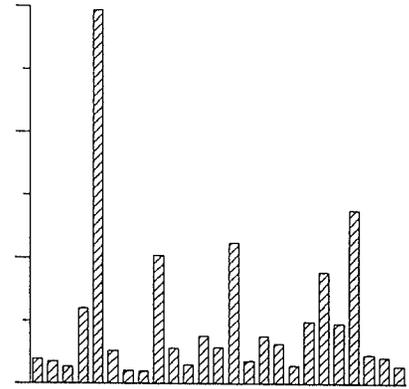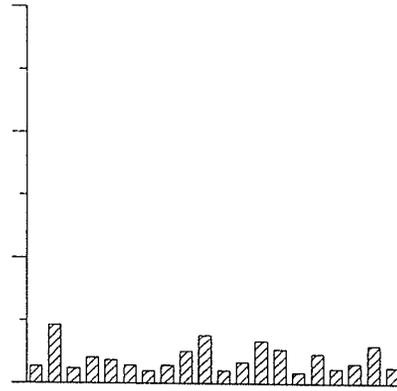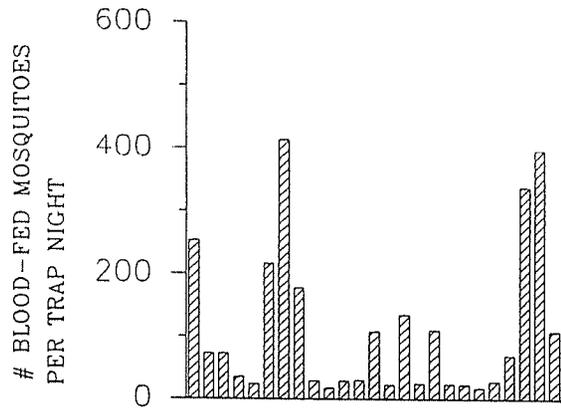
TRAP NIGHTS

Figure 4. Index of interrupted blood meals (see text for definition) on each of rubidium-marked and cesium-marked quail for each trap night during which ≥10 rubidium-marked mosquitoes and ≥10 cesium-marked mosquitoes were collected (top graphs). The bottom graphs contain the ratio of the larger index to the smaller from the top graph. Each bar represents one trap night. A) 15 trap nights, B) 14 trap nights, and C) 11 trap nights. The first two bars in the bottom graph of A and the first bar of the bottom graph of B are missing because the denominator of the ratio in each of these cases is 0, so the ratio could not be calculated. The last bar in the bottom graph of B and the last two bars in the bottom graph of C represent values much greater than 10.
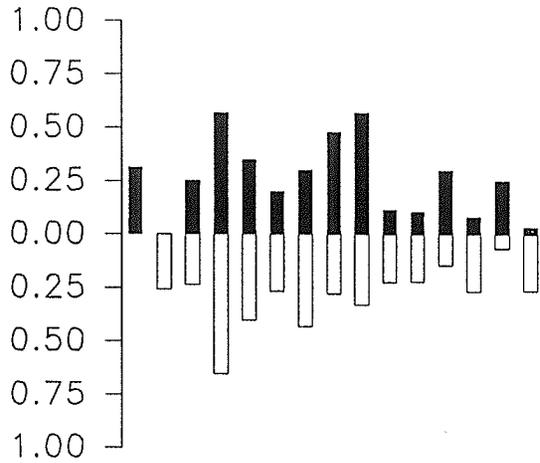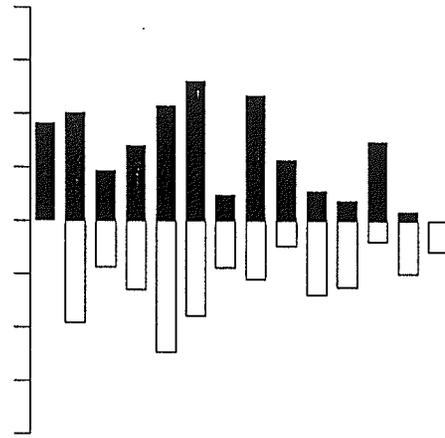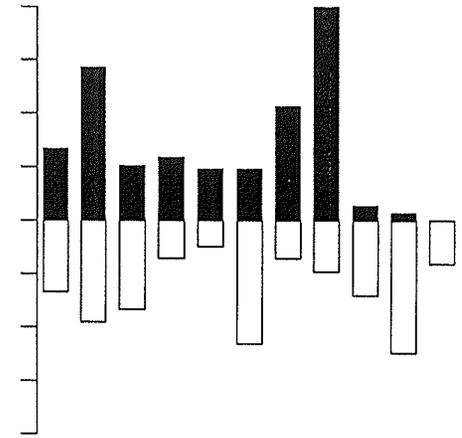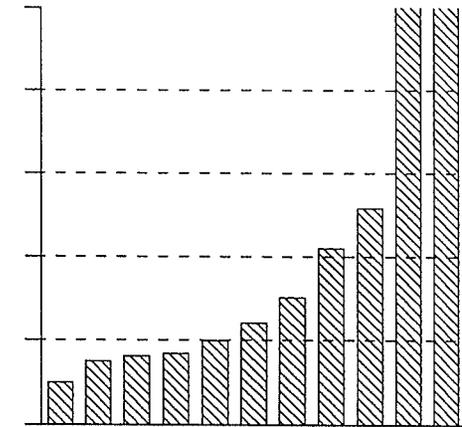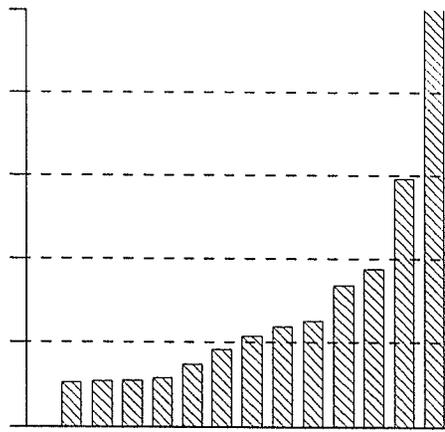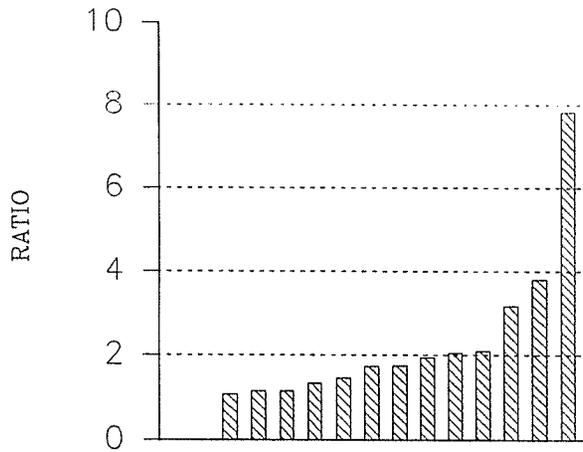
Figure 5. Relationship between the index of the probability that a given quail would interrupt blood-feeding mosquitoes and the probability that the same quail would be fed on for each trap night with ≥ 10 cesium-marked mosquitoes and ≥ 10 rubidium-marked mosquitoes. The data for cesium-marked quails are given in the top graph (regression is significant p=0.0038, r^2=0.20, slope=-0.30) and the data for the rubidium-marked quails are given in the bottom graph (regression is significant p=0.0057, r^2=0.19, slope=-0.41). Symbols for both graphs; (T) *Cx. tarsalis* collected in 1991, (T/R) mixed *Cx. tarsalis/restuans* collections from 1993, and (N) *Cx. nigripalpus* collected in 1992.
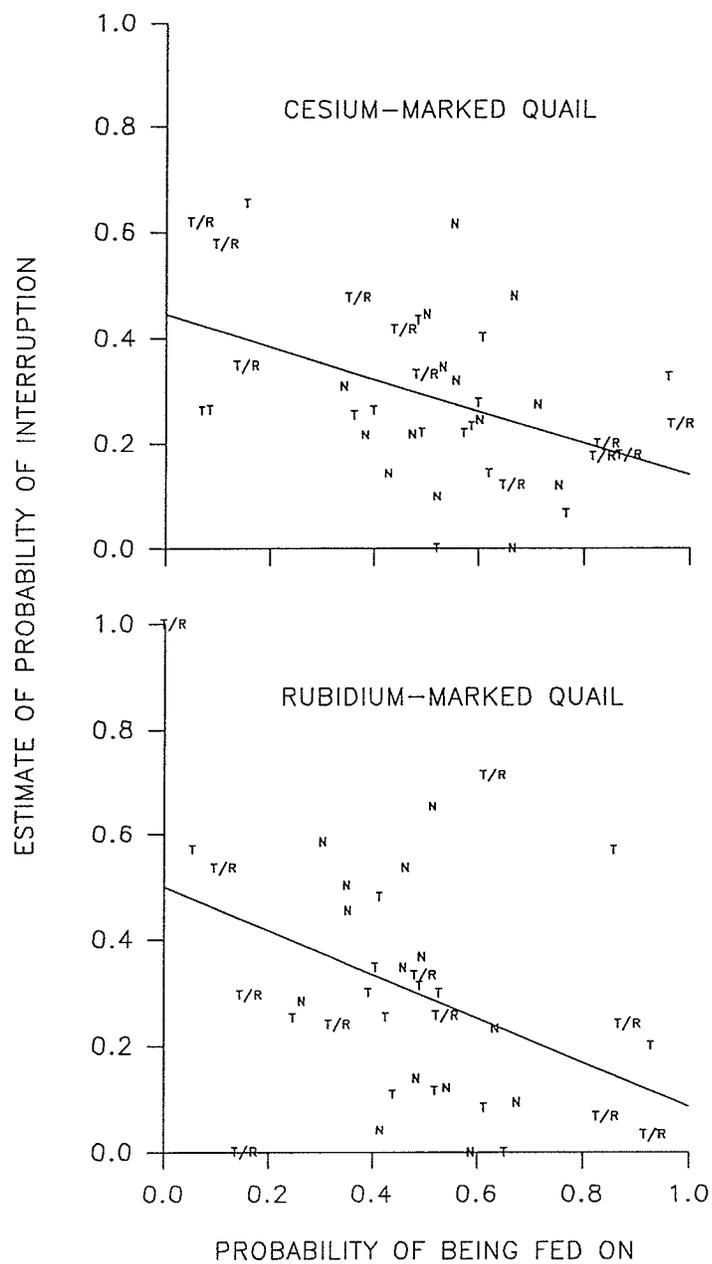
Figure 6. Relationship between probability of multiple
feeding and the degree of deviation from an equal
distribution of blood meals from each bird for each trap
night with $\geq$17 blood-fed, marked mosquitoes. Symbols are as
in Fig.5. Regression is significant (p<0.0001, r^2=0.26,
slope=-0.16).

DEVIATION FROM EQUAL PROBABILITY
THAT EACH BIRD IS FED ON
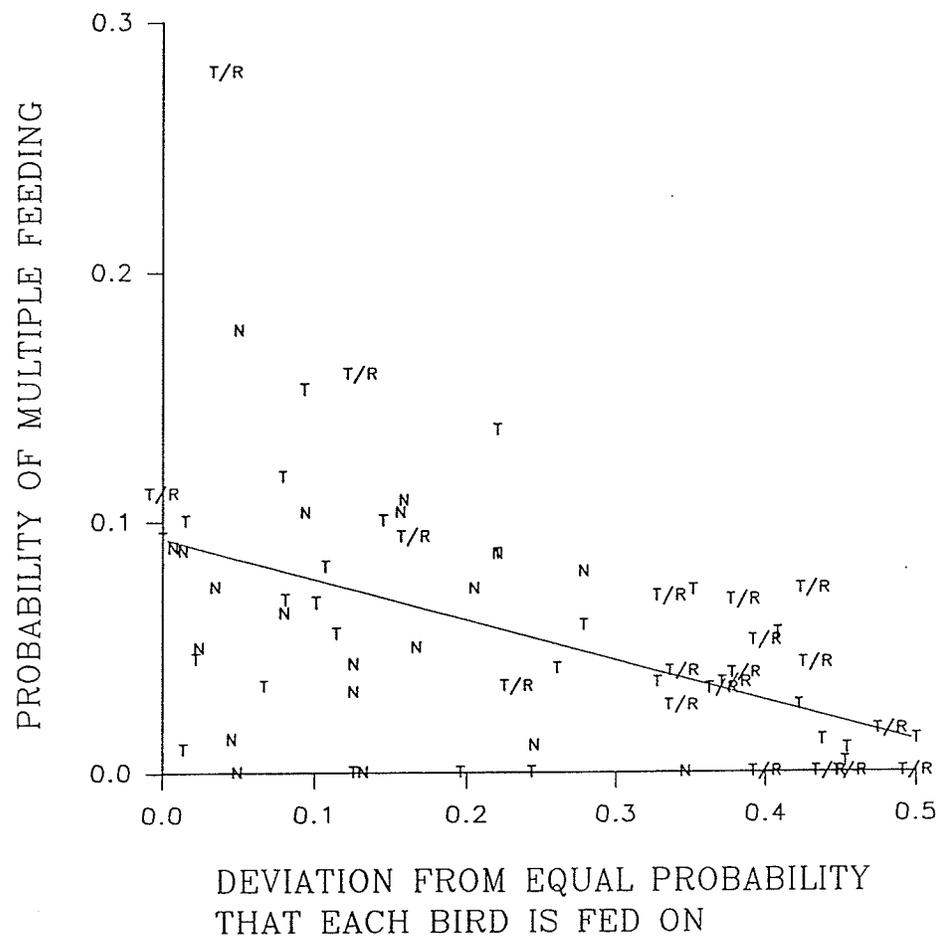
CHAPTER 3

Blood-Feeding Success of *Aedes aegypti* and *Culex nigripalpus*
(Diptera: Culicidae) in Relation to Defensive Behaviour of
Japanese Quail (*Coturnix japonica*) in the Laboratory.

ABSTRACT

Laboratory studies were conducted to determine the degree to
which individual Japanese quail vary with respect to
intensity and relative prevalence of several categories of
anti-mosquito behaviour when exposed to *Ae. aegypti* or *Cx.
nigripalpus* in the laboratory. Quail behaviour was
videotaped prior to and during exposure to mosquitoes. Five
categories of behaviour (head shakes, foot stamps, pecks,
feather fluffs and changes in body position) were observed.
In response to mosquito attack, the frequency of each of
these behaviours increased. The overall intensity of anti-
mosquito activity, as measured by all categories combined,
varied significantly between birds in each pair and was
inversely related with the proportion of blood-fed
mosquitoes ($p=0.049$, $r^2=0.267$ for *Ae. aegypti*, $p=0.0022$,
$r^2=0.38$ for *Cx. nigripalpus*) in each cage that had fed on
the corresponding bird.

INTRODUCTION

Host-seeking mosquitoes face uncertainty in locating
vertebrate hosts in complex habitats. Once located, not all
vertebrates are necessarily equally accessible because some
are less tolerant than others of mosquito attack (Edman et
al. 1972, Kale et al. 1972). Edman and Scott (1987) listed
host species, size, age, health, and individuality as
factors likely to account for variation in mosquito feeding
success on different hosts. Individuality (differences not
related to species, size, age, and health status) was
accorded the least importance. However, published studies
on differences in defensive behaviour among species may not
have included enough replicate animals to document
thoroughly the degree of variation among hosts of the same
physical status (Edman et al. 1972, Kale et al. 1972, Webber
and Edman 1972). Ornithophilic mosquito species, including
Cx. tarsalis, may vector arboviruses such as Western Equine
Encephalomyelitis Virus (WEEV) among relatively homogeneous
populations of birds, for example, house sparrows (*Passer
domesticus* (L.)) (Holden et al. 1973). In situations such
as these, differences in behaviour such as tolerance to
mosquito attack among otherwise similar host individuals may
influence feeding success, and thus, vectorial capacity of
attacking mosquitoes (Smith 1987, Dye 1992).

