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I tested a theoretical model proposing that anemia favors transmission of blood-borne parasites to vectors by accelerating the blood-feeding rate. Using Aedes aegypti, the yellow fever mosquito, initially, I was not able to confirm this phenomenon either in an artificial or a live system; anemia did not correlate with blood-feeding rate, time or volume. I then analyzed the feeding rate over different time intervals to examine the possibility that inconsistent feeding rates masked the expected results; a comparison of the blood-feeding rate at one particular time period supports the theory.

I then continued investigating the influence of anemia on egg production of mosquitoes and found that anemia has negative influence on vector's fecundity. I conclude that although anemia has opposite influences on

mosquitoes in blood-feeding rate and egg production, they can get, at least under certain conditions, benefit from blood-feeding on anemic hosts.

Influence of Host Anemia on Blood-Feeding Rate and Egg Production of *Aedes aegypti* (L.) (Diptera: Culicidae)

by

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INFLUENCE OF HOST ANEMIA ON BLOOD-FEEDING RATE AND EGG PRODUCTION OF Aedes aegypti (L.) (DIPTERA: CULICIDAE)

Introduction

Mosquitoes transmit many of the most important pathogens of human beings and animals. They not only cause considerable irritation, but also are responsible for significant morbidity and economic loss. Mosquitoes are the only vectors that transmit the pathogens of malaria, yellow fever, and dengue to human beings, and they are also the most important vectors of filariases and viral encephalitides to human beings (Harwood and James, 1979). Arthropod-borne viruses infecting and causing disease in vertebrate hosts number about 125; epidemiological and environmental investigations would suggest that at least 100 are mosquito-borne (Maramorsch, 1962). The economic losses on cattle and milk production in the United States during 1965, caused by mosquitoes, were estimated at 25 million and 10 million dollars respectively (Steelman, 1976).

Ronald Ross, the first to elucidate the life cycle of malaria, developed a model of the basic reproduction rate of pathogens. This model had been elaborated by George Macdonald in 1958 and is probably the most famous model

for estimating disease transmission. This model describes the number of secondary disease cases arising from one primary disease case introduced into a human population. Recently, parasites have been shown to modify many of the parameters in this model (Molyneux and Jefferies, 1986). One particularly intriguing suggestion has been that the anemia associated with blood-borne parasites favors pathogen transmission by vectors.

Daniel and Kingsolver (1983) developed a model of sucking insects. According to this model, blood-feeding rate is a monotonically decreasing function of hematocrit value. If mosquitoes were to feed at a faster rate on anemic blood (lower hematocrit level) than on normal blood (higher hematocrit level), this might favor, in addition to the parasite, the vector as well because the shortened contact with a host may offset a lower protein intake. If so, then the biting habit and survival rate of vectors would be changed by parasites. Therefore, the estimation of pathogen transmission from the Ross-Macdonald model would be modified.

If anemic blood has no influence on egg production of the vector, the parasite benefits overall with no vector loss. It is likely, however, that anemic blood does affect the fecundity of blood-feeding vectors. Thus, the influence of blood-feeding on an anemic host becomes of

relative importance in the relationships among vectors, parasites and hosts.

This study, accordingly, sought to:

1. determine the relationships between anemia and vector feeding behavior (rate), and assess the design of Daniel and Kingsolver's model;
2. investigate the influence of anemia on egg production of mosquitoes;
3. weigh the gains and losses in the relationships among vectors, parasites and hosts.

Review of Literature

Feeding behavior of mosquitoes

Adult mosquitoes can only feed on fluids, because the food canal in both male and female is a fine capillary tube which allows only liquid to pass. Water, sugar and blood are the three most important fluid requirements for mosquitoes to maintain life. Doubtless, water is the easiest one to take and appears necessary for both female and male mosquitoes (Owen and Reinholz, 1968).

The most common and important energy source for both male and female mosquitoes is sugar (nectar and honeydew). Downes (1958) reported that it is more common for female mosquitoes to feed on nectar than on blood. There are many reports of mosquitoes visiting flowers, Aedes being the most common; other species of Anopheles, Culex, Mansonia, Psorophora and Toxorhynchites also are reported as feeding on nectar.

Although sugar is the most important energy source for adult mosquitoes, blood is the most important protein source for female mosquitoes to produce eggs. The protein content of blood is an essential nutrient for the development of eggs and serves as an energy source. A few days after emergence, they are physiologically ready for blood-feeding and mating (Porter *et al.*, 1986), although it has been suggested that sugar-feeding occurs prior to blood-feeding.

The feeding responses of mosquitoes to fluids are complex. Blood-feeding is the most complicated. There are at least 3 differences between blood and sugar-feeding. First, it is more convenient and easier for mosquitoes to feed on sugar solutions than on blood. They can feed on many kinds of liquid foods which are found in plants, for example nectar, honeydew, fruit juices, and "oozes" from injured or diseased areas of the plant. In contrast, blood-feeding follows a complex procedure that starts with host finding. Before landing on the host (Jones and Pillitt, 1973) or during the blood-feeding session, mosquitoes are interrupted often by host defensive behavior.

Second, the physiology of mosquitoes has different influence on blood-feeding and sugar-feeding. Most adult mosquitoes start sugar feeding on day 2 after emergence (Pimentel and Rossignol, 1990), and start blood-feeding on 2.6 days (63 h) after emergence (Porter *et al.*, 1986). Also, different diets end up in different organs. A blood meal ends up in the midgut whereas a sugar meal ends up in the crop. The final organ to store these two different materials is controlled solely by chemical signals in the diet, which means they have different feeding modes on different food resources.

Third, the mechanics of blood-feeding are more complex than those of sugar-feeding. Dethier (1957)

suggests that the procedures of blood-feeding include detecting and finding the host, alighting, probing (move stylets of mouthparts towards the surface of host skin), piercing or penetrating (insert stylets of mouthparts into skin), locating blood (either in a blood vessel or from a hemorrhage), ingesting blood, and ceasing blood-feeding. Each step is affected by many factors.

Factors affecting blood-feeding

In general, blood-feeding in most mosquito species is specific, i.e., they take blood only from a specific host. Forattini *et al* (1988) reported that Aedes mosquitoes feed blood mainly on mammalian hosts, while most Culex species feed on avian hosts. Mosquitoes typically feed more than once. The frequency of blood-feeding has a profound influence on vectorial capacity (Macdonald, 1957).

If the blood meal is below a critical volume, then eggs fail to develop (Spielman and Wong, 1974), and the mosquito will look for another host (Klowden and Lea, 1978); if the blood meal volume is above a critical volume, then two mechanisms regulate their host-seeking behavior preventing blood-feeding before eggs are laid. The first inhibition mechanism is induced by distention of the abdomen, which is a neural mechanism. It inhibits host-seeking behavior of mosquitoes until distention is below a critical volume. The second inhibition is induced by oocytes, and is a humoral mechanism. It is initiated

by the ovaries, but results from a substance produced by the fat bodies during vitellogenesis (Klowden, 1987).

Many factors affect blood-feeding of mosquitoes. The relationships among these factors are complex. Host defensive behavior may be the most important factor that affects blood-feeding in mosquitoes. Vertebrates have developed their own defensive mechanisms. In fact, mosquitoes feed more successfully on inactive hosts than on active hosts (Day and Edman, 1984). Waage and Nondo (1982) reported that after an initial exposure to mosquitoes, hosts could learn how to increase their efficient defensive behavior to repel mosquitoes. Some vertebrates, especially birds, develop behavioral responses to avoid the annoyance from the bite of mosquitoes (Webber and Edman, 1972). Although vertebrate hosts do not totally avoid the attacks of mosquitoes, their anti-mosquito behavior at least reduces the volume of blood-loss (Klowden and Lea, 1979).

There are many factors that ultimately affect blood-feeding of mosquitoes in the preliminary host finding and probing steps. To find a host, host body temperature is unquestionably the most important factor (Peterson and Brown, 1951) while olfactory and visual signals also are used (Hocking, 1971). In host probing, heat is an important factor, (Christophers, 1960), while CO_2 (Burgess, 1959) and moisture (Khan and Maibach, 1971) are

cofactors. Chemical and mechanical stimuli to the tarsi and proboscis of mosquitoes are other important factors that affect host recognition (Rutledge *et al.*, 1964).

The activation of pumping and the destination of the blood-meal is determined by chemical and osmotic properties of the diet (Galun *et al.*, 1971). The readiness to blood-feed and the sensitivity to test the food are affected by mosquito age, food state, water supplement and the previous blood-meal (Khan and Maibach, 1970). The termination of blood-feeding is initiated by abdominal stretch receptors which act in concert and signal the presence of optimal blood meal volume to the brain (Gwadz, 1969).

The physiology of both mosquito and host influence blood-feeding behavior of mosquitoes. Salivary function, specifically apyrase activity of mosquitoes inhibits platelet aggregation of hosts, enhancing blood vessel location (Ribeiro *et al.*, 1984). Hemostasis, consisting mostly of platelet aggregation, vasoconstriction and coagulation, is a major hurdle for all blood-feeders and numerous strategies have been developed to overcome this defense. Platelet aggregation seems to be the principal target that mosquitoes have focused on, although a vasodilator (Pappas *et al.*, 1986) and an anticoagulant (Ribeiro *et al.*, 1985) are present in saliva.

Diuresis

When mosquitoes blood-feed, they face the problem of how to maintain water and salt balance, as well as concentrate blood. Diuresis is the way that insects solve the problem. Recent studies have elucidated the endocrine control of diuresis in the blood-feeding of Aedes aegypti. Adult females take 2-5 min to consume a blood meal which is about twice of their body mass (Petzel *et al.*, 1987). Diuresis starts approximately 2 min after the beginning of blood-feeding (diuresis may occur when a mosquito is still feeding). The diuresis rate rises up to the peak 4-8 min later, and in most cases it largely finished within 20 min after the beginning of blood-feeding. However, the procedure of diuresis may continue at a greatly reduced rate for up to 2 hours (Williams *et al.*, 1983). During the first hour of diuresis, water content of blood meal was removed and the protein content was concentrated (Nijhout and Carrow, 1978).

It is suggested that diuretic peptides stored in the head may be released after a blood meal is taken and control diuresis (Wheelock *et al.*, 1988). Beyenbach and his co-workers (Petezel *et al.*, 1987; Williams and Beyenbach, 1984) have used HLPC to separate peptide material from the extracts of head and have isolated three fractions which affect the transepithelial voltage of the isolated Malpighian tubules. These three fractions have

the same characteristics in that they are heat-stable, pronase-degradable, and are small molecular weight peptides; but they have different functions: fractions I and II depolarize the transepithelial voltage, whereas fraction III firstly depolarizes, and then hyperpolarizes the voltage.