During a field study of blood-feeding success of several
species of wild mosquitoes on pairs of apparently identical
quail (Chapter 2), I observed that blood feeding often was
distributed unevenly between the two hosts (Chapter 2). I
assumed that differences in the probability of each bird
being fed on were due to corresponding differences in the
intensity of defensive behaviour exhibited by each quail as
has been demonstrated for other avian species (Edman et al.
1972, Kale et al. 1972). I hypothesized that the

probability of a quail being fed on was inversely proportional to the degree of defensive behaviour of that quail relative to the other in the cage. In this paper, I report the results of experiments conducted to evaluate the hypothesis that the observed distributions of blood meals in previous field studies were due to differences in behaviour of the quails used as bait.

MATERIALS AND METHODS

I conducted laboratory experiments with two species of mosquitoes. First, Japanese quail, *Coturnix japonica*, were exposed to *Ae. aegypti*. Fifteen pairs of quail were used; six pairs were exposed to 100 mosquitoes and nine pairs were exposed to 200 mosquitoes. Each pair was exposed twice, each time to the same number of mosquitoes. First and second exposures were separated by no more than eight hours to minimize variation in the hunger status of mosquitoes drawn from the same cohort. Lab-reared female *Ae. aegypti* were three to five days old and maintained on 3% sucrose until 48 hours prior to use, when only water was provided. The photoperiod of *Ae. aegypti* was adjusted so that the mosquitoes were allowed to feed during their photo phase because the peak feeding activity of this species is during the day. In the second experiment, 20 new pairs of Japanese quail of the same line were exposed to *Cx. nigripalpus*. The photoperiod of *Cx. nigripalpus* was adjusted so that experimental exposure to quail coincided with the scoto phase of this mosquito species, the time when it naturally blood feeds in the wild. Each pair of quail was exposed once to 100 *Cx. nigripalpus* (F13), a Vero Beach strain colonized from wild females collected at the Florida Medical Entomology Laboratory in 1992. Female *Cx. nigripalpus* were 7 to 12 days old and were maintained on the same sugar and

water regimen as *Ae. aegypti* used in the first experiment.

For the first experiment, 15 quail were injected with rubidium and 15 with cesium so that I could identify the blood source(s) of each engorged mosquito (Anderson et al. 1990). Each injected bird was labeled on the leg with a piece of masking tape printed with its marker and a number. Quail were exposed in pairs. The birds in each pair were selected randomly by reaching into the flock cage and catching each individual by hand without regard to the label. Rubidium-injected quail were paired with cesium-injected quail, but without regard to bird number. The bird number was used to keep the bird pairs consistent from the first exposure to the second. The quails were placed in 30 cm by 30 cm by 30 cm clear, acrylic cages (Fig.7). The cages were divided internally with wire mesh (mesh size, 13 mm by 13 mm) so that the quail were allowed to stand on the same type of mesh as in their normal holding cage and so that the space under the mesh (again similar to that present in normal holding cages) could serve as a refuge for mosquitoes from the quail. The quail were separated by one layer of the same mesh to prevent them from interacting directly during the experiment, but they were otherwise unrestrained.

Quail behaviour was recorded on video tape prior to and during exposure to the mosquitoes. Illumination for videotaping was provided by a soft-white flourescent lamp 30 cm long, placed approximately 10 cm above the cage and covered by a red cellophane filter as red light has been reported not to affect mosquito feeding behaviour (Webber and Edman 1972). The light and dark periods of each species of mosquito were adjusted so that experiments were carried out during the photo phase of *Ae. aegypti* and the scoto phase of *Cx. nigripalpus* to match the active period of each species of mosquito. Mosquitoes were placed in an acrylic

tube separated from the main cage by a remotely-controlled sliding door. The quail were placed in the cage approximately 30 minutes before video taping began, to allow them to habituate to the cage. This period of time was determined from preliminary observations of the quail in their holding cages to be more than sufficient for quail to return to eating and grooming behaviour after a person entered the room. All manipulations performed by the experimenter, including activation of video recorders, were done from behind a black cloth screen to minimize disturbance of the quail. Experiments were conducted in well-insulated, controlled-environment chambers to minimize external noise. Mosquitoes were released into the cage five minutes after the cameras were activated. After 55 minutes of exposure to the mosquitoes, the quail were removed from the cage. The number of dead mosquitoes in each cage was recorded. The cages were then placed in a freezer to kill the remaining mosquitoes. After freezing, mosquitoes were counted and sorted according to the presence and amount of blood and then analyzed for rubidium and cesium (Anderson et al. 1990).

The proportion of blood-fed mosquitoes in each cage was calculated as the number of mosquitoes with blood from at least one quail divided by the number of mosquitoes released into the cage. The probability of a given quail being fed on was estimated directly from the proportion of all meals in that cage obtained from that quail. The proportion of blood meals from a given quail in a cage was calculated as the number of meals with the corresponding mark divided by the total meals on both quail (number of simple meals from both birds + two times the number of mosquitoes with multiple meals). For the purposes of this calculation, mosquitoes with one marker were assumed to have taken one meal, while those positive for both markers (multiple meals)

were considered to have taken two meals, thus the factor of two in the denominator above. The proportion of mosquitoes with multiple blood meals was calculated as the number of mosquitoes with blood from both quail divided by the number of blood-fed mosquitoes.

Head shakes, pecks, feather fluffs (rapid erection followed by relaxation of part of the plumage), change of body position and foot stamps were counted for analysis. Categories of behaviour were decided upon from trial observations of ten quail before and during exposure to mosquitoes prior to the actual experiments. Quail used for the experiments were different individuals from those used for preliminary observations and, thus, had not been exposed previously to mosquitoes. The categories listed above were those that accounted for most of the quail activity and that changed most markedly in intensity during exposure to mosquitoes. Quail behavioural counts were transcribed from the video tapes to data sheets. All activity within the five described categories was counted for the 5-minute interval prior to mosquito release. As well, all activity was counted for a 10-minute interval (beginning at five minutes after mosquito release and ending at 15 minutes after mosquito release) during mosquito exposure. The tape was played at regular speed and behaviours ticked off on a data sheet. Rapid series of movements were recorded by pausing the tape between each different behaviour. The frequency of each type of activity per minute was calculated. The rates of each behaviour before and during exposure to mosquitoes were compared by the paired t-test (Snedecor and Cochran 1976). An overall index of activity for each quail in each cage was calculated by dividing the total number of movements (all categories combined) by one quail by the total of all movements of both quail. Thus, the activity index for each quail was a proportion of all

activity for the corresponding cage. This index was regressed against the proportion of blood meals attributable to the corresponding quail for each of the two species of mosquito.

RESULTS

The mean proportion of blood-fed mosquitoes was highest for cages with 100 *Ae. aegypti* (exposure 1) and lowest for cages with 200 *Ae. aegypti* (exposure 2) (Fig.8). The mean proportion of blood-fed mosquitoes decreased significantly from exposure 1 to exposure 2 for cages with 200 *Ae. aegypti*. The mean proportion of multiple meals was highest for cages with 200 *Ae. aegypti* (exposure 2) and lowest for cages with 100 *Ae. aegypti* (exposure 1), although no differences were statistically significant (Fig.8).

Before mosquito exposure, pecks and foot stamps occurred at a greater frequency than activities in the other behavioural categories (Fig.9). During exposure to mosquitoes, pecks, foot stamps and head shakes occurred at the greatest frequency. The mean number of head shakes per minute and the mean number of pecks per minute from before mosquito exposure were significantly different from the frequencies of these behaviours during mosquito exposure for the birds exposed to *Cx. nigripalpus*. The mean number/min of head shakes, pecks, foot stamps and changes in location during mosquito attack were significantly different from the corresponding values prior to mosquito attack for cages with 100 *Ae. aegypti* for both exposures. Only head shakes, pecks and foot stamps increased significantly during mosquito attack for cages with 200 *Ae. aegypti* during the first exposure, whereas all five behaviours increased significantly during the second exposure (Fig.9).

The proportion of *Ae. aegypti* that obtained blood from a

given quail in each cage was inversely related to the proportion of all activity in the cage by the correspondingly marked quail (p=0.049). The regression is based on the proportion of blood meals taken from the rubidium-marked birds and the proportion of all activity in a given cage exhibited by the rubidium-marked bird. The corresponding proportions recorded for the cesium-marked quail are constrained to be 1 minus the proportions for the rubidium-marked birds. Thus, it was unnecessary and statistically invalid to include both sets of proportions on the same regression. Similarly, the proportion of *Cx. nigripalpus* that obtained blood from the rubidium-marked quail in each cage was inversely related to the proportion of all activity in the cage by the correspondingly marked quail (p=0.0022) (Fig.10).

DISCUSSION

The rate at which Japanese quail shook their heads, stamped their feet, pecked, fluffed their feathers and moved from one place to another increased when the birds were exposed to mosquito attack (Fig.9). All five categories of behaviour occurred to some extent before the quail were exposed to mosquitoes, although pecks and foot stamps were dominant. Pecking, when it is not foraging related, and scratching are natural behaviour for many species of galliformes, including quail and may represent comfort movements (Kilgour and Dalton 1984). The mean number/min of head shakes increased the most dramatically in response to mosquitoes (Fig.9). Overall, the apparent effect of mosquito attack was to induce quail to increase the mean number/min of pre-existing behaviours. Although I could not unequivocally determine the number of mosquitoes attacking different parts of the quail, my qualitative impression was

that most mosquitoes fed on heads and feet of the quails.
This may explain why foot stamps also were one of the
dominant categories during mosquito exposure (Fig.9). Most
of the dead mosquitoes, about ¼ of all *Ae. aegypti* and about
½ of *Cx. nigripalpus*, were missing and presumed eaten as
they could not escape from the cages. This was likely
because pecks were a relatively dominant category of
behaviour (Fig.9) during mosquito exposure. I observed that
quail were able to catch flying mosquitoes. The high amount
of mortality observed in this study is evidence that blood
feeding is risky behaviour.

Although the mean number and proportion of blood-fed
mosquitoes decreased significantly from exposure 1 to
exposure 2 only in cages with 200 *Ae. aegypti* (Fig.8), the
same trend is apparent for cages with 100 *Ae. aegypti*
(Fig.8). It appears that blood feeding success of *Ae.*
aegypti was negatively affected by the previous exposure of
the host quail.

The observed proportion of two-host meals taken by *Ae.*
aegypti in my study (between 5% and 14%) compares well with
data for *Ae. aegypti* published by Scott et al. (1993), who
found that about 7% of blood-fed mosquitoes had taken
multiple meals. The rate of multiple feeding by *Cx.*
nigripalpus observed in this study (7%) is consistent with
the rate of multiple feeding (6%) observed for *Cx.*
nigripalpus collected in the field in cages baited with two
quail, although of a different species (Chapter 2).

Japanese quail were extremely variable with respect to
the overall rates at which they defended themselves from
mosquito attack (Fig.10). The proportion of all activity in
a given cage attributable to one quail varied from near 0 to
near 1 and this was inversely related to the proportion of
blood meals attributable to the corresponding quail. These
data support my hypothesis that variation in blood-feeding

success of mosquitoes on individual quail hosts is due, at
least in part, to differences in tolerance to mosquito
attack (Chapter 2). When mosquitoes were allowed to attack
two hosts, relative feeding success of mosquitoes increased
on the host least able or willing to defend itself (Day et
al. 1983, Edman et al. 1974). Other authors have noted
variation in the proportion of blood-fed mosquitoes
attracted to different individuals of the same avian species
(Blackmore and Dow 1958, Dow et al. 1957). In the context
of my experiments with Japanese quail and those of Edman et
al. (1972) with several ciconiiform species, it is likely
that the variation in engorgement rates on different
individuals of the same avian species observed by Blackmore
and Dow (1958) and by Dow et al. (1957) was due to
differences in the intensity of anti-mosquito behaviour.

Kale et al. (1972) observed considerable variation
among some individuals of the same ciconiiform species in
the proportion of activity in each behavioural category.
Significant variation in defensive behaviour among
individual avian hosts of the same species, size, age, sex
and health status may influence feeding success and thus
vectorial capacity of mosquitoes to a larger extent than
previously believed (Edman and Scott 1987). Further
research in this area with other avian hosts such as
passerine birds and different species of mosquitoes adapted
to a greater or lesser extent to feeding on birds may
produce insight into the behaviours most important in
limiting blood-feeding success of mosquitoes.

Figure 7. Cage used to expose quail to *Ae. aegypti* or *Cx. nigripalpus*. The mosquitoes were released by remote control from the cylinder at the bottom of the front of the cage. The quail were separated from each other by wire mesh (13 mm by 13 mm). The clear acrylic front and sides of the cages permitted video recording of quail behaviour.
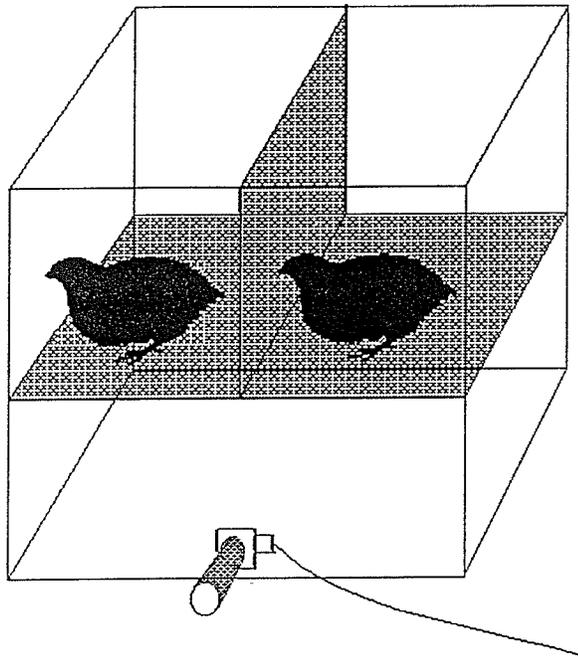
Figure 8. Proportion of blood-fed mosquitoes and mosquitoes with multiple blood meals for each of the quail groups exposed to *Cx. nigripalpus* or *Ae. aegypti*. Significant differences between adjacent bars (p<0.05) by 2 sample t-test (Snedecor and Cochran 1976) are indicated by horizontal, double-headed arrows.
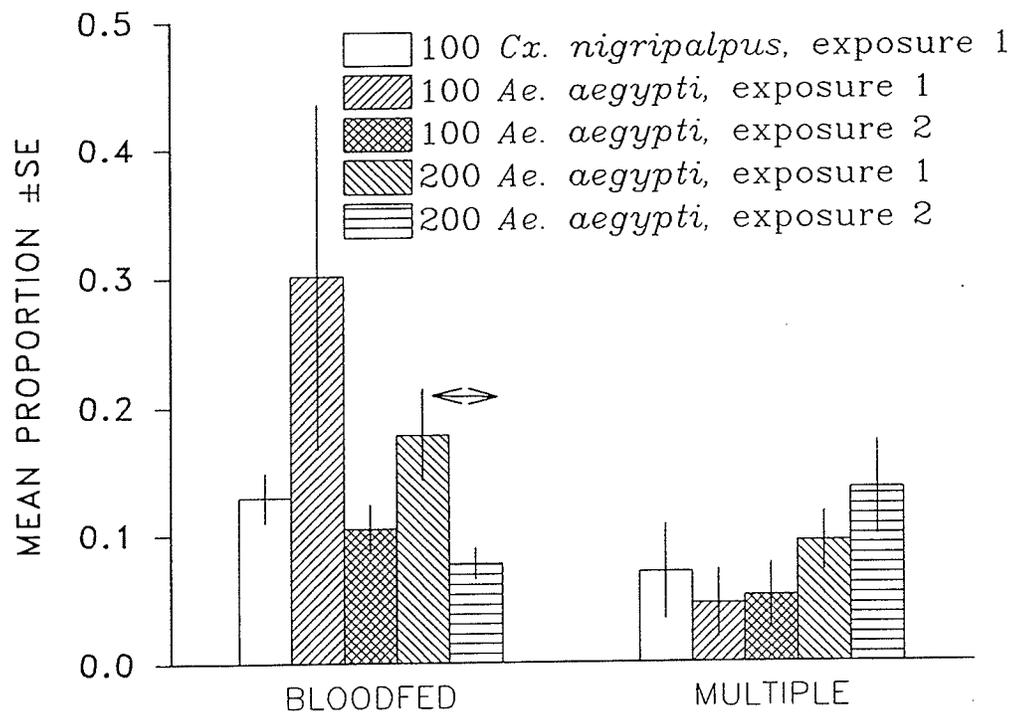
Figure 9. Comparison of rate of head shakes, pecks, foot stamps, feather fluffs, and change in location per minute by Japanese quail before and during exposure to mosquitoes. The species and number of mosquitoes used and experience of the quail (naive-first exposure, experienced-second exposure) is given at the top of each graph. Significant differences between adjacent bars (p<0.05) by paired t-test (Snedecor and Cochran 1976) are indicated by horizontal, double-headed arrows.

MEAN #(±SE)/MIN

0 2 4 6 8 10

100 *Culex nigripalpus*, exposure 1
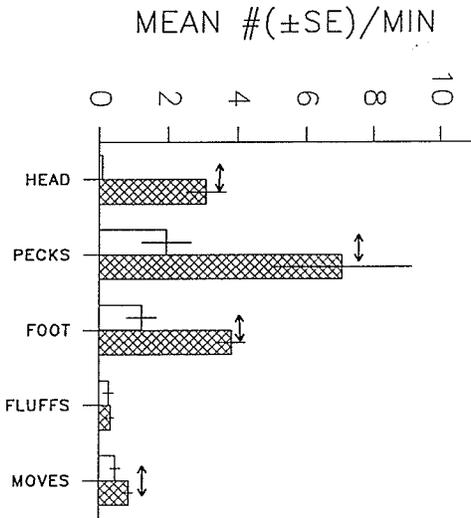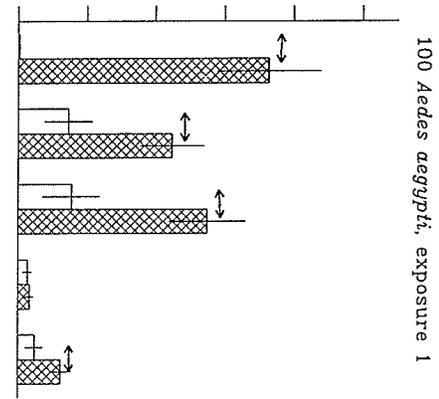
HEAD
PECKS
FOOT
FLUFFS
MOVES

Before exposure to mosquitoes
During exposure to mosquitoes

MEAN #(±SE)/MIN

0 2 4 6 8 10

100 *Aedes aegypti*, exposure 1

HEAD
PECKS
FOOT
FLUFFS
MOVES

MEAN #(±SE)/MIN

0 2 4 6 8 10

100 *Aedes aegypti*, exposure 2

HEAD
PECKS
FOOT
FLUFFS
MOVES

200 *Aedes aegypti*, exposure 1

HEAD
PECKS
FOOT
FLUFFS
MOVES

200 *Aedes aegypti*, exposure 2

HEAD
PECKS
FOOT
FLUFFS
MOVES

79
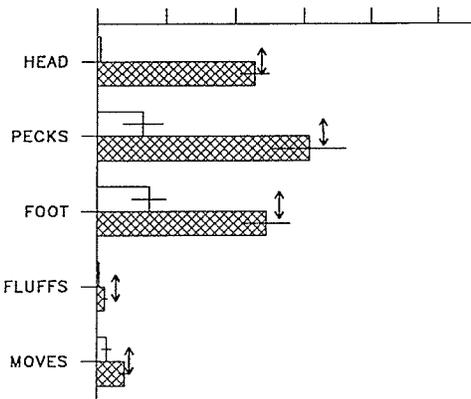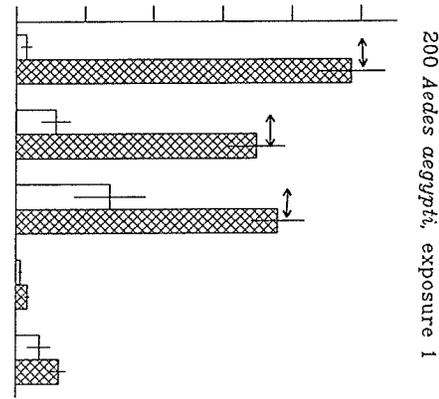
Figure 10. Relationship between the proportion of all activity in a cage attributable to the rubidium-injected quail and the proportion of blood meals attributable to the same quail. The solid straight line represents the regression and the curved lines represent the 95% confidence limit of the regression. Top graph: quail exposed to *Ae. aegypti* (combined for two densities and two exposures). Bottom graph: quail exposed to *Cx. nigripalpus* (100 mosquitoes, first exposure).

Ae. aegypti

$Y = 1.07 - 0.99X$
$R^2 = 0.267$
$P = 0.049$
Confidence Limits of Slope
Upper = -0.389
Lower = -1.64

Cx. nigripalpus

$Y = 0.852 - 0.741X$
$R^2 = 0.381$
$P = 0.0022$
Confidence Limits of Slope
Upper = -0.362
Lower = -1.12

PROPORTION OBTAINING BLOOD FROM RB–MARKED QUAIL

PROPORTION OF ALL ACTIVITY
IN CAGE ATTRIBUTABLE TO
RB–MARKED QUAIL
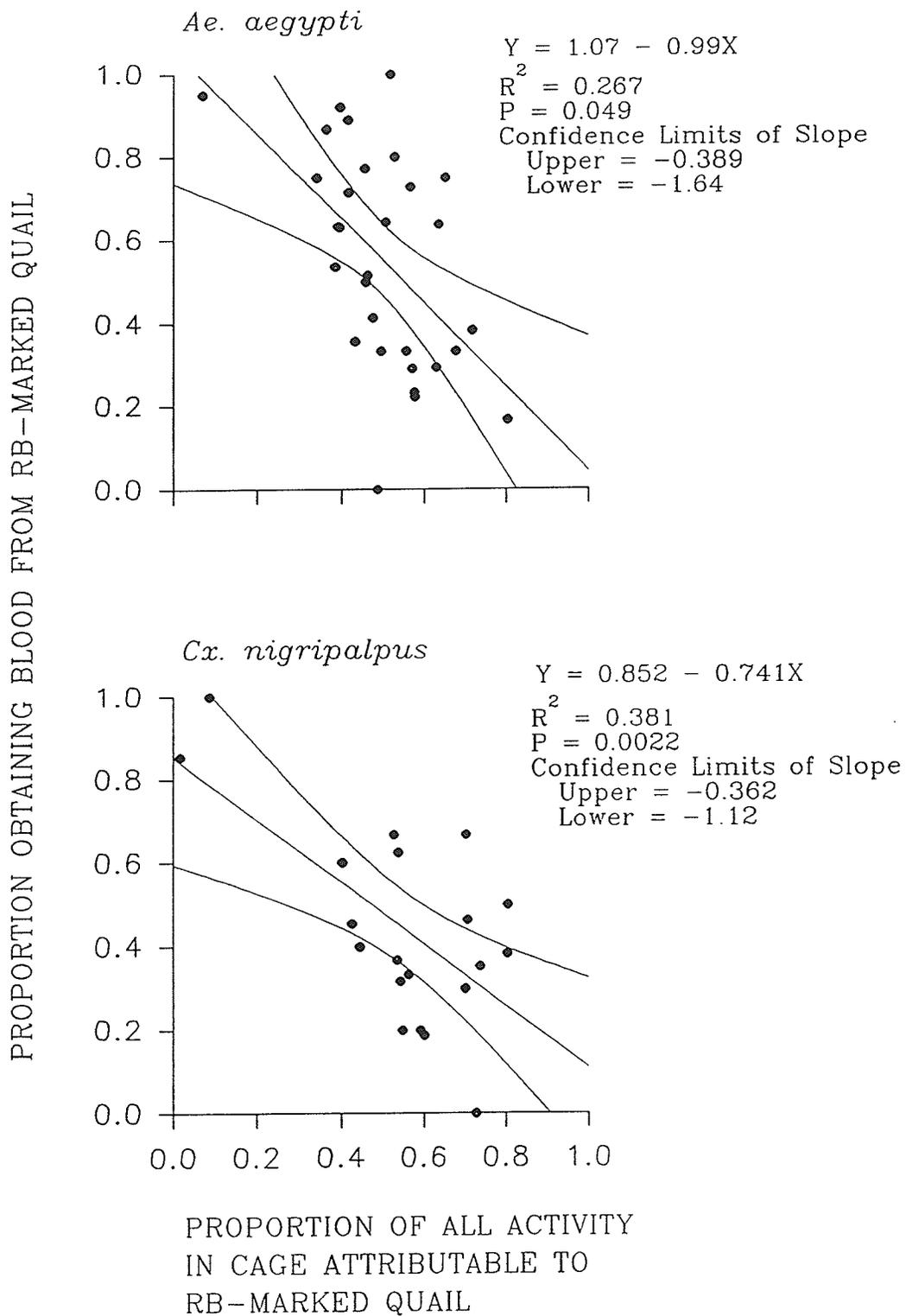
CHAPTER 4

Modeling the Trade-off Between Mortality Associated with
Blood Feeding and Increased Fitness from Multiple Feeding by
Culex nigripalpus (Diptera: Culicidae).

ABSTRACT

Data from a field experiment are presented to document
interrupted meals, multiple meals, and host-induced
mortality associated with blood feeding of *Cx. nigripalpus*,
an important pest and vector. A total of 1617 *Cx.
nigripalpus* were collected during the field experiment, of
which 706 (44%) were blood-fed. Of the fed mosquitoes, 571
(81%) were fully fed (92% of which had single meals), and
134 (14%) had partial meals. Of the partially-fed females,
64 (9% of total blood feds) had taken ½ meals, 36 (5% of
total blood feds) had taken ¼ meals, and 34 (5% of total
blood feds) had taken trace meals. Approximately 7% of all
blood meals contained blood from two hosts. The refeeding
rate for mosquitoes with partial meals was 26%. Quail hosts
killed or ate approximately 24% of *Cx. nigripalpus* that
entered field cages. I used a simulation model to examine
conditions under which multiple feeding would be favoured
despite host-induced mortality associated with each feeding
attempt by the mosquitoes. Within the parameters evaluated
for this model, multiple feeding was favoured as a
behavioural strategy under conditions of low, feeding-
associated mortality (probability of dying ≤ 0.5), but this
pattern was reversed when the probability of feeding success
was high (≥ 0.95). I suggest that variation in the observed
frequency of multiple feeding among different species of
mosquitoes may be due to differences in the risk that
different hosts pose to their mosquito parasites.

INTRODUCTION

The fecundity of anautogenous mosquitoes in each gonotrophic cycle is a function of the amount of blood obtained (Briegel 1985, 1990, Downe and Archer 1975, Edman and Lynn 1975, Jalil 1974, Woke et al. 1956). This means that fitness is closely tied to blood-feeding success. In addition to fully fed and empty individuals, female mosquitoes with partial meals (Magnarelli 1977) and with multiple meals (Chapter 1, Boreham and Garrett-Jones 1973, Boreham et al. 1978, Edman and Downe 1964, Rempel et al. 1946) were observed often in collections of blood-fed mosquitoes. These observations were consistent with the hypothesis that some mosquitoes were interrupted before they fed to repletion, and that some of the partially fed individuals fed again, presumably to increase blood intake, at least to the point beyond which further increase in blood-meal volume did not increase fitness (Edman et al. 1975).

Many vertebrate hosts resist attack and, in so doing, limit the amount of blood imbibed by the mosquito (Edman et al. 1972, Chapters 2,3). Reduced fitness due to partial feeding is not the only cost associated with host contact. Mosquitoes may be killed by the defensive behaviour of their hosts (Edman et al. 1984, Kale et al. 1972, Webber and Edman 1972). Vertebrates may also become the predators and have been observed to consume attacking mosquitoes (Corbet and Downe 1966, Day and Edman 1984) or to have mosquitoes in their digestive tracts (Busby and Sealy 1978, Guinan and Sealy 1986). The tenacity with which mosquitoes persist in blood feeding is likely modified by available carbohydrate reserves that are used during host location and feeding (Walker and Edman 1985b). In as much as there are significant costs to the mosquito associated with each

blood-feeding attempt, increased fecundity from additional meals (Briegel and Horler 1993) represents a tradeoff against the decreased probability of survival and consequently, reproduction, with each host contact. Such a tradeoff has been modeled for feeding-related mortality of tsetse flies (Hargrove and Williams 1995, Randolph et al. 1992), but not for mosquitoes.

Estimates of feeding-associated mortality derive largely from laboratory studies in which known numbers of mosquitoes have been permitted to attack caged vertebrate hosts (Day and Edman 1984, Edman et al. 1972, Kale et al. 1972, Webber and Edman 1972). The mortality estimates from these studies were calculated from the difference between the number of mosquitoes at the beginning of the experiment and the number remaining alive at completion. Under uncontrolled conditions in nature, it is difficult to count directly the mosquitoes killed and consumed by hosts without knowing the number that have attacked. Point estimates of attack rates may be made by collecting the mosquitoes attracted to bait animals, but the act of sampling may seriously bias subsequent behavioural data from the same individual mosquitoes. Mosquitoes are too small and often occur at such high densities as to make it impossible to count them in a way that does not affect their behaviour.

An objective of my paper was to estimate the probability of feeding-associated mortality for *Cx. nigripalpus* attracted to quail-baited traps in a field situation. Another objective was to use a stochastic simulation model based on published aspects of feeding biology of *Cx. nigripalpus* and observations from my field study to examine possible tradeoffs between feeding-associated mortality and increases in lifetime production of offspring associated with multiple feeding.

MATERIALS AND METHODS

I developed a trapping scheme to estimate the proportion
of wild, blood-feeding *Cx. nigripalpus* killed by caged
northern bob-white. The field study was carried out in the
hardwood hammock surrounding the Florida Medical Entomology
Laboratory at Vero Beach, FL. Northern bob-white were used
as model avian hosts to attract host-seeking *Cx. nigripalpus*
to box traps described in Chapter 1. A pair of quail
(either both males or both females) was placed in each box
trap (n = 10 traps per night). One quail in each pair was
injected with rubidium chloride and the other was injected
with cesium chloride according to the method of Anderson et
al. (1990). With this method, it was possible to determine
whether one or both quail were fed on by each of the blood-
fed mosquitoes collected.

The box traps were hung approximately 1.5 m above ground
from trees in the hammock approximately 30 min before
sunset. Two treatments were established. Treatment 1: Two
hours after the cages were placed in the field, the no-
return entrances on the underside of these traps were
sealed, the quail were removed, the traps were returned to
the laboratory and the number of dead or moribund mosquitoes
on the bottom of each cage was counted. The traps were
placed in the freezer at -20 C. to kill the remaining
mosquitoes. Treatment 2: Two hours after the cages were
placed in the field, the no-return entrances on the
underside of these traps were sealed, but the traps with
quail were left in place for an additional two hours. At
the end of four hours from the start of the experiment, the
cages were handled as in Treatment 1. I expected that the
mean number of mosquitoes per cage in Treatment 1 would
provide an estimate of the mean number of mosquitoes per
cage in Treatment 2 at the two hour mark when the cages in

Treatment 2 were closed to eliminate recruitment of more mosquitoes over the next two hours. Each treatment was replicated 20 times. On each night, the cages were assigned randomly to specific collection sites within the study area without respect to the treatment assigned to each cage. The experiment began on 12 August and terminated on 20 August, 1992. Trapping was carried out on six nights within this period. The cages were of identical construction and were cleaned thoroughly after each use.

The mosquitoes were counted and identified to species. The amount of blood in each blood-fed individual was scored as a trace, ¼, ½, or full meal, modified from Edman et al. (1975). The ¾-meal category of Edman et al. (1975) was combined with the full category in our study because the data presented by Edman and Lynn (1975) were consistent with the conclusion that there was no significant difference in the refeeding potential of Cx. nigripalpus in these two categories. The proportion of engorged Cx. nigripalpus for each cage was calculated as the number of blood-fed, marked (with either or both rubidium or cesium in the blood meal) individuals divided by the total number of females collected. The proportion of multiple meals was calculated as the number of blood-fed mosquitoes with blood from both quail divided by the total number of marked, blood-fed mosquitoes per cage. The proportion of blood meals in each of the trace, ¼, ½, and full categories for each cage was calculated as the number of marked blood meals in each category divided by the total number of marked blood meals.