Blood meal digestion

Autogenous mosquitoes are those that can lay eggs without taking any blood meal, whereas anautogenous mosquitoes are unable to lay eggs before they have taken a blood meal. A few mosquitoes are autogenous for their first egg batch and subsequently are anautogenous. No species is known to be autogenous beyond the first oogonicycle. After blood-feeding, the ingestion of blood in anautogenous mosquitoes is known to trigger a series of physiological events which leads to the development of eggs. In Aedes aegypti, most intact proteins disappear 24-48 hr after blood-feeding (Irby and Apperson, 1989; Gooding, 1966). The proteinase activity in the midgut of Aedes aegypti reaches a maximum 24-36 hr after blood-feeding (Gooding, 1966). Several essential amino acids for egg development were found to increase sharply after a blood-meal. These amino acids are Ileu, Leu, Lys, Try, Val, His and Glu (Lea *et al.*, 1956). The peaks of these free amino acid concentrations start to rise in the hemolymph 4 h after blood-feeding. They reach the highest

level at 12-24 h, and then gradually return to the preceding level 5 days after a blood-feeding (Thayer *et al.*, 1971; Uchida *et al.*, 1990). Uchida and others (1990) observed that the concentration of these amino acids declined slightly first during the first 1-2 h and then increased sharply.

These amino acids appearing in mosquitoes after blood-feeding may be a factor influencing host specificity and disease transmission ability of vectors. Thayer and others (1971) reported that the changes of most free amino acid titers in Aedes aegypti are almost the same after an ingestion of blood-meal in both an avian and a human host. These amino acids are Tau, Ser, Thr, Asp(NH_2), and/or Glu(NH_2), Pro, Glu, Leu, Tyr, Val, Lys and His. Their concentration is significantly increased following a blood-meal. However, the changes of some free amino acid titres, usually Asp, and Ileu, are different after a blood meal on human or avian host. It is believed that such differences may be the most important factor in host specificity of an insect and its ability to transmit certain diseases caused by parasites.

There are many factors which affect blood-meal digestion. Irby and Apperson (1989) reported that the digestion rate and pattern of the principal proteins in serum (albumin and immunoglobulin G) might be different, and influenced by different blood sources. Light is one

of the factors that affects blood meal digestion rate. The longer the light exposure, the faster the blood meal digestion occurs. Age, mating and parity are also factors that affect the blood-meal digestion rate of female Aedes aegypti. Thayer and others (1971) suggested that both young and old mosquitoes may have the same blood digestion rate, but the metabolism of free amino acids in old mosquitoes was not as rapid as that in young mosquitoes. Downe (1975) showed that in Aedes aegypti, blood-meal digestion rate of females which were inseminated or injected with matrone (component of male accessory glands) is more rapid than that of virgin females; however, Thayer and others (1971) reported that insemination has no significant influence on tissue-free amino acids, and that there is no effect of mating in both quality of free amino acids and blood meal digestion rate, as judged by free amino acids patterns.

Relationship among vectors, hosts and parasites

The relationship among vectors, hosts and pathogens is complex, each of them is influenced both by each other and by environmental conditions to varying degrees (Smith, 1971). Epidemiologically, arthropod vectors are the most important factor in the spread of pathogen transmission in tropical areas. Two factors determine the epidemiological significance of vectors: internal characteristics of the vector itself, that provides a developmental environment

for pathogens to develop to infective stage, and the type of relationship that exists between vector and vertebrate host.

Vector-parasite relationship

All the factors which affect a vector's ability to serve as a host for pathogens are named vector competence. Vector competence is affected by both extrinsic factors and physiology of the vector itself. The ability of pathogens to exploit one particular vector may be measured by the following factors: (1) uptake by vector, (2) development in vector body, once they have penetrated in vector, and (3) the number of infectious pathogens output and delivered to final host.

Uptake of pathogens is potentially affected by the diverse physiological characters of vector, host and pathogen itself. Circadial, seasonal periodicity and tropism affect uptake (Kilama, 1976). McGreevy and others (1978) reported that after microfilariae were ingested by mosquitoes, they might be destroyed by the pharyngeal teeth of certain mosquitoes. Young *et al* (1990) reported that during the early stages when mosquitoes infected with parasite Bacillus sphaericus in their gut, mosquitoes have the potential to inhibit these pathogens. However, as reviewed by Molyneux and Jefferies (1986), pathogens have their own way to enhance uptake by vectors.

There are many factors which affect the development, movement and transmission cycle of pathogens once ingested by a vector. The parasites must travel to a suitable tissue where they can have sufficient nutrition for development, and then they must penetrate into or gain access to a specific site from which they will exit. The peritrophic membrane of arthropods probably is the first factor to limit the movement of pathogens. Rudzinska and coworkers (1982) have reported that peritrophic membrane of vectors may limit the passage of microfilariae and Babesia. The midgut wall may serve as a barrier to penetration by virus (Kramer *et al.*, 1981) and microfilariae (Schrater *et al.*, 1982). Mellor and Boorman (1980) reported that bluetongue virus can penetrate a vector's midgut best in association with the infection of microfilariae which disrupt the membrane. Because parasites develop in the host body, therefore the transmission cycle of arthropod-borne animal viruses are dependent upon the intrinsic interrelationships that exist between the virus and the vector, the invertebrate host (Monath, 1980).

Intensity of disease in human hosts may be related to the pathogen output from the vector. The output of Chagas disease pathogens is determined by the timing and placement of vector's feces. In tsetse-trypanosome interactions, the pathogen forms rosettes around sensory

structures in the food canal of the infected fly, thereby physically obstructing the flow of ingested food, which is believed to enhance pathogen delivery. Aedes triseriatus infected with La Crosse virus (LAC) tended to probe more and engorge less than uninfected mosquitoes (Craig *et al.*, 1980). Arthropod-transmitted parasites, notably malaria, may enhance their transmission by modifying vector blood-feeding behavior (Ribeiro *et al.*, 1985). In vertebrate hosts, blood-borne parasites induce hemostatic changes so that mosquitoes locate blood faster in the infected hosts than the uninfected hosts (Ribeiro *et al.*, 1985).