The mean number of mosquitoes per cage for Treatment 1 was assumed to represent the attack rate per pair of quail over two hours of exposure. The mean number of mosquitoes per cage for Treatment 2 (after four hours elapsed time) divided by the mean number of mosquitoes/cage for Treatment 1 was used as an estimate of survival of mosquitoes in

Treatment 2 for the two hours after the cage entrances were closed because no additional mosquitoes could enter the cages after the entrances were sealed. One minus survival of mosquitoes in Treatment 2 times 100 equals the per cent mortality over two hours. The proportion of mosquitoes that had been interrupted before satiation (number partially fed divided by number of blood-fed mosquitoes) per cage was calculated for each treatment. The proportion of mosquitoes with multiple meals (number with multiple meals divided by the number of blood-fed mosquitoes) per cage was also calculated for each treatment. I expected that the proportion of interrupted meals would decrease and the proportion of multiple meals would increase with time of exposure to the quail.

THE MODEL

I used estimates of feeding success based on blood-meal volume from the field study as well as published literature to set behavioural rules (Figs.11,12) for two simulation models, one of which simulated mosquitoes that took multiple blood meals (Fig.12a) and the other that simulated mosquitoes that took only simple meals (Fig.12b). For the models, I calculated an index of fitness as the total number of eggs laid by a female mosquito during her lifetime. I compared two hypothetical populations of mosquitoes. One was characterized as persistent (multiple feeders) in that females interrupted with ½ a blood meal attempted to refeed (Edman et al. 1975) once the same night. The other was characterized as non-persistent (non-multiple feeders) in that fed females stopped feeding after one attempt, irrespective of the amount of blood obtained.

I designed two simulation models to calculate the expected lifetime fecundity of individuals that differ in

whether a second blood meal is attempted, given that the first is partial ($\leq\frac{1}{2}$). For one model, individuals were assumed to be persistent. I explicitly defined the value of persistence to mean that if a mosquito was interrupted before it obtained $>\frac{1}{2}$ a blood meal, there was a 90% chance that it would attempt to feed once more the same night (Fig.11). The value of 90% was chosen because Edman et al. (1975) showed that approximately this percentage of partially-fed *Cx. nigripalpus* resumed probing up to 1 hour after being interrupted. For the second model, non-persistence was defined to mean that a mosquito would feed once only, provided that it obtained some blood. All mosquitoes of both types that obtained no blood on the first try, attempted to feed once more (Fig.11) because Edman and Lynn (1975) demonstrated that fecundity was zero for female *Cx. nigripalpus* that did not obtain blood and nearly 100% of unfed mosquitoes immediately refed. The maximum number of feeding attempts per night was set at two for both models because the number of hosts available to mosquitoes in my field studies (Chapters 1,2) and thus, the number of measurable blood meals per mosquito was limited to two.

For both models, the probability of each blood-meal size (trace=0.05,$\frac{1}{4}$=0.05, $\frac{1}{2}$=0.10, and full=0.8) was set to approximate the observed proportion of simple meals for each of these categories in the field experiment (Table 4). These probabilities were kept constant for each reproductive cycle of each mosquito. The relationship between fecundity and blood meal size was derived from the study of Edman and Lynn (1975), in which the fecundity of *Cx. nigripalpus* was found to increase in a nearly linear fashion from trace to $\frac{1}{2}$ a blood meal, that is, trace blood meals supported the production of approximately 24 eggs, $\frac{1}{4}$ blood meals about 49 eggs,$\frac{1}{2}$ blood meals about 67 eggs and full meals about 219. Some of the partially-fed mosquitoes in the study of Edman

and Lynn (1975) did not produce eggs, especially those with very small, trace meals, however in the model, I assumed, for the sake of simplicity that all mosquitoes that had stopped feeding after a partial meal (all non-persistent individuals, 10% of persistent individuals and those still partially fed after two attempts) developed eggs. Full meals were assumed to result in 200 eggs, ½ meals 100 eggs, ¼ meals 50 eggs and trace meals 25 eggs.

Partially blood-fed, mosquitoes in the persistent model were assumed to attempt a second blood meal only within the same night because oocyte-induced inhibition of blood feeding has been shown to take effect within six to 24 hours of blood uptake (Edman et al. 1975, Klowden and Lea 1979a). Both models were structured so that mosquitoes with no blood at the end of a night (two attempts) would attempt to blood feed on the next night (Fig.12). Mortality was assumed to occur at each feeding attempt with some probability. For both types of mosquitoes, egg development was assumed to take three days (Nayar and Knight 1981), so that, in the models, oviposition occurred on the fourth evening after feeding (Appendix 1). After eggs were laid, the mosquito was allowed to feed the next night, providing that it survived through the intervening 24 hours (Fig.12). Mosquitoes were allowed to survive for a maximum of three reproductive cycles or 20 days whichever came first (Nayar and Knight 1981).

Three parameters, daily survival, feeding-associated mortality and probability of obtaining blood were allowed to vary. Daily survival was included to account for background mortality in the interval between feeding periods (Randolph et al. 1992). Feeding associated mortality was included so that the risk associated with different numbers of feeding attempts could be assessed separately from all other mortality (Randolph et al. 1992). Probability of obtaining

blood was included to reflect the fact that some proportion of mosquitoes may be prevented from obtaining any blood during a given period (Table 4). Each of these variables was allowed to vary and each unique combination of variable values was tested so that general trends in the effect of and the interaction among these variables on the relative fitness of two feeding strategies could be examined. Each variable was incremented by a value of 0.15 from 0.05 to 0.95.

At each of the unique combinations of the three variables, the models were run 10 times. In a run, the lives of 1000 mosquitoes were simulated. A mosquito's status was evaluated after each event (denoted by the boxes in Fig.12 with text ending in a question mark). The events denoted with a question mark employed stochastic elements as follows. During each event, the computer program generated a uniform random number between 0 and 1. Decision rules in the program were written as mathematical inequalities representing the relationship between the value of the variable in question (probability of daily survival, feeding-associated mortality or obtaining blood) and the random number generated at that point. If the inequality was evaluated as true, the mosquito would proceed to the next event as indicated by the arrows marked `yes' in Fig. 12. If the inequality was false, the mosquito would proceed to the next event indicated by the `no' arrow.

At the end of each run, the number of eggs laid each day (by all ovipositing mosquitoes) were calculated. The total number of eggs laid by each mosquito was also calculated. For each run, the average lifetime fecundity per mosquito was calculated by dividing the total eggs laid by all females by 1000. The average of this value was calculated for the 10 runs. The effects of the three variables and mosquito persistence on lifetime fecundity were assessed by

analysis of variance. The ratio of mean lifetime fecundities of mosquitoes simulated by each model were also presented in a payoff matrix to compare the relative benefit that resulted from each of the two behavioural strategies. The ratio was calculated for each combination of daily survival and probability of obtaining blood by dividing the fecundity of the superior type by the fecundity of the inferior type. This ratio was calculated only for those cases in which more than two eggs were laid by both types of mosquitoes. Two was chosen as a cutoff value for meaningful data because a female mosquito must produce more than two offspring on average for population growth to occur. Results below this were considered trivial. A payoff matrix was presented for each of the probabilities of feeding-associated mortality.

The simulation models were written as computer code in QBasic® (Appendix 1). Prior to final use of the simulation models, the computer programs were extensively validated and tested for accuracy by evaluating the performance of test 'mosquitoes'. I printed out all of the random numbers and the status of each mosquito associated with each step in the programs. This approach ensured that the mosquitoes were behaving as expected and that lifetime egg production accurately reflected the ecological circumstances set by any particular values of the variables used.

RESULTS

FIELD EXPERIMENT

The overall numbers of mosquitoes collected in the field experiment and their blood fed status are given in Table 4. The mean number of mosquitoes per cage declined from Treatment 1 to Treatment 2, although this difference was not

statistically significant (Table 5). The percentage of partial meals declined, while the percentage of multiple meals increased from Treatment 1 to Treatment 2, although these differences were not statistically significant (Table 5).

THE MODEL

The mean number of eggs/female lifetime varied between zero and approximately 300 for both persistent and non-persistent females depending on the value of 'X' (probability of dying during blood feeding), 'Y' (probability of obtaining blood) and 'S' (probability of surviving each 24 hour period) (Figs.13-19).

Persistent mosquitoes tended to lay more eggs during their lifetime than did non-persistent mosquitoes at lower probabilities of blood feeding-associated mortality. The exceptions to this trend occurred at the highest probabilities of obtaining blood. Non-persistent mosquitoes tended to achieve higher fecundity than persistent individuals more frequently at higher probabilities of feeding-associated mortality (Figs.17-19). Also, the magnitude of the relative payoff to non-persistent mosquitoes at higher levels of feeding mortality is not as great as for the relative payoff to persistent individuals at low levels of feeding mortality. Absolute differences in numbers of eggs produced by persistent and non-persistent mosquitoes were greatest at higher daily survival probabilities. Persistent mosquitoes tended to do better than non-persistent mosquitoes at lower probabilities of obtaining blood (Figs.13-19).

DISCUSSION

Most mosquito species must feed on vertebrate blood to reproduce and there is a direct link between observed feeding behaviour and fitness, indexed here as lifetime fecundity. Feeding persistence may affect the amount of blood obtained, which, in turn affects fecundity. The species, age, gender and health status (Edman and Scott 1987) of vertebrate hosts has been found to affect the feeding success of mosquitoes. In my study, feeding success of mosquitoes also was found to vary depending on the individual host attacked, even when hosts were alike with respect to species, gender, age and health (Chapters 1, 2). This was due, at least in part to variation in the degree to which hosts tolerate mosquito attack (Chapter 3). Hosts defend themselves and, in so doing, they may inflict considerable mortality (Edman et al. 1984). I have presented suggestive evidence from a field study that quail hosts killed and/or consumed almost 25% of the *Cx. nigripalpus* to which they were exposed. This estimate may not reflect the actual probability of host-induced mortality for mosquitoes feeding in the field because the cage design may have limited the ability of mosquitoes to escape from the quails. This is especially true for mosquitoes that may have rested on the wire cages within the box traps. In this situation, the quail would have been able to catch the mosquitoes. Also, the study used adult quails, so that conclusions made here may not be applicable to natural situations in which nestling passerines are attacked.

I hypothesized that, if the quail were consuming mosquitoes, the mean number per cage in Treatment 2 should be less than the estimate derived from Treatment 1. Although the decrease in mean number of mosquitoes per cage from Treatment 1 to Treatment 2 was not statistically

significant, the trend is consistent with my hypothesis that some mosquitoes were killed and eaten by the quail.

It is important to examine the link between feeding persistence and fecundity on one hand and between persistence and mortality on the other hand as a tradeoff that may have been important in the origin of feeding persistence and the maintenance of such behaviour. This relationship is important because each feeding attempt by a mosquito may be associated with a substantial probability of death and thus the chance that the fitness gained with a first meal may be lost during a second feeding attempt, a classic question in behavioural ecology (Krebs and Davies 1991, Lima and Dill 1990).

Within the constraints imposed by the assumptions of my model and for the variables used, multiple feeding sometimes increased the lifetime egg production of mosquitoes relative to that of mosquitoes that did not take multiple meals. This was especially evident at lower probabilities of feeding-associated mortality. The magnitude of the fecundity advantage to persistent mosquitoes at daily survival probabilities below 0.5 may have been an artifact of the low numbers of eggs produced under those conditions (Figs.13-19). Even at lower probabilities of feeding mortality, non-persistent mosquitoes sometimes laid more eggs, especially in those cases in which the probability of obtaining blood was high. The mosquitoes had a good chance of obtaining blood in the next reproductive cycle, so it would not have made sense to put current investment at risk for a modest increment in fecundity.

Although most mosquitoes were able to obtain some blood on their first attempt, the persistent mosquitoes tended to die during the second attempt and before they could lay the eggs that would have developed from the first, partial meal. In this situation, the non-persistent females were able to

lay some eggs because they did not put the fitness from the first partial meal at risk by attempting another. At high probabilities of feeding-associated mortality, the non-persistent females generally did better than the multiple feeders because the high probability of feeding-associated mortality virtually assured that multiple feeders would die either during their first or second feeding attempts. Feeding-associated mortality exacted a much heavier toll on the more persistent mosquitoes. A prediction arising from this model is that multiple feeding should be most beneficial when hosts inflict relatively low mortality, but the strength of this correlation may be modified by the probability of imbibing blood and the magnitude of the increment in fecundity, i.e. depending on whether the cumulative fitness curve is linear, accelerating, or decelerating. My study was based on a linear fitness curve.

The information generated from the simulation model may be used as a theoretical framework to help explain the observation that many mosquito species take multiple blood meals in nature (Chapter 1, Boreham and Garrett-Jones 1973, Boreham et al. 1978, Edman and Downe 1964, Rempel et al. 1946). Based on serological studies of mosquito-feeding patterns, other authors concluded that multiple feeding is the exception, rather than the rule (Washino and Tempelis 1983). It may have been that of mosquitoes that successfully fed, the greatest proportion did so to repletion on the first attempt and were in no need of a second meal. My data for *Cx. nigripalpus* caged with hosts (91.8% of full meals had been obtained from 1 host) support this conclusion, but this figure may have included meals interrupted and resumed on the same host, a phenomenon I could not measure directly.

Partially fed mosquitoes (20% of simple meals in Table 4) may have arrived at their observed status in several

ways. The data listed in Table 4 were, at best a snapshot of the status of the mosquitoes after a period of time. The experimental design could not produce any information on the activities of individual mosquitoes, except for the hosts fed on, however, one can speculate about some possibilities.

First, partially fed mosquitoes may have represented mosquitoes that had been interrupted and that had not yet attempted a second meal. The observed increase in the percentage of multiple meals and the decrease in the percentage of incomplete meals from Treatment 1 to 2 in my field study were consistent with this possibility. It seems likely that a proportion of females with partial meals after two hours of exposure took second meals during the additional two hours available in Treatment 2 (relative to Treatment 1). This process would effectively increase the proportion of multiple meals observed from Treatment 1 to 2.

Second, partially fed mosquitoes collected in the absence of data on host availability (Magnarelli 1977) may be interpreted as evidence that a second host was not available or that a second attempt was unsuccessful. My experimental design answers the first, but not the second concern. Another possibility is that mosquitoes with partial meals may have obtained them on second or third attempts after the first was completely unsuccessful. Again, my experimental design could not address this issue.

It is safe to say that serological evidence from feeding studies (Washino and Tempelis 1983) must be interpreted with caution with regard to estimating the true frequency of multiple feeding. Also, if one considers the percentage of all interrupted meals (partial + multiple) that were multiple in my study (25.9%), the probability that *Cx. nigripalpus* would refeed after interruption was much higher than the overall proportion of multiple meals (6.6%) suggested. These figures also do not address the

proportions of mosquitoes that were interrupted and resumed feeding on the same bird, a parameter that was not directly measurable with the techniques that I used.

The information presented in this study was consistent with the hypothesis that mosquitoes may experience substantial mortality associated with blood feeding. However, the extra fecundity that accrues from repeated host contacts during a single gonotrophic cycle appears to outweigh the risk of mortality, at least under some of the conditions in my model. There were some conditions under which the non-persistent behaviour resulted in greater potential fitness than that from persistent behaviour. From these data, I conclude that there may be a real tradeoff in cost and benefit from feeding persistence and that this tradeoff will determine the ecological conditions under which one strategy might be selected over the other. The observed probability of feeding-associated mortality from my field work fell in the lower range of feeding associated mortalities used in the simulation model, although the caged conditions of the mosquitoes necessitates a cautious reliance on the actual value of the mortality estimates derived from this study. This was also the range in which mosquitoes that took multiple meals derived greatest potential reproductive benefit, relative to mosquitoes that did not take multiple meals.

The greatest benefit of the modeling approach used here was to suggest ecological conditions under which one might expect to find feeding persistence. Persistence is likely to have been selected for mosquitoes associated with hosts intolerant of and likely to interrupt blood feeders. Within this framework, mosquito specialists on non-insectivorous hosts might be expected to be more persistent because of the low probability of being eaten by their hosts. From the standpoint of size, large vertebrates are more successfully

fed on than small species (Edman and Scott 1987).
Specifically, species such as *Ae. flavescens* Müller (Hearle
1929) and *Psorophora ciliata* (Fabricius) (Edman and Downe
1964), both of which feed primarily on large ungulates,
might be expected to be more persistent and thus have higher
rates of multiple feeding than species such as *Cx. tarsalis*,
which feeds primarily on small birds (Washino and Tempelis
1983). In fact, the frequency of multiple feeding by *Ae.*
flavescens was higher (11%, Anderson and Brust unpublished
data) than that of *Cx. tarsalis* (6%) when collected under
the same conditions (Chapter 1). Similarly, the observed
frequency of multiple feeding by Ps. *ciliata* (62%) exceeded
the frequency observed for *Cx. tarsalis* (22%) by nearly
three times in the same study (Edman and Downe 1964). These
examples support the hypothesis that feeding persistence is
likely to be found in species of mosquitoes for which the
primary host poses little threat. However, in order to
examine this idea rigorously, it would be desirable to
measure the persistence of many species of mosquitoes under
controlled conditions and correlate such information with
data on the risk of mortality posed by their respective
hosts. The feeding persistence of *Ae. triseriatus* under
varying nutritional conditions has been experimentally
tested in the laboratory by Walker and Edman (1985b), but a
full study incorporating the question of persistence and
host range has not been done for any species of mosquito.

The model I used was based on the assumption that female
mosquitoes would be either persistent or non-persistent for
their entire life span. In fact, older females are more
likely to take multiple meals than younger individuals
(Klowden 1988, Xue et al. 1995). Additional experiments
should be carried out to determine if individual mosquitoes
may behave differently (i.e. increase their risk by
refeeding more frequently) with respect to feeding

persistence as they age and remaining lifetime fecundity decreases. Such a change in behaviour has been modeled (Clark 1993) for a number of organisms, including the parasitic wasp, *Leptopilina heterotoma* (Thompson). This wasp subjects its offspring to greater risk of failure by superparasitizing hosts when life expectancy and thus future reproductive potential is decreased (Roitberg et al. 1993). The extension of such a behavioural model to mosquitoes awaits the demonstration that mosquitoes can use information about the immediate risk from a host and their own reproductive potential to alter their feeding strategy.

It is important to note that the models I designed were based on a simplistic representation of feeding behaviour, for example, my decision to limit feeding attempts to two per night and the exclusion of a parameter to account for the effect of carbohydrate nutrition on feeding behaviour (Klowden 1988). Additionally, I assigned unvarying values to the probabilities of taking a second meal, given a first partial meal, and the probability of each blood meal size class. It is worth keeping in mind that the objective of this study was to consider possible tradeoffs in benefit and cost of feeding persistence given that blood feeding is a risky undertaking for mosquitoes. The method for assessing the possibility of such a tradeoff was to compare lifetime fecundity of individual mosquitoes as a function of the presence or absence of multiple feeding. Thus, the important piece of information is which strategy did better and under what circumstances. The absolute values of lifetime fecundity that resulted from each model may not have reflected actual values because of the simplifying assumptions of the model. The effect of varying those parameters held constant in this study can be explored in the future.

Table 4. Blood-feeding success[1] of *Cx. nigripalpus* attracted
to Northern bob-white.

Blood fed status	#	% of engorged	% of interrupted
trace	34	4.9	18.8
¼	36	5.1	19.9
½	64	9.1	35.4
multiple	47	6.6	25.9
Total interrupted	181	25.6	100
full	573	81.0	--
Total engorged	707	100	--
No meal	910	--	--
Total collected	1617	--	--

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[1]Data represent all mosquitoes collected during 20 trap
nights and three treatments combined.

Table 5. Change in partial and multiple feeding by *Cx.*
nigripalpus, and the mean[1] number of females of this species
attacking Northern bob-white over a two hour period.

Measurement	Treatment 1	Treatment 2	%Change
Mean/cage(±SE)	26.5(±5.2)	20.1(±4.0)	-24.34
% partial	25.8	15.9	-9.9
% multiple	5.6	7.4	+1.8

[1]Mean of 20 trap nights per treatment.

Figure 11. Decision rules governing feeding behaviour of
mosquitoes in the simulation model. The crosshatched area
within the silhouette drawings represents blood meal size.
Per cents beside each vertical arrow give the proportion of
mosquitoes in each blood-meal size category that behave as
designated at the top of the arrow.

PERSISTENT MOSQUITOES

NON-PERSISTENT MOSQUITOES

EGG DEVELOPMENT

EGG DEVELOPMENT

100%

100%

FULL

REFEED
90%

10%

100%

HALF

90%

10%

100%

QUARTER

90%

10%

100%

TRACE

100%

100% REFEED

NO BLOOD
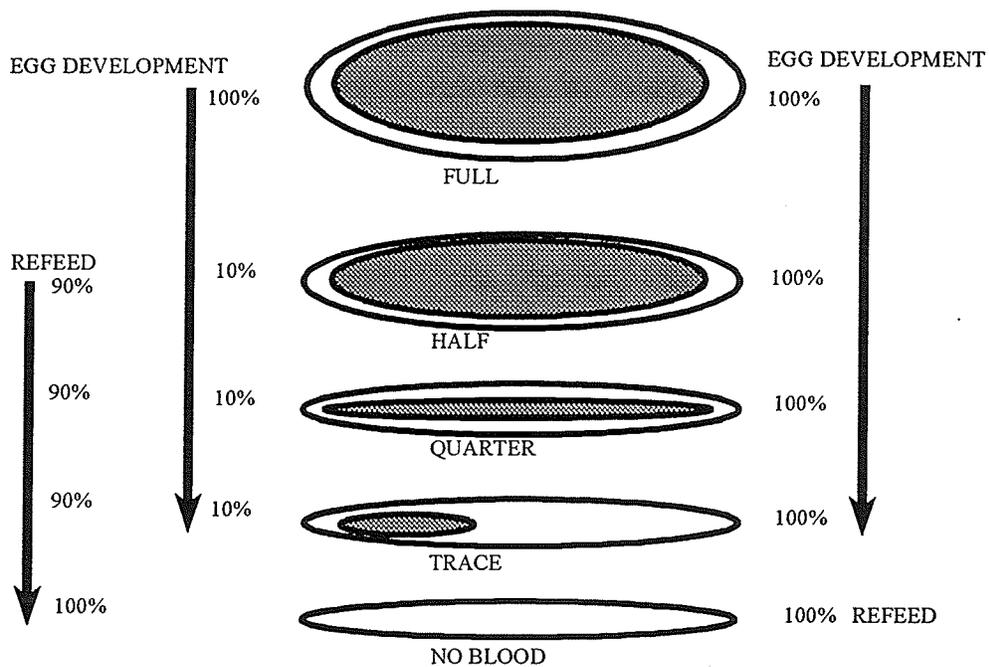
Figure 12. Flow chart of the simulation model for persistent
mosquitoes (a) and for non-persistent mosquitoes (b).
Dotted arrows denote additional blood-feeding opportunities
of persistent mosquitoes relative to non-persistent
mosquitoes. Each text box with a question mark signals an
event decided by comparison of a uniform random number
(between 0 and 1) and the value of the appropriate variable.
The corresponding decision rules in the simulation are
structured as inequalities so that when evaluated as true,
the mosquito proceeds along the pathway indicated by the
'yes' arrow and when evaluated as false, along the 'no'
arrow. Probabilities associated with different blood meal
volumes are as given in Fig.11.

a
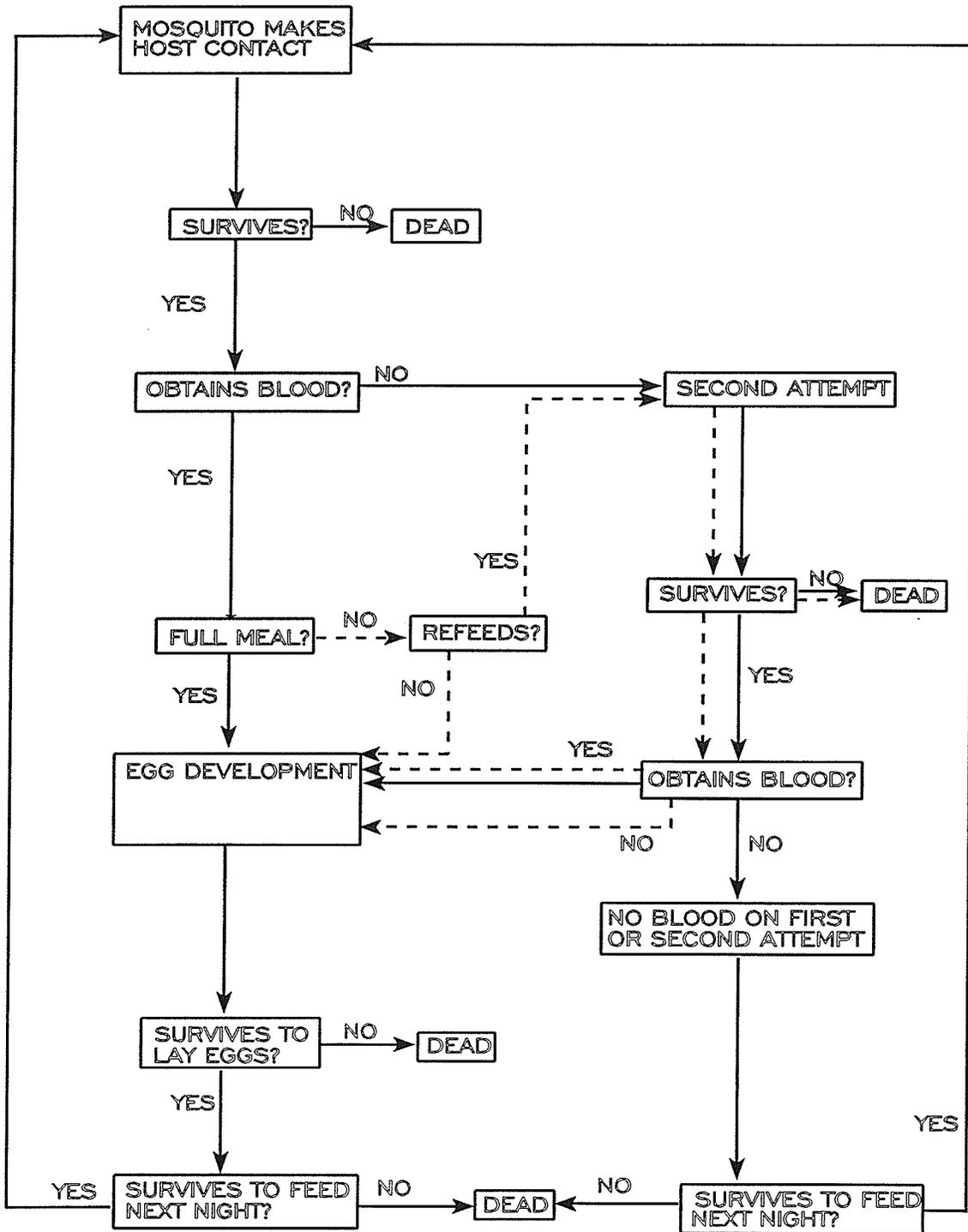
b

Figure 13. Mean-life time fecundity (for feeding associated mortality =0.05) of persistent mosquitoes (a.), and of non-persistent mosquitoes (b.) and relative payoff matrix (c.) according to probability of daily survival and probability of obtaining blood. Symbols in matrix denote the feeding behaviour that is better for each combination of daily survival and blood-feeding success (■-persistent, □-non-persistent). Symbol size (equal to the ratio of the higher to lower average, lifetime fecundities) is proportional to the relative fitness advantage of the better strategy. Blank spaces are values for which the model returned trivial results (fecundity ≤2 for both feeding strategies).

X=0.05

(a.)

(b.)

(c.)

Figure 14. Mean-life time fecundity (for feeding associated mortality =0.20) of persistent mosquitoes (a.), and of non-persistent mosquitoes (b.) and relative payoff matrix (c.) according to probability of daily survival and probability of obtaining blood. Symbols in matrix denote the feeding behaviour that is better for each combination of daily survival and blood-feeding success (■-persistent, □-non-persistent). Symbol size (equal to the ratio of the higher to lower average, lifetime fecundities) is proportional to the relative fitness advantage of the better strategy. Blank spaces are values for which the model returned trivial results (fecundity ≤2 for both feeding strategies).

X=0.20

(a.)　　　　　　　　　　(b.)　　　　　　　　　　(c.)

Figure 15. Mean-life time fecundity (for feeding associated mortality =0.35) of persistent mosquitoes (a.), and of non-persistent mosquitoes (b.) and relative payoff matrix (c.) according to probability of daily survival and probability of obtaining blood. Symbols in matrix denote the feeding behaviour that is better for each combination of daily survival and blood-feeding success (■-persistent, □-non-persistent). Symbol size (equal to the ratio of the higher to lower average, lifetime fecundities) is proportional to the relative fitness advantage of the better strategy. Blank spaces are values for which the model returned trivial results (fecundity ≤2 for both feeding strategies).