Many workers believe that parasites might have some degree of influence on their hosts and vectors. Some of them have reported that the longevity of mosquitoes is affected by Plasmodium parasites (Klein *et al.*, 1982), and that mosquitoes feed more often on vertebrate hosts infected with arboviruses than on uninfected hosts. Hacker (1971) demonstrated that when the population of Aedes aegypti infected with Plasmodium gallinaceum Brumpt, their fecundity was reduced.

Other studies suggested that parasites have no detrimental effect on the longevity of their hosts. Sweeney and others (1989) reported that there was no influence on egg number and hatch ability in Culex annulirostris, when they infected with the microsporidian parasite, Amblyospora dyxenoides. Nayar and Bradley

(1987) also reported that Aedes taeniorhynchus infected with Dirofilaria immitis (Leidy) has no influence on their egg development. When adult birds infected with parasites, they did not reduce their ability to repel mosquitoes.

Vector-host relationship

From Macdonald's model, entomological components include population density of vectors, longevity and their biting habits, together termed vectorial capacity. It includes all the variables which affect the ability of a vector to transmit disease. Doubtlessly, host behavior and weather conditions have detrimental effect on vector abundance and its biting habits (Freier, 1990), but the effect of abiotic factors is indirect and through a parameter of vectorial capacity. The effect of various biotic and abiotic factors on transmission has been reviewed (Burkot *et al.*, 1989).

Recent studies have shown that animals infected with blood-borne parasites are both more susceptible and more attractive to mosquitoes (see rev. by Molyneux and Jefferies, 1986). Anti-mosquito behavior of mice ceased in animals infected with the parasites of Plasmodium berghei, P. yoelii, or P. chabaudi (Day and Edman, 1983). Mosquitoes (Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus) could engorge on all restrained mice hosts easily, but they only could successfully engorge on

unrestrained mice when they were infected with malaria or SLE (Day and Edman, 1984). Mosquitoes located blood more rapidly in malaria or RVF virus-infected animals than in noninfected animals and the median duration of probing (blood location) on infected rodents was reduced by at least 1 min as compared to noninfected rodents (Rossignol *et al.*, 1985).

However, vertebrates have developed their own defensive behavior. Host defensive behavior could affect feeding pattern (Edman *et al.*, 1974) and feeding volume of mosquitoes (Klowden and Lea, 1979). Also, host immune responses to mosquitoes have a significant influence on mosquitoes' life cycle. When female Aedes aegypti feed on immunized rabbits with Aedes aegypti whole-mosquito homogenate, their egg production was reduced by 24 to 31% (Wikel, 1982).

Parasite-host relationship

The relationship between blood-borne parasites and their hosts is subtle. Anemia is one example. It is measured as hemoglobin per unit volume of blood which is below the normal level. The causes of anemia are multiple and diverse, blood-borne parasites being one biofactor to cause anemia. Generally speaking, the anemia caused by parasites does not directly cause the death of their hosts. Martin *et al* (1988) reported that the severity of

anemia highly correlated with the number of parasites present in blood.

Daniel and Kingsolver (1983) developed a mathematical model of blood-sucking in insects. According to the model, the blood-feeding time is a monotonically increasing function of hematocrit level. As a consequence, mosquitoes could get an advantage from infected hosts, because they could take the anemic blood at a faster rate. If so, then anemia would have an important implication for the evolution of disease transmission. This model has been challenged on a theoretical basis (Kesavan and Reddy, 1985).

Based on theoretical considerations, Daniel and Kingsolver suggested that if a parasite reduced host hematocrit, a vector could engorge more rapidly on such an infected host. If duration of vector-host contact was reduced and if gains from this reduction outweighed any detrimental effects of infection, parasite and vector would both benefit from an encounter.

Nutritional requirements for egg reproduction

Protein is the primary required nutrient for egg development in mosquitoes. Klowden (1987) reported that without blood, anautogenous mosquitoes could not produce eggs. Sugar might not be a required nutrient for mosquitoes in egg production, but it has influence on egg number. Klowden (1986) reported that sugar and

carbohydrates significantly affect egg production of mosquitoes after a blood meal. Eiakad and Humphreys (1990) also suggested that sugar-feeding following by a complete blood meal by Anopheles pharoensis favors egg production.

In addition, salt influences egg production. When salts containing sodium or potassium are added to the diet, the number of eggs is twice that of a salt-free diets (Dimond *et al.*, 1958). However, the concentration of vitamins, nucleic acid or sterols has little or no influence on egg number (Clements, 1963).

The quantity of nutrients which is already stored in mosquitoes might be an important factor in determining what kinds and how much additional nutrient must be taken before previtellogenic development. If mosquitoes developed under a crowded environment during the larval stage, then they would produce an adult of smaller body size and these mosquitoes are believed to store less nutrients than the larger mosquitoes which developed from a sufficient food and space environment (Nayar and Sauerman, 1970). Eiakad and Humphreys (1990) also reported that the diet in the larval stage has influence on the ovarian development in adult Anopheles pharoensis.

Model of egg development of Aedes aegypti

It has been confirmed that once protein is digested, the amino acids produced and stimulated egg production.

The blood meal thus triggers oogenesis, and host-seeking behavior is usually inhibited until the batch of eggs is laid (Klowden and Lea, 1979; Klowden, 1981). Recent reports show that many factors, including at least two environmental signals (emergence and the blood meal) and three hormones (JH, EDNH and ecdysone), interact to control egg development in Aedes aegypti.

Emergence causes the release of JH (Gwadz and Spielman, 1973; Hagedorn *et al.*, 1977), which acts on three target tissues. First, it causes changes in feeding behavior (Meola and Petralia, 1980) and mating. Simultaneously, JH affects the post-emergence follicle growth which proceeds to the resting stage (Gwadz and Spielman, 1973; Hagedorn *et al.*, 1977), but follicle growth is interrupted until a blood meal is ingested. Finally, JH makes the fat body competent to take up nutrients.