X=0.35
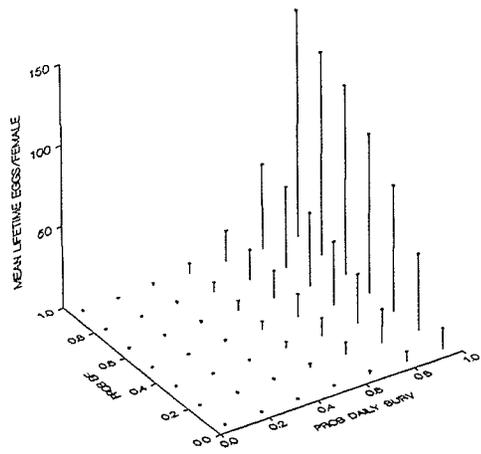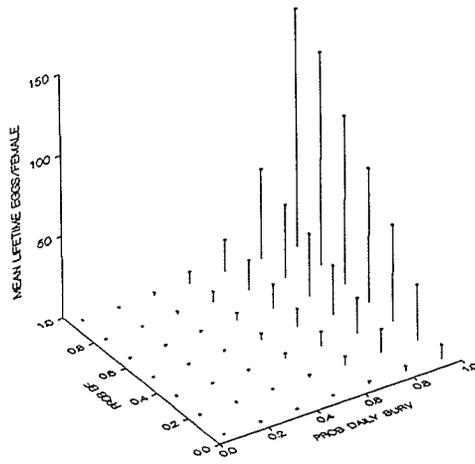
(a.) (b.) (c.)
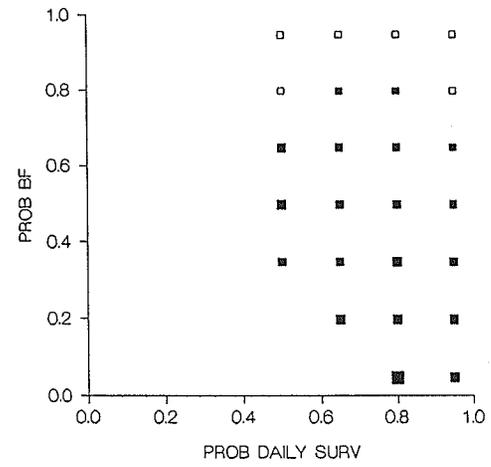
Figure 16. Mean-life time fecundity (for feeding associated mortality =0.50) of persistent mosquitoes (a.), and of non-persistent mosquitoes (b.) and relative payoff matrix (c.) according to probability of daily survival and probability of obtaining blood. Symbols in matrix denote the feeding behaviour that is better for each combination of daily survival and blood-feeding success (■-persistent, □-non-persistent). Symbol size (equal to the ratio of the higher to lower average, lifetime fecundities) is proportional to the relative fitness advantage of the better strategy. Blank spaces are values for which the model returned trivial results (fecundity ≤2 for both feeding strategies).
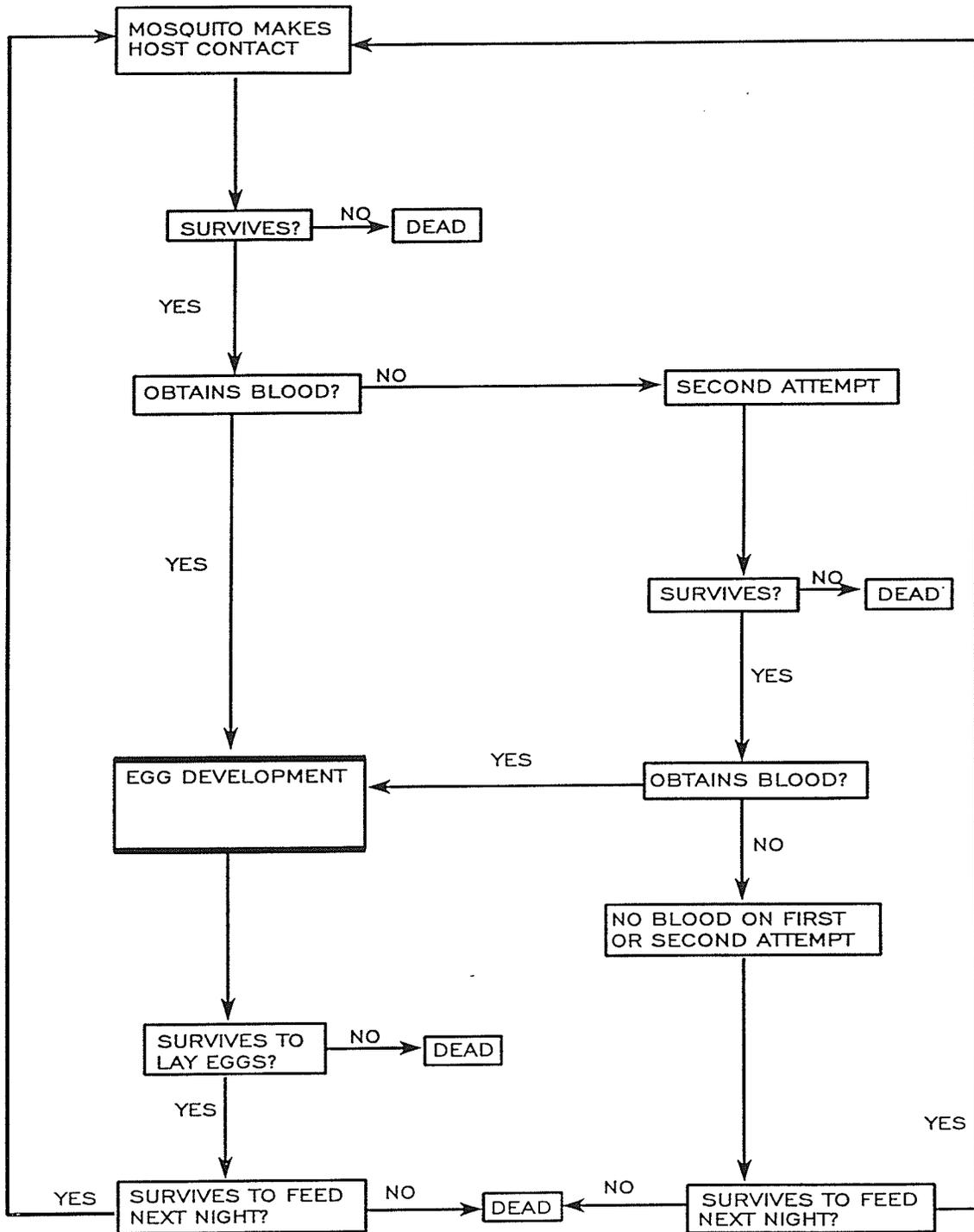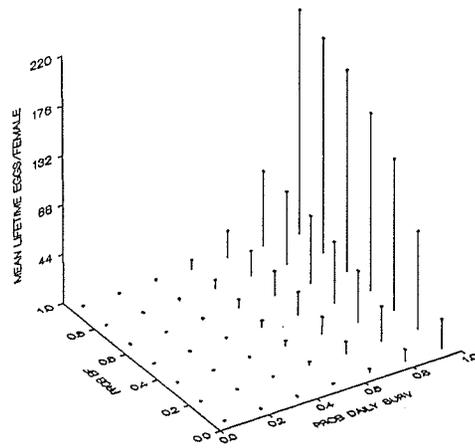
X=0.5

(a.)
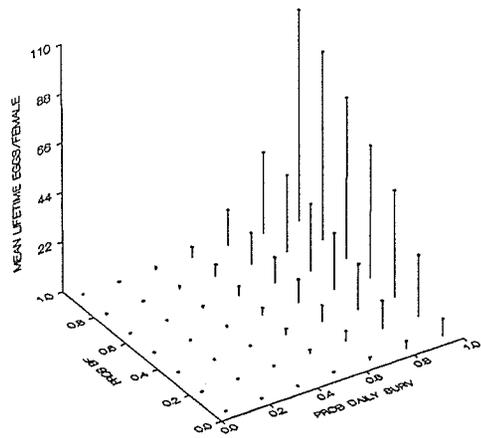
(b.)
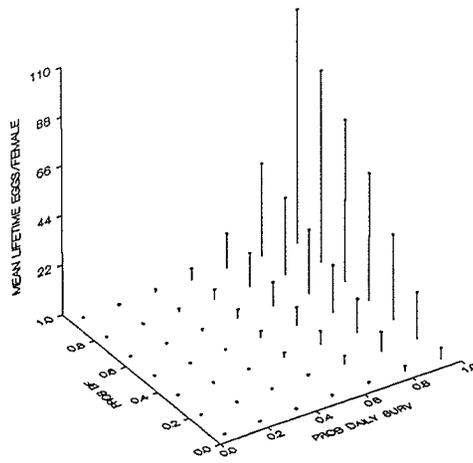
(c.)
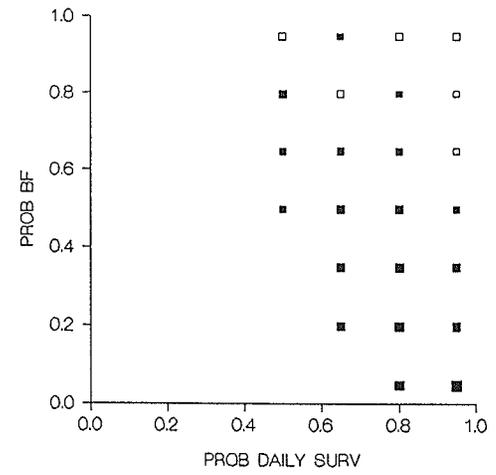
Figure 17. Mean-life time fecundity (for feeding associated mortality =0.65) of persistent mosquitoes (a.), and of non-persistent mosquitoes (b.) and relative payoff matrix (c.) according to probability of daily survival and probability of obtaining blood. Symbols in matrix denote the feeding behaviour that is better for each combination of daily survival and blood-feeding success (■-persistent, □-non-persistent). Symbol size (equal to the ratio of the higher to lower average, lifetime fecundities) is proportional to the relative fitness advantage of the better strategy. Blank spaces are values for which the model returned trivial results (fecundity ≤2 for both feeding strategies).

X=0.65

(a.)
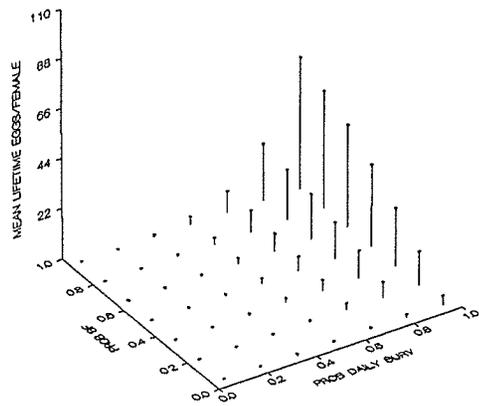
(b.)
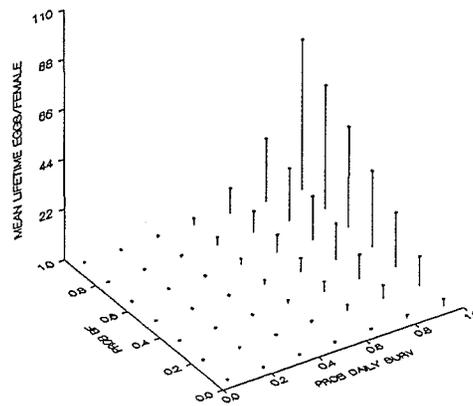
(c.)
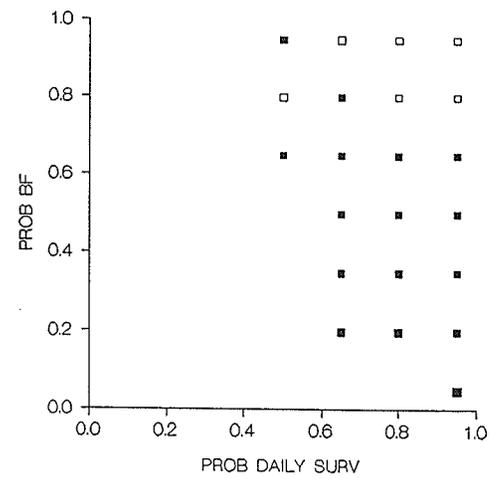
Figure 18. Mean-life time fecundity (for feeding associated mortality =0.80) of persistent mosquitoes (a.), and of non-persistent mosquitoes (b.) and relative payoff matrix (c.) according to probability of daily survival and probability of obtaining blood. Symbols in matrix denote the feeding behaviour that is better for each combination of daily survival and blood-feeding success (■-persistent, □-non-persistent). Symbol size (equal to the ratio of the higher to lower average, lifetime fecundities) is proportional to the relative fitness advantage of the better strategy. Blank spaces are values for which the model returned trivial results (fecundity ≤2 for both feeding strategies).
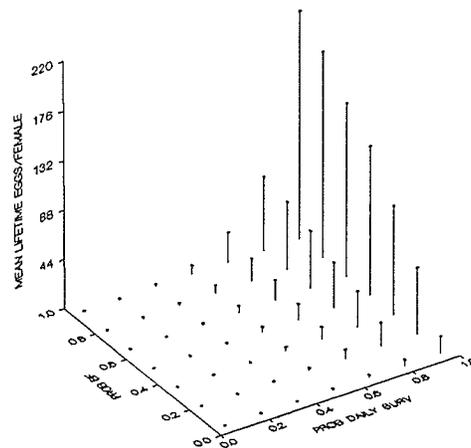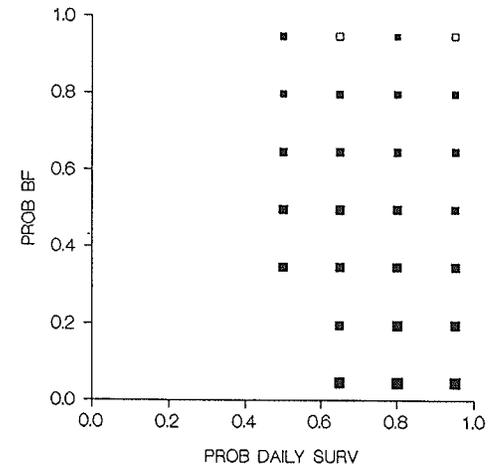
X=0.80

(a.)
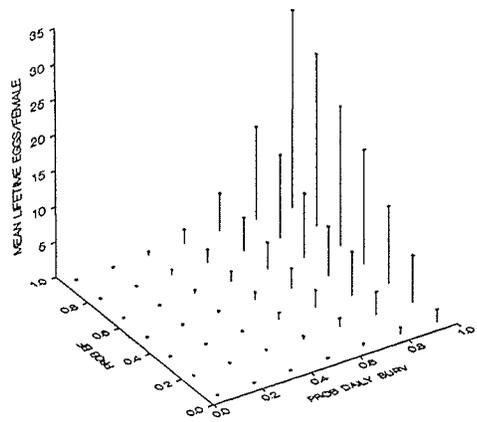
(b.)
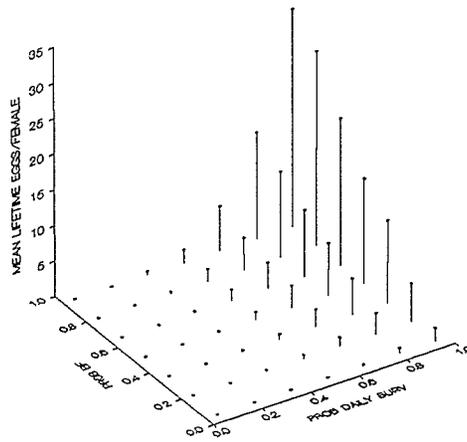
(c.)
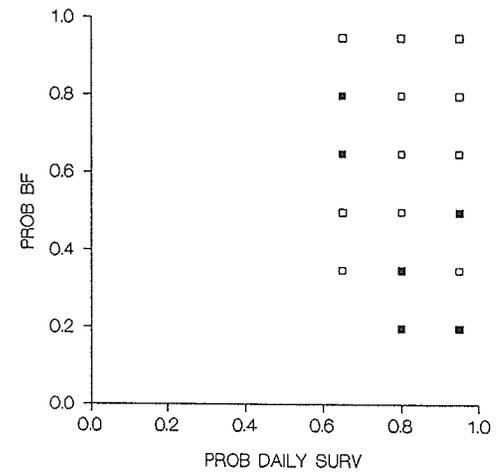
Figure 19. Mean-life time fecundity (for feeding associated
mortality =0.95) of persistent mosquitoes (a.), and of non-
persistent mosquitoes (b.) and relative payoff matrix (c.)
according to probability of daily survival and probability
of obtaining blood. Symbols in matrix denote the feeding
behaviour that is better for each combination of daily
survival and blood-feeding success (■-persistent, □-non-
persistent). Symbol size (equal to the ratio of the higher
to lower average, lifetime fecundities) is proportional to
the relative fitness advantage of the better strategy.
Blank spaces are values for which the model returned trivial
results (fecundity ≤2 for both feeding strategies).

X=0.95
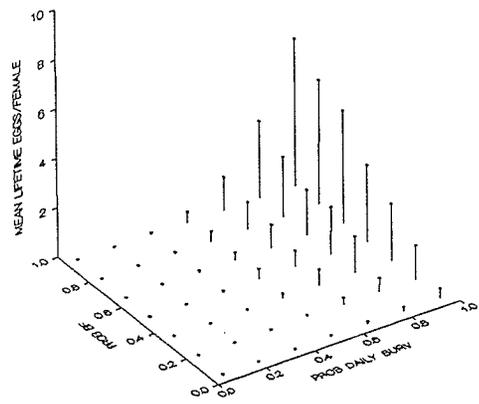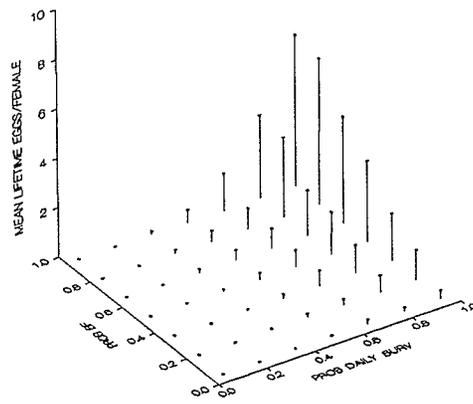
(a.) (b.) (c.)
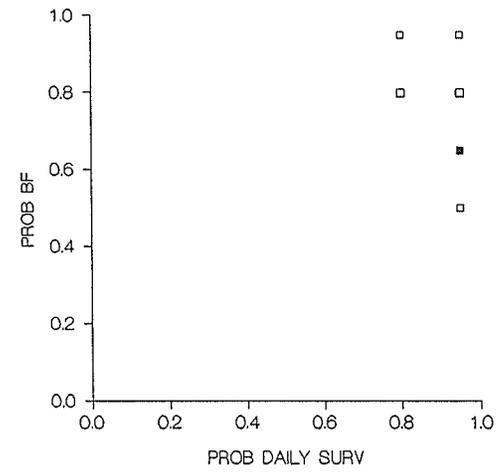
CHAPTER 5

GENERAL DISCUSSION

Edman and Downe (1964) documented that most mixed meals contained blood from at least one host not commonly present in single-host meals. They speculated that multiple meals occurred because mosquitoes were unlikely to feed to repletion on a less-acceptable host. More recently, support for an alternative hypothesis to explain multiple feeding, at least for gonotrophically concordant genera such as *Culex* has been recorded. Mosquitoes of this type are most frequently interrupted by host defensive behaviour (Edman and Scott 1987, Klowden 1988) and they seek more blood, most likely because their fecundity is increased as a result (Edman and Lynn 1975, Jalil 1974, Woke et al. 1956). The source of subsequent meals taken to augment the first, partial meal is dependent on a number of factors, not the least of which is the availability of another host in the event that the first is refractory to further attack because of irritation associated with the first. For species with a limited host range, multiple feeding on closely related vertebrates may be more significant than that which involves unrelated hosts because the most available sources of second or subsequent meals may be of the same host type as the one on which an initial meal was interrupted. This is especially true for colonial or social avian hosts, for example, house sparrows (Holden et al. 1973) or red-winged blackbirds (Weatherhead 1981, 1983).

Most studies in which multiple feeding has been documented were based on serological methods of identifying the vertebrate source(s) of blood meals (Washino and Tempelis 1983). Elucidating the host range of medically important mosquitoes was the primary objective of most early

serological studies. The detection of multiple feeding was a secondary consideration. However, these methods were likely to miss multiple feeding on closely related hosts because, in general, serology lacks specificity below the family level. More recently, other methods have been used to demonstrate that multiple feeding may often occur when several individuals of one type of host are available (Anderson et al. 1990, Boreham et al. 1978), but few species of mosquitoes have been studied in this way.

In this thesis, I have documented that field populations of *Cx. tarsalis*, *Cx. restuans*, and *Cx. nigripalpus* take multiple meals when offered two quail hosts simultaneously in overnight exposure. In fact, there was circumstantial evidence that multiple feeding may not have been limited to two meals per mosquito even though the method of detecting multiple feeding was designed to measure feeding on up to two hosts. My ability to detect multiple feeding was repeatable with three species of mosquitoes, two species of hosts and in two different geographical locations. I interpret this evidence, as well as published reports of multiple feeding by over 60 species of mosquitoes (Xue and Edman 1991), to mean that multiple feeding is a widespread phenomenon among mosquitoes. My observations accord well with estimates from other studies of multiple feeding by species of *Culex* (Boreham et al. 1978, Cupp and Stokes 1976, Edman and Downe 1964) even though these other studies were based on standard serological methods.

Although multiple feeding was documented in several studies, it was not possible to conclude with absolute certainty why some mosquitoes took two or more meals. It may have been that one type of host was relatively unacceptable to feeding mosquitoes so that the mosquitoes desisted of their own accord. Alternatively, multiple meals may have resulted because one host resisted attack and

interrupted the feeding process. The two quail in each cage
in my study were of the same species, size, age, sex and
health so that potentially confounding effects of these
parameters on the attractiveness of individual hosts were
controlled (Edman and Scott 1987). I could not absolutely
rule out differences in attractiveness of individual quail
as a determining factor in the observed patterns of blood
feeding. However, I demonstrated that feeding success of
mosquitoes on individual quail was negatively related to the
relative activity of the same quail, a relationship that has
been demonstrated for the feeding success of Cx. *nigripalpus*
on other avian hosts (Edman et al. 1972). These two pieces
of information, when considered together, support the notion
that defensive behaviour of avian hosts is one important
determinant of feeding success and thus of feeding
frequency.

There was considerable variation in the observed
frequency of multiple feeding by each of the species I
studied, even after I controlled for the inflationary effect
of small sample size on proportions by eliminating
observations with small numbers of mosquitoes. I had
expected that mosquitoes would blood feed with equal
frequency on both quail within a given cage because quail
within a pair were of the same species and similar to each
other with regard to weight, gender, and age. However, the
distribution of blood meals within a cage was skewed
significantly toward one quail or the other in most cases.
I was able to show that there was an inverse relationship
between the probability that a given quail would be fed on
and the relative intensity of the defensive behaviour
exhibited. Multiple feeding was highest in those cages in
which the two quail were fed on in more or less equal
proportion and lowest for quail pairs where one quail was
fed on to a much greater extent than the other. Although

this could have resulted from any asymmetry between quails that might have affected attraction, it is reasonable to conclude that defensive behaviour of the quail was one of the important determinants of the probability that a mosquito would be interrupted, and, consequently, feed more than once.

In addition to significant variation in the overall intensity of defensive behaviour among individual quail, I have shown that the least active quail were more likely to have been fed on and less likely to interrupt mosquitoes. Thus, such quail might be considered as better quality food resources for hungry mosquitoes. An important implication of the variation I observed among individual hosts is that host-seeking mosquitoes are faced with uncertainty during host selection, even after the effect of different host species is removed. This contradicts the view (Edman and Scott 1987) that behavioural variation among otherwise identical hosts is the least important determinant of mosquito feeding success. Resource heterogeneity has not been examined with regard to the evolution of specific mosquito foraging behaviours, but it has been found to affect foraging behaviour of other organisms (Krebs and Davies 1991).

Interrupted blood meals and the associated decrease in fecundity are not the only costs to mosquitoes of host defensive behaviour. I found that approximately 24% of *Cx. nigripalpus* were killed (probably eaten) by quail in a field study. The dynamics of behavioural interactions between mosquitoes and insectivorous hosts such as birds (Busby and Sealy 1978, Corbet and Downe 1966, Day and Edman 1984, Guinan and Sealy 1986) are different from many other predator/prey relationships because both mosquitoes and their hosts may prey on each other. Predation risk associated with foraging activities has been documented for

many animals (Lima and Dill 1990), however, the predators in these cases were not the food item of the forager. For species of mosquitoes such as *Cx. tarsalis*, *Cx. nigripalpus*, and *Cx. restuans*, that commonly feed on birds, each feeding attempt carries with it a significant risk of mortality, over and above that to be expected from purely defensive behaviour. Thus, the potential extra fecundity from second and subsequent blood meals may not be realized, and the fecundity from the first, small meal may be jeopardized if a mosquito is killed by its host during a second meal.

I used a simulation model to examine the potential tradeoff of cost and benefit from two different levels of feeding persistence. I defined the more persistent mosquitoes to be those that took multiple blood meals, in contrast to the less persistent females that did not attempt a second feed after some blood had been imbibed during the first. I found that multiple feeding often was the better strategy, but only when the probability of feeding associated mortality was low. Multiple feeding has been demonstrated for many species of mosquito in many genera (Xue and Edman 1991) and so I speculate that it has been selected as a feeding strategy in the evolution of mosquito behaviour. However, the probability of occurrence of multiple feeding is subject to the complex interplay of physiological condition of the mosquito (Klowden 1988) and ecology and behaviour of the vertebrate host (Edman and Scott 1987). As such, it will be difficult to formulate a simple model of where and when multiple feeding should occur.

From an epidemiological perspective, multiple blood feeding may increase the vectorial capacity of mosquitoes for pathogen transmission, especially during the amplification phase of viruses that are maintained in bird populations. To date, most models of disease transmission

have been based on the assumption that mosquitoes feed on one host per gonotrophic cycle. Data on multiple feeding may be used to challenge this assumption. Multiple host contacts during a single gonotrophic cycle may provide additional opportunities for mosquitoes to acquire pathogen infection, assuming that the probability of an infection is the same for small meals as it is for full meals. Multiple feeding is probably of more importance once a mosquito is infective as the pathogen may be transmitted to several vertebrates by a single mosquito within a short period of time. The fact that *Cx. tarsalis* (Henderson et al. 1979) and *Cx. nigripalpus* (Day and Edman 1988) are infective for life once they have acquired WEEV and SLEV respectively, supports this hypothesis. Multiple feeding may be important in the rapid spread and focal nature of some arbovirus transmission patterns (Scott et al. 1993). It is of more interest that there is some evidence that arboviruses may interfere with salivary physiology of mosquitoes such that they feed less successfully and so more frequently, which may, in turn, enhance the probability of transmission (Grimstad et al. 1980). This means that multiple feeding may be one of the most important factors that influence the ecological relationship between mosquitoes, their vertebrate hosts and the pathogens that infect both.

SUMMARY AND CONCLUSIONS

Multiple blood feeding on conspecific avian hosts was documented for field populations of three species of *Culex*, all of which are primary or secondary vectors of arboviruses in North America. I concluded that multiple feeding may be an important consideration in models of disease transmission because multiple host contacts within a single gonotrophic cycle may provide additional opportunities for mosquitoes to acquire and transmit pathogenic organisms. In this field study, I observed that individual quail varied in the degree to which they were fed on by attacking mosquitoes and patterns of interrupted and multiple blood feeding by mosquitoes could be related to the overall pattern of blood-feeding success. Patterns of host-utilization observed in the field may have been a result of differences among individual quail in the intensity of their defensive behaviour against attacking mosquitoes. This hypothesis was supported by data from a laboratory study. Some categories of defensive behaviour were affected by the species of mosquito, the density of attacking mosquitoes and by previous exposure to mosquitoes.

Data from a field study were used to estimate feeding-associated mortality to which mosquitoes attracted to my quail were subject. In my study, quail consumed approximately 24% of attacking mosquitoes. I also used data on feeding success (as indexed by the proportion of blood fed mosquitoes and blood meal size) to construct a stochastic simulation model of feeding behaviour with which I examined potential tradeoffs in cost and benefit to mosquitoes of feeding persistence. In general, data from this simulation model were consistent with the hypothesis that multiple feeding results in increased fitness relative to not taking multiple meals when the probability of feeding

associated mortality is low, but that the reverse is true when feeding associated mortality is high.

With regard to the objectives of my research, I conclude that:

1) multiple feeding on conspecific hosts of the same sex, age, size and health status is part of the behavioural repertoire of *Cx. tarsalis*, *Cx. restuans*, and *Cx. nigripalpus*. I also conclude that multiple feeding may be a more important behavioural phenomenon in relation to pathogen transmission than previously thought, especially for ornithophilic species of mosquitoes.

2) variation in anti-mosquito, defensive behaviour among individual quail is responsible for the patterns of host utilization, feeding success and multiple feeding observed in my initial field studies.

3) there are potential tradeoffs between costs and benefits of feeding persistence, such that multiple feeding may result in greater fitness under a limited range of ecological conditions, but that the overwhelming weight of evidence from the literature supports the hypothesis that multiple feeding has been selected as a behavioural strategy in most species of mosquitoes.