After a blood meal is ingested, the egg development neurosecretory hormone (EDNH) is released (Chang and Judson, 1977; Van and Lea, 1984), and acts on the ovaries, and stimulates them to synthesize ecdysone (Hagedorn *et al.*, 1979). After ecdysone is converted to 20-hydroxyecdysone (Hagedorn *et al.*, 1975,) the fat body responds to it (Flanagan and Hagedorn, 1977), and stimulates the synthesis of vitellogenin. The latter acts on ovaries and makes their development completed.

Egg number and egg size

Blood quantity, quality and female body mass are the most important factors that affect egg number and size. Once mosquitoes feed on a blood meal, the egg number is mainly affected by blood quantity and quality. Steinwascher (1982) found that the fecundity of female Aedes aegypti is highly correlated with pupal body mass because larger females mature more eggs than smaller females. Therefore, he suggested that an important strategy for female mosquitoes is to maximize body mass at pupation.

Egg number produced by the mosquitoes mainly correlates with the volume of the blood meal digested (Nayar and Sauerman, 1975; Feinsod and Spielman, 1980). The minimum quantity of blood necessary for any egg maturation in Aedes aegypti is 0.40 mg (Colless and Chellapah, 1960). Above the minimum volume, the egg number correlated with the blood volume ingested, but it no longer increases if the blood volume ingested is above 3.0 μl (Klowden 1987).

In addition, the age of female mosquitoes might be another factor to affect the egg number. Eiakad and Humphreys (1988) reported that the number of egg was decreased at a successive oviposition of female mosquitoes.

The egg size of mosquitoes varies, even within the same species complex. Damrongphol and Baimai (1989) reported that there is a difference in egg size and shape in the Anopheles dirus complex. The relationship between the body size and egg size is still obscure. It is generally believed that there is a positive relationship between offspring fitness and egg size (Smith and Frewell, 1974; Begon and Parker, 1986). Steinwascher (1982) reported that if females hatch from large eggs, they grew at a faster rate and attain a larger adult body size which allows them to take a larger blood volume, lay more and larger eggs than females hatched from smaller eggs. However, some entomologists (Wiklund and Persson, 1983; Wiklund and Karlsson, 1984) found that the correlation between them is not high; it is very difficult to find a critical minimum egg size (Begon and Parker, 1986; Steinwascher, 1984).

Chapter 1: Influence of host anemia on blood-feeding rate of mosquitoes

Introduction

Many parasites enhance transmission by modifying the behavior or physiology of their vectors and hosts. Recent work suggests that hosts infected with blood parasites are both more susceptible, and possibly even more attractive, to mosquitoes and numerous vectors (see rev. by Molyneux and Jefferies, 1986).

One particularly intriguing suggestion has been that anemia, associated with blood-borne parasites, favors pathogen transmission. Daniel and Kingsolver (1983) developed a mathematical model of blood sucking in insects, based on previous work on nectar feeding by Lepidoptera. According to this model, blood-feeding rate is a monotonically decreasing function of red blood cell level. As a consequence, mosquitoes derive a benefit from anemic hosts because they can take anemic blood at a faster rate. If so, anemia caused by blood-borne parasites would have important implications for the evolution of disease transmission.

This experiment accordingly was conducted to determine whether or not mosquitoes feed faster on anemic or normal blood in both an artificial and a natural system.

Material and Methods

Mosquito rearing:

Mosquitoes used in these experiments were the yellow-fever mosquitoes, Aedes aegypti (L), Georgia strain. Eggs were stored at room temperature. When needed, they were put in a glass finger-bowl of 4" radius and 3" depth to be hatched. Larvae were fed pelleted Hartz gerbil and hamster food.

Pupae were taken from the finger-bowl and put in a plastic cup containing water. Upon emergence, both male and female mosquitoes were moved and kept in a rearing cage. Adults were fed dry sucrose; water was provided. Mosquitoes were held at room temperature with a 12/12 hr light/dark cycle. Only female mosquitoes at the age of 4-9 days after emergence were used.

Mosquito weighing:

Cold-anaesthetized mosquitoes were weighed individually before and after blood-feeding. First, the mosquito was put into a glass test-tube, and then the tube was placed in a basket containing ice cubes. After the mosquito was immobilized, it was weighed on an electronic balance with sensitivity of 10^{-5}g. The difference between before and after blood-feeding is considered to be the net blood weight the mosquito had taken.

Hematocrit determination:

Rabbit blood used in this study was provided by Western Oregon Rabbit Company (Philomath, Oregon). Red blood cell (RBC) levels used in the experiment of artificial membrane apparatus were 0%, 25%, 50% 75% and 100% which are equivalent to the hematocrit values of 0%, 10%, 20%, 30% and 40%, respectively.

To avoid the lysis of blood, rabbit blood was centrifuged at 2200 rpm for 15 min (in a hematocrit centrifuge). Each different RBC level treatment was achieved by removing a suitable proportion of RBC. ATP (10^{-4}M) was added to each blood treatment in the artificial membrane apparatus. The temperature of the feeding chamber containing rabbit blood was kept at 37^{o}C by a water bath circulator. The membrane used to cover the feeding chamber was "Beaudruche" (Long and Long, Belleville, NJ).

To minimize any effect due to hemosedimentation, the feeding chamber which containing blood was shaken after blood-feeding by the mosquito or every 5 to 10 minutes if no feeding occurred.

Blood-feeding time measurement:

Duration of blood-feeding was taken to start when the stylets of mosquito had penetrated into host skin or membrane and the palpi had stopped moving until the stylets were withdrawn from the host or membrane.

Rabbit treatment:

The white rabbits used in the study were raised in Laboratory Animal Resource of Oregon State University. Both rabbits were males and matched for weight and age. They were made anemic by withdrawing approximately 20% of estimated blood volume. The blood-feeding site on the host was the ear.

Results

First, I compared the blood-feeding rate of mosquitoes on rabbit blood at different hematocrit levels in blood-feeding chamber covered by an artificial membrane. There was no correlation between blood-feeding rate and hematocrit level. The slope of the regression line is 0.0012, R^2 is 0.03 (Fig. 1).

Next, I compared the blood-feeding behavior of mosquitoes on 100% rabbit blood with and without centrifuging (Table 1). There was no significant difference in the blood-feeding time and weight on these two treatments. There was however a significant difference in the blood-feeding rate. These data suggested that the method I used, centrifugation, to make these blood treatments had no influence on the blood-feeding time and weight, but had influence on the blood-feeding rate.

Fig 1 Regression line of blood-feeding rate of Aedes aegypti on different RBC levels in an artificial system

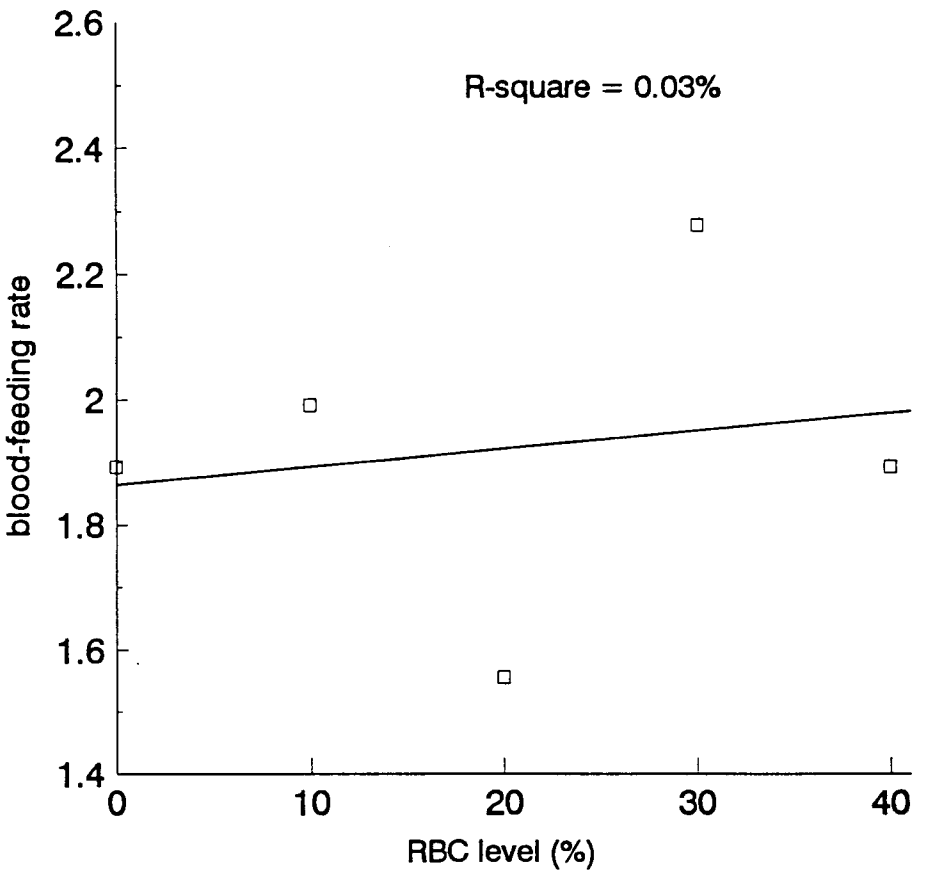


Table 1 Comparison of blood-feeding time, weight and rate of Aedes aegypti on rabbit blood with and without centrifugation in an artificial system

	100% RBC Treatment		P-value
	Centrifuged	noncentrifuged	
Time (sec)	106.71 ± 8.6	108.07 ± 4.7	0.89
Weight (10^{-5}g)	208.90 ± 22.8	252.08 ± 16.9	0.14
Rate (10^{-5}g/sec)	1.89 ± 0.22	2.47 ± 0.26	0.04

I then compared the blood-feeding rate of mosquitoes on serum treatment (0% RBC) with that on 100% RBC treatment without centrifuging. I found that the blood-feeding rate of mosquitoes on the serum treatment was not faster than on 100% R.B.C. treatment, which is contrary to expectation (Table 2). It seems that hematocrit level might not play a role in blood-feeding rate, because mosquitoes did not feed faster on serum (0% RBC).

Possibly, the blood-feeding through an artificial membrane apparatus might not represent the true blood-feeding behavior of mosquitoes. Therefore, I compared the blood-feeding rate of mosquitoes on live rabbits. The hematocrit value of the anemic rabbit used in this experiment was 29%, that of the normal rabbit was 42%. No significant differences were found between these two rabbit treatments in rates, time, and ingested weight either (Table 3).

During the time I did these experiments, I observed that mosquitoes fed on blood at different rates over the blood-feeding session. Hematocrit level may still play a role, but was masked by mosquito's behavior. Therefore, I analyzed the blood-feeding rate of mosquitoes on a live normal rabbit at 20 second intervals. To obtain these data, I measured the mean ingested weight per time for the first 20 seconds by interrupting feeding; then, the second group of mosquitoes were fed for only 40 seconds,

Table 2 Comparison of blood-feeding rate on different RBC treatments in an artificial system

Treatment (% RBC)	Blood-feeding rate (10^{-5}g/sec)
100 (noncentrifuged)	2.47 ± 0.16
0 (serum)	1.89 ± 0.20

Table 3 Comparison of blood-feeding on normal and anemic rabbits

	Treatment		P-value
	normal	anemic	
Time (sec)	120 ± 7	127 ± 10	0.56
Weight (10^{-5}g)	316 ±26	321 ± 29	0.90
Rate (10^{-5}g/sec)	2.61 ± 0.19	2.65 ± 0.26	0.89

and the mean of the previous group subtracted from this last one, and so on. I found that the blood-feeding volume of mosquitoes was fairly constant for the first 80 seconds, and then fell suddenly (Fig. 2), as did the blood-feeding rate (Table 4). When I compared the blood-feeding rate of 126 sec treatment (the whole feeding session) with that of live rabbit treatments, I found that the rates were almost the same, they are 2.61 ± 0.19, 2.65 ± 0.26 and 2.76 ± 0.20 x 10-5 g/sec, respectively (Table 3 and 4). These results imply that the blood-feeding rate of mosquitoes declined after they had fed for 80 sec in both anemic and normal rabbits.

To circumvent the possible error in averaging introduced by time periods following 80 seconds, I compared blood-feeding rate of mosquitoes on normal (41% hematocrit) and anemic (33% hematocrit) rabbits only over the first 40 seconds. Under this condition, I noted a significant difference between them, the blood-feeding rate are 4.2 ± 0.28 and 3.51 ± 0.22 x 10^{-5} g/sec, respectively, P = 0.04 (Table 5). The blood-feeding weights are different, being 140 ± 8.8 and 168 ± 11.2 (10^{-5}g) respectively. As an additional control, the blood-feeding rate on the anemic rabbit was tested when its hematocrit level was normal, and was found to be similar to the normal one, blood-feeding rate is 3.4 ± 0.28 (10^{-5} g/sec), P = 0.03 (Table 5). Besides, the

Fig 2 Mean blood-feeding weight at different time intervals

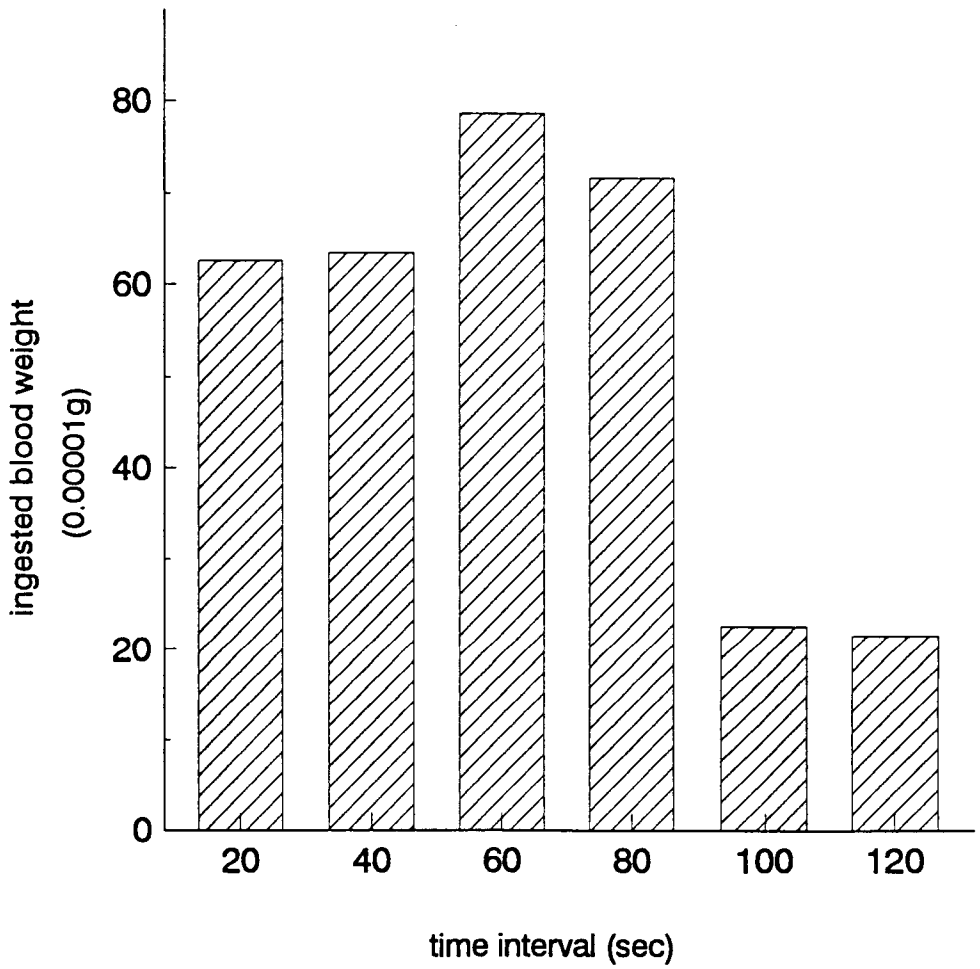


Table 4 Mean blood-feeding rate over different time intervals

Treatment	Blood-feeding rate (10^{-5}g/sec)
0-20 sec	3.13 ± 0.24
0-40 sec	3.51 ± 0.23
0-60 sec	3.41 ± 0.21
0-80 sec	3.45 ± 0.16
0-100 sec	3.02 ± 0.27
0-126 sec	2.76 ± 0.20

Table 5 Comparison of blood-feeding rate on normal and anemic rabbits over the first 40 seconds

	Rabbit 1	Rabbit 2	
	Normal (41%)	Anemic (33%)	Normal (42%)
Feeding rate (10^{-5}g/sec)	3.51 ± 0.22	4.2 ± 0.28	3.4 ± 0.28

ratio between these observed values is 1.1966 which essentially identical to the expected ratio of 1.1716 that I had calculated from the Daniel and Kingsolver's model (Table 6). Anemia, therefore, influences blood-feeding rate, but its impact may be masked by vector or host behavioral parameters.

In addition, I compared the blood-feeding behavior of mosquitoes in an artificial system (using 100% RBC) with that on a live animal (using normal rabbit). No significant difference was found between the treatments in the feeding rate, time, and weight (Table 7).

Finally, I checked the relationships between body weight and blood-feeding rate and weight. Table 8 shows that the R-square of the regression line of blood-feeding rate on body weight is low in each treatment in both an artificial apparatus and in a live rabbit system. These results suggest that there is no correlation between body weight and blood-feeding weight at least in Aedes aegypti. Also, there is no correlation between body size and blood-feeding rate at different time interval treatments in a live animal system (Table 9).

Table 6 Relative duration of blood-feeding at different hematocrits calculated from Daniel and Kingsolver's model

Hematocrit value (%)	Duration of Blood-feeding (expected)	Blood-feeding rate (observed)
0	1.0	
10	1.1882	
20	1.4290	
30	1.6415	4.20
40	1.9231	3.51

1. Expected ratio of blood-feeding time between hematocrit value of 30% and 40% is 1.6415/1.9231 = 0.8536 (i.e., the ratio of blood-feeding rate is 1/0.8536 = 1.1716)

2. Observed ratio of blood-feeding rate between anemic and normal rabbits is 4.20/3.51 = 1.1966

Table 7 Comparison of blood-feeding time, weight and rate in a live animal and an artificial system

	Artificial	Live animal	P-value
Sample size	65	16	
Feeding rate (10^{-5}g/sec)	2.47 ± 0.16	2.64 ± 0.19	0.46
Feeding weight (10^{-5}g)	252.08 ± 16.9	316.31 ± 26	0.17
Feeding time (sec)	108.07 ± 4.7	120 ± 7	0.25

Table 8 Regression analysis of blood-feeding weight on body weight in different RBC treatments

Treatment	Sample size	Slope	P-value	R-square (%)
Blood-feeding on an artificial system				
Serum	55	-0.08	0.92	0.02
25% RBC	35	1.21	0.30	3.43
50% RBC	71	0.58	0.23	2.09
75% RBC	47	0.63	0.36	1.88
100% RBC	30	1.36	0.15	7.41
Blood-feeding in a live rabbit				
normal rabbit	16	0.89	0.37	5.73
anemic rabbit	16	0.73	0.52	3.03

Table 9 Regression analysis of blood-feeding rate on body weight in different time interval treatments

Treatment (sec)	Sample size	Slope	P-value	R-square (%)
20	30	-0.24	0.32	3.56
40	26	0.43	0.37	3.36
60	31	-0.46	0.40	2.43
80	22	0.40	0.55	1.79
100	14	-0.37	0.75	0.89
126	17	1.42	0.41	4.48

Discussion

From Daniel and Kingsolver's blood-sucking model, the physical character of blood is one of the factors that determine blood-feeding rate. In this study, it was found that the blood-feeding rate of mosquitoes was affected by the treatments used to modify the blood. The blood-feeding rate slowed when mosquitoes fed on the blood treatments obtained by centrifuging normal blood. It might be that the red blood cells were lysed by centrifugation resulting in a lower feeding rate. However, this is unlikely because the blood-feeding rate on 0% RBC treatment was not higher than that on 100% RBC treatment.

If mosquitoes were allowed to feed to repletion, either through an artificial membrane or on a live animal, they did not display a correlation between anemia (hematocrit level) and blood-feeding rate. Statistical deviation in the data was quite large and led me to suspect that other factors were masking the theoretically expected results. On verifying this possibility, I noted that the blood-feeding rate was constant only for the initial minute and a half or so, after that it fell sharply. This observation may be due to either an actual change in the blood-feeding rate or the losses of diuresis.

Stobbart (1977) suggested that when measuring blood weight ingested by mosquitoes, it must be corrected for the weight loss from fluid excreted and transpired during the blood-feeding session (5 min or less), the total corrected values being 0.073 mg. Compared with the weight gained from the blood meal, 2.63 mg, (Stobbart, 1977), this is relatively small and might have little effect on the feeding weight that I calculated. Therefore, the difference in the feeding rates over the whole blood-feeding session do exist.

When this pattern was circumvented experimentally, I noted that a significant difference in the blood-feeding rate did exist between normal and anemic animals, and that this difference closely matched theory. I conclude that the Daniel and Kingsolver's model is correct in its premise and design, at least for Aedes aegypti, but that the inconsistent blood-feeding rate may restrict its implications. Until the cause of these changes is determined, be they due to diuresis or behavior, it will be difficult to determine whether or not the model possesses epidemiologic relevance. I did determine, however, that when such factors are minimized, the model is strongly predictive.

Many authors mentioned the relationships between body size and feeding amount. MacDonald (1956) suggested that smaller size female mosquito take a smaller size

blood meal. Steinwascher (1982) also suggested that females hatching from larger eggs grew faster, and attain a larger adult body size which allowed them to be able to take a larger blood meal. However, in this study, I found that there is no correlation between body weight and blood-feeding weight and rate in Aedes aegypti.

The egg number produced by mosquitoes was mainly determined by the volume of blood meal ingested (Woke *et al.*, 1956; Feinsod and Spielman, 1980). If blood meal amount is above a critical volume, then egg number is proportional to the blood meal volume ingested. However, if the blood meal amount is above 3.0 μl, the egg number is no longer increased with blood meal amount (Woke *et al.*, 1956; Klowden, 1987). Therefore, an amount greater than 3.0 μl is not crucial for mosquitoes to mature a batch of eggs. In my observations, any size of female mosquitoes could feed