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APPENDIX 1

QBASIC@ Code for Stochastic Simulation of Persistent
(Multiple) Blood Feeding

```
OPEN "c:\path\filename.ext" FOR OUTPUT AS #1
REM Declare Constants
maxmos = 1000
REM `MAXMOS' =  # of mosquitoes to simulate
maxdays = 24
REM `MAXDAYS' = maximum lifespan of mosquitoes
maxlays = 3
REM `MAXLAYS' = maximum # of gonotrophic cycles
REM Define Storage
DIM totalmoseggs(1 TO maxdays) AS DOUBLE
DIM totalmosalive(1 TO maxdays) AS INTEGER
REM run simulation for all possible values of each variable
x = .05
REM `X' = prob. of dying during feeding attempt
WHILE x <= .95
  y = .05
REM `Y' = probability of imbibing blood
  WHILE y <= 1
REM `S' = probability of living thru 24 hr period
    s = .05
    WHILE s <= 1
REM `cd' = current day, `cm' = current mosquito
REM `totalmosalive(cd)'=# mosq. alive at end of a day
REM `totalmoseggs(cd)'=eggs laid on a given day
        FOR cd = 1 TO maxdays
           totalmosalive(cd) = 0
           totalmoseggs(cd) = 0
        NEXT cd
        FOR cm = 1 TO maxmos
REM Mosq. enters simulation, set initial status to alive
           mosalive = 1
           mosaliveday = 1
           mostimeseggslaid = 0
           cd = 1
REM Determine if mosq. has lived to end of lifespan
           WHILE (cd <= maxdays)
moseggs = 0
IF mosalive = 1 THEN
REM `URN' = uniform random number
REM seeds random number generator
RANDOMIZE TIMER
urn = RND
IF urn <= x THEN
REM mosq. dies feeding
```

```
      mosalive = 0
      mosaliveday = 0
  ELSE
    urn = RND
    IF urn <= y THEN
REM mosq. feeds 1st attempt, meal size given by f1-4
      urn = RND
       IF urn <= .05 THEN
         f1 = .125
      ELSEIF urn <= .1 THEN
          f1 = .25
      ELSEIF urn <= .2 THEN
          f1 = .5
      ELSE
          f1 = 1
      END IF
REM begin loop for mosq. with full meal, 1st attempt, no
REM 2nd meal, oogenesis begins
      IF urn > .2 THEN
        urn = RND
REM determine if mosq. lives 4 days to lay eggs
        IF urn <= s THEN
           mosalive = 1
           totalmosalive(cd) = totalmosalive(cd) + 1
           cd = cd + 1
           IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
             urn = RND
             IF urn <= s THEN
               mosalive = 1
               totalmosalive(cd) = totalmosalive(cd) + 1
               cd = cd + 1
               IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
                 urn = RND
                 IF urn <= s THEN
                   mosalive = 1
                   totalmosalive(cd) = totalmosalive(cd) + 1
                   cd = cd + 1
                   IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
                     urn = RND
                     IF urn <= s THEN
                       mosalive = 1
                       moseggs = f1 * 400
                     totalmoseggs(cd)=totalmoseggs(cd)+moseggs
REM Mosquito has laid eggs for the nth time
                       mostimeseggslaid = mostimeseggslaid + 1
                       IF mostimeseggslaid >= maxlays THEN
REM Mosquito has laid eggs more than MAX times, it dies
                         mosalive = 0
```

```
                            ELSE
                              urn = RND
                              IF urn > s THEN
                                mosalive = 0
                              ELSE
REM  If URN <= S, try to feed next night
                              END IF
                            END IF
                        ELSE
REM If URN > S, then stop
                          mosalive = 0
                          mosaliveday = 0
                            END IF
                      ELSE
REM passed maxdays
                          mosalive = 0
                          END IF
                    ELSE
REM if URN > S, then stop
                        mosalive = 0
                        mosaliveday = 0
                      END IF
                  ELSE
REM passed maxdays
                      mosalive = 0
                      END IF
                ELSE
REM If URN > S, then stop
                    mosalive = 0
                    mosaliveday = 0
                  END IF
              ELSE
REM passed maxdays
                  mosalive = 0
                  END IF
            ELSE
REM If URN > S, then stop
                mosalive = 0
                mosaliveday = 0
              END IF
          ELSE
            urn = RND
            IF urn > .9 THEN
REM Begin loop that calculates eggs for partially-fed
REM mosq. that do not refeed
              urn = RND
              IF urn <= s THEN
REM Begin determination of daily survival to oviposition
                mosalive = 1
                totalmosalive(cd) = totalmosalive(cd) + 1
                cd = cd + 1
```

```
            IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
            urn = RND
            IF urn <= s THEN
               mosalive = 1
               totalmosalive(cd) = totalmosalive(cd) + 1
               cd = cd + 1
               IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
                  urn = RND
                  IF urn <= s THEN
                     mosalive = 1
                     totalmosalive(cd) = totalmosalive(cd) + 1
                     cd = cd + 1
                     IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
                        urn = RND
                        IF urn <= s THEN
                           mosalive = 1
                           moseggs = f1 * 200
                        totalmoseggs(cd)=totalmoseggs(cd)+moseggs
REM Mosquito has laid eggs for the nth time
                        mostimeseggslaid=mostimeseggslaid+1
                        IF mostimeseggslaid >= maxlays THEN
REM Mosquito has laid eggs more than MAX times; it dies
                           mosalive = 0
                           ELSE
                           urn = RND
                           IF urn > s THEN
REM If URN > S, mosquito dies, so stop
                              mosalive = 0
                              ELSE
REM If URN <= S, go to beginning, mosquito feeds again
                           END IF
                        END IF
                     ELSE
REM If URN > S, mosquito dies, so stop
                        mosalive = 0
                        mosaliveday = 0
                        END IF
                  ELSE
REM passed maxdays
                     mosalive = 0
                     END IF
               ELSE
REM If URN > S, mosquito dies, so stop
                  mosalive = 0
                  mosaliveday = 0
                  END IF
            ELSE
REM passed maxdays days
```

```
                        mosalive = 0
                        END IF
                ELSE
REM If URN > S, mosquito dies, so stop
                    mosalive = 0
                    mosaliveday = 0
                END IF
            ELSE
REM passed maxdays days
                mosalive = 0
                END IF
          ELSE
REM If URN > S, mosquito dies, so stop
            mosalive = 0
            mosaliveday = 0
          END IF
        ELSE
REM Begin 2nd attempt for mosq. partially fed 1st attempt
          urn = RND
REM check to see if mosq. has died during feeding attempt
          IF urn <= x THEN
REM If URN <= X, mosquito is killed, so stop
            mosalive = 0
            mosaliveday = 0
          ELSE
REM check to see if mosquito gets blood
            urn = RND
            IF urn <= y THEN
REM determine size of blood meal
              urn = RND
              IF urn <= .05 THEN
                f3 = .125
              ELSEIF urn <= .1 THEN
                f3 = .25
              ELSEIF urn <= .2 THEN
                f3 = .5
              ELSE
                f3 = 1
              END IF
REM add up blood from 2 meals to max. of 1 meal equiv.
              f4 = f1 + f3
              IF f4 > 1 THEN
                f4 = 1
              END IF
REM determine if fed mosq. lives thru next 4 days
              urn = RND
              IF urn <= s THEN
                mosalive = 1
                totalmosalive(cd) = totalmosalive(cd) + 1
                cd = cd + 1
                IF cd <= maxdays THEN
```

```
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
                urn = RND
                IF urn <= s THEN
                   mosalive = 1
                   totalmosalive(cd) = totalmosalive(cd) + 1
                   cd = cd + 1
                   IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
                     urn = RND
                     IF urn <= s THEN
                        mosalive = 1
                        totalmosalive(cd)=totalmosalive(cd)+1
                        cd = cd + 1
                        IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
                          urn = RND
                          IF urn <= s THEN
                             mosalive = 1
REM calculate number of eggs to be laid
                             moseggs = f4 * 200
REM Mosquito has laid eggs for the nth time
                             mostimeseggslaid=mostimeseggslaid+1
                             IF mostimeseggslaid >= maxlays
                                THEN
REM If mosq. is about to lay more than MAX times, it dies
                                mosalive = 0
                             ELSE
                                urn = RND
                                IF urn > s THEN
                                   mosalive = 0
REM mosq. has layed eggs and starts feeding again
                                ELSE
                                END IF
                             END IF
                          ELSE
REM mosq has died, so stop
                             mosalive = 0
                             mosaliveday = 0
                          END IF
                        ELSE
REM passed maxdays days
                          mosalive = 0
                        END IF
                     ELSE
REM mosq has died, so stop
                        mosalive = 0
                        mosaliveday = 0
                     END IF
                  ELSE
REM passed maxdays days
                mosalive = 0
```

```
                          END IF
                    ELSE
REM mosq has died, so stop
                        mosalive = 0
                        mosaliveday = 0
                    END IF
                ELSE
REM passed maxdays days
                    mosalive = 0
                  END IF
              ELSE
REM mosq. has died, so stop
                mosalive = 0
                mosaliveday = 0
              END IF
          ELSE
REM Determine daily survival to ovip. for mosq. with
REM partial 1st meal, no success on 2nd attempt
              urn = RND
REM check daily survival
              IF urn <= s THEN
                mosalive = 1
                totalmosalive(cd) = totalmosalive(cd) + 1
                cd = cd + 1
REM check total age of mosquito
                IF cd <= maxdays THEN
                  urn = RND
                  IF urn <= s THEN
                    mosalive = 1
                    totalmosalive(cd) = totalmosalive(cd) + 1
                    cd = cd + 1
                    IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
                      urn = RND
                      IF urn <= s THEN
                        mosalive = 1
                        totalmosalive(cd)=totalmosalive(cd)+1
                        cd = cd + 1
                        IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
                          urn = RND
                          IF urn <= s THEN
                            mosalive = 1
                            moseggs = f1 * 200
                    totalmoseggs(cd)=totalmoseggs(cd)+moseggs
REM Mosquito has laid eggs for the nth time
                            mostimeseggslaid=mostimeseggslaid+1
                            IF mostimeseggslaid >= maxlays
                            THEN
REM Mosquito has exceeded allowed # of cycles; it dies
                              mosalive = 0
```

```
                                    ELSE
                                      urn = RND
                                      IF urn > s THEN
REM If URN > S, mosquito has died, so stop
                                            mosalive = 0
                                      ELSE
REM If URN <= S, mosq lives to feed again
                                            END IF
                                      END IF
                                 ELSE
REM If URN > S, mosq. has died, so stop
                                      mosalive = 0
                                      mosaliveday = 0
                                      END IF
                               ELSE
REM passed maxdays
                               mosalive = 0
                               END IF
                          ELSE
REM If URN > S, mosq. has died, so stop
                          mosalive = 0
                          mosaliveday = 0
                     END IF
                   ELSE
REM passed maxdays
                     mosalive = 0
                     END IF
                 ELSE
REM If URN > S, mosq has died, so stop
                   mosalive = 0
                   mosaliveday = 0
               END IF
             ELSE
REM passed maxdays
               mosalive = 0
               END IF
           ELSE
REM If URN > S, mosq. has died, so stop
             mosalive = 0
             mosaliveday = 0
           END IF
         END IF
       END IF
     END IF
   END IF
 ELSE
REM If URN > Y, then imbibed zero blood on first attempt
REM determine if it survives second attempt
   urn = RND
   IF urn <= x THEN
REM If URN <= X, mosq. dies trying to feed, so stop
```

```
          mosalive = 0
          mosaliveday = 0
      ELSE
REM If URN > x, mosq. lives thru feeding attempt
REM determine if mosq. imbibes blood 2nd attempt
          urn = RND
          IF urn > y THEN
REM If URN > Y, mosq. still empty after 2 attempts
REM determine if mosquito lives thru next 24 hours
            urn = RND
            IF urn > s THEN
REM If URN > S, mosq. has died, so stop
              mosalive = 0
              mosaliveday = 0
            ELSE
REM If URN <= S, mosq. lives to feed again next night
            END IF
        ELSE
REM Mosq. feeds 2nd time after none on 1st
REM determine size of blood meal
          urn = RND
          IF urn <= .05 THEN
            f2 = .125
          ELSEIF urn <= .1 THEN
            f2 = .25
          ELSEIF urn <= .2 THEN
            f2 = .5
          ELSE
            f2 = 1
          END IF
REM determine daily survival until eggs can be laid
          urn = RND
          IF urn <= s THEN
            mosalive = 1
            totalmosalive(cd) = totalmosalive(cd) + 1
            cd = cd + 1
            IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
              urn = RND
              IF urn <= s THEN
                mosalive = 1
                totalmosalive(cd) = totalmosalive(cd) + 1
                cd = cd + 1
                IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
                  urn = RND
                  IF urn <= s THEN
                    mosalive = 1
                    totalmosalive(cd) = totalmosalive(cd) + 1
                    cd = cd + 1
                    IF cd <= maxdays THEN
```

```
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
                        urn = RND
                        IF urn <= s THEN
                           mosalive = 1
REM calculate number of eggs
                           moseggs = f2 * 200
                     totalmoseggs(cd)=totalmoseggs(cd)+moseggs
REM Mosquito has laid eggs for the nth time
                           mostimeseggslaid=mostimeseggslaid+1
REM determine if last possible egg batch
                           IF mostimeseggslaid >= maxlays THEN
REM Mosquito has laid max. egg batches allowed, it dies
                              mosalive = 0
                              ELSE
                              urn = RND
                              IF urn > s THEN
REM If URN > S, mosq. has died during next 24 hr, so stop
                                 mosalive = 0
                              ELSE
REM If URN <= S, mosq. has lived to feed again
                              END IF
                           END IF
                        ELSE
REM if URN > S, mosq has died, so stop
                           mosalive = 0
                           mosaliveday = 0
                        END IF
                     ELSE
REM passed maxdays days
                        mosalive = 0
                     END IF
                  ELSE
REM if URN > S, mosq. has died, so stop
                     mosalive = 0
                     mosaliveday = 0
                  END IF
               ELSE
REM passed maxdays days
                  mosalive = 0
               END IF
            ELSE
REM If URN > S, mosq. has died, so stop
               mosalive = 0
               mosaliveday = 0
            END IF
         ELSE
REM passed maxdays days
            mosalive = 0
         END IF
      ELSE
REM If URN > S, mosq. has died, so stop
```

```
                mosalive = 0
                mosaliveday = 0
              END IF
            END IF
          END IF
        END IF
END IF
ELSE
  mosalive = 0
  mosaliveday = 0
  END IF
REM determine if mosq. has passed max. lifespan
IF cd <= maxdays THEN
  totalmosalive(cd) = totalmosalive(cd) + mosaliveday
END IF
                cd = cd + 1
              WEND
            NEXT cm
REM Initialize the tables
          PRINT #1, x; y; s;
          FOR cd = 1 TO maxdays
            PRINT #1, totalmosalive(cd); totalmoseggs(cd);
          NEXT cd
          PRINT #1,
REM increment each variable
          s = s + .15
      WEND
      y = y + .15
  WEND
  x = x + .15
WEND
PRINT "End of Simulation"
CLOSE #1
END
```

QBASIC@ Code for Stochastic Simulation of Non-Persistent
Blood Feeding

```
REM OPEN "c:\pathname\filename.ext" FOR OUTPUT AS #1
REM Declare Constants
maxmos = 1000
REM `MAXMOS' =  # of mosquitoes to simulate
maxdays = 20
REM 'maxdays' = maximum lifespan of mosq.
maxlays = 3
REM 'maxlays' = maximum # of gonotrophic cycles
REM Define Storage
DIM totalmoseggs(1 TO maxdays) AS DOUBLE
DIM totalmosalive(1 TO maxdays) AS INTEGER
REM run simulation for all possible values of each variable
x = .05
REM 'X'=probability of dying during each feeding attempt
WHILE x <= .95
 y = .05
REM 'Y'=probability of obtaining blood
   WHILE y <= 1
     s = .05
REM 'S'=probability of living through each 24 hr period
     WHILE s <= 1
REM `cd' = current day, `cm' = current mosquito
REM `totalmosalive(cd)'=# mosq. alive at end of a day
REM `totalmoseggs(cd)'=eggs laid on a given day
       FOR cd = 1 TO maxdays
          totalmosalive(cd) = 0
          totalmoseggs(cd) = 0
       NEXT cd
       FOR cm = 1 TO maxmos
REM Mosq. enters simulation, set initial status to alive
          mosalive = 1
          mosaliveday = 1
          mostimeseggslaid = 0
          cd = 1
REM determine if mosq. has passed max. lifespan
          WHILE (cd <= maxdays)
REM set egg load at beginning of the day to zero
moseggs = 0
IF mosalive = 1 THEN
REM `URN' = uniform random number
REM 'RANDOMIZE TIMER' seeds random number generator
RANDOMIZE TIMER
urn = RND
IF urn <= x THEN
REM mosq. dies feeding
  mosalive = 0
  mosaliveday = 0
```

```
ELSE
REM mosq. lives through feeding attempt
  urn = RND
  IF urn <= y THEN
REM mosq. gets blood
     urn = RND
REM 'f1-4' sets blood meal size
    IF urn <= .05 THEN
       f1 = .125
    ELSEIF urn <= .1 THEN
       f1 = .25
    ELSEIF urn <= .2 THEN
       f1 = .5
    ELSE
       f1 = 1
REM If mosq. is fed, goes on to oviposition
    END IF
    urn = RND
    IF urn <= s THEN
REM Determine if mosq. lives through next 4 days
       mosalive = 1
       totalmosalive(cd) = totalmosalive(cd) + 1
       cd = cd + 1
       IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
          urn = RND
          IF urn <= s THEN
             mosalive = 1
             totalmosalive(cd) = totalmosalive(cd) + 1
             cd = cd + 1
             IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
                urn = RND
                IF urn <= s THEN
                   mosalive = 1
                   totalmosalive(cd) = totalmosalive(cd) + 1
                   cd = cd + 1
                   IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
                      urn = RND
                      IF urn <= s THEN
                         mosalive = 1
                         moseggs = f1 * 200
                         totalmoseggs(cd)=totalmoseggs(cd)+moseggs
REM Mosq. has laid eggs for the nth time
                         mostimeseggslaid = mostimeseggslaid + 1
                         IF mostimeseggslaid >= maxlays THEN
REM Determine if mos. has laid eggs more than MAX times
                            mosalive = 0
                         ELSE
                            urn = RND
```

```
                              IF urn > s THEN
                                mosalive = 0
                              ELSE
REM 1. If mosq. lives through 24 hr after ovip. feeds again
                            END IF
                          END IF
                        ELSE
                          mosalive = 0
                          mosaliveday = 0
                        END IF
                      ELSE
REM passed maxdays
                        mosalive = 0
                      END IF
                    ELSE
                      mosalive = 0
                      mosaliveday = 0
                    END IF
                  ELSE
REM passed maxdays
                    mosalive = 0
                  END IF
                ELSE
                  mosalive = 0
                  mosaliveday = 0
                END IF
              ELSE
REM passed maxdays
                mosalive = 0
              END IF
            ELSE
              mosalive = 0
              mosaliveday = 0
            END IF
          ELSE
REM If mosq. did not get blood 1st, try 2nd
            urn = RND
            IF urn <= x THEN
REM Mosq. dies on 2nd attempt
              mosalive = 0
              mosaliveday = 0
            ELSE
REM Mosq. lives through 2nd attempt
              urn = RND
              IF urn > y THEN
REM Mosq. has no blood after 2nd attempt
                urn = RND
                IF urn > s THEN
REM Mosq. (unfed) dies during next 24 hrs.
                  mosalive = 0
                  mosaliveday = 0
```

```
              ELSE
REM Unfed mosq. lives to feed next night
          END IF
       ELSE
REM Mosq. gets blood on 2nd attempt
REM Blood meal size given by 'f2'
          urn = RND
          IF urn <= .05 THEN
             f2 = .125
          ELSEIF urn <= .1 THEN
             f2 = .25
          ELSEIF urn <= .2 THEN
             f2 = .5
          ELSE
             f2 = 1
          END IF
REM Does mosq. live to lay eggs
           urn = RND
          IF urn <= s THEN
            mosalive = 1
            totalmosalive(cd) = totalmosalive(cd) + 1
            cd = cd + 1
            IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
              urn = RND
             IF urn <= s THEN
               mosalive = 1
               totalmosalive(cd) = totalmosalive(cd) + 1
               cd = cd + 1
               IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
                 urn = RND
                IF urn <= s THEN
                  mosalive = 1
                  totalmosalive(cd) = totalmosalive(cd) + 1
                  cd = cd + 1
                  IF cd <= maxdays THEN
REM DO NOT PASS MAX NUMBER OF DAYS FOR SIMULATION
                    urn = RND
                   IF urn <= s THEN
                     mosalive = 1
                     moseggs = f2 * 200
REM Check gonotrophic cycle
                     mostimeseggslaid=mostimeseggslaid+1
                     IF mostimeseggslaid >= maxlays THEN
REM Mosq. has laid last batch of eggs; it dies
                       mosalive = 0
                       ELSE
                       urn = RND
                       IF urn > s THEN
REM After ovip., does mosq. live to feed again?
```

```
                                        mosalive = 0
                                   ELSE
REM Mosq. starts next gonotrophic cycle
                                      END IF
                                   END IF
                                ELSE
                                   mosalive = 0
                                   mosaliveday = 0
                                   END IF
                             ELSE
REM passed maxdays
                                   mosalive = 0
                                   END IF
                          ELSE
                             mosalive = 0
                             mosaliveday = 0
                             END IF
                       ELSE
REM passed maxdays
                             mosalive = 0
                             END IF
                    ELSE
                       mosalive = 0
                        mosaliveday = 0
                       END IF
                    ELSE
REM passed maxdays
                       mosalive = 0
                       END IF
                 ELSE
                    mosalive = 0
                    mosaliveday = 0
REM Close feeding loop for 2nd attempt
              END IF
           END IF
        END IF
      END IF
   END IF
ELSE
   mosalive = 0
   mosaliveday = 0
   END IF
IF cd <= maxdays THEN
   totalmosalive(cd) = totalmosalive(cd) + mosaliveday
END IF
              cd = cd + 1
REM Increment Day
           WEND
         NEXT cm
REM Start next mosq.
REM Initialize the tables
```

```
        PRINT #1, x; y; s;
        FOR cd = 1 TO maxdays
           PRINT #1, totalmosalive(cd); totalmoseggs(cd);
        NEXT cd
        PRINT #1,
REM Increment variables
        s = s + .15
    WEND
    y = y + .15
  WEND
  x = x + .15
WEND
PRINT "End of Simulation"
CLOSE #1
END
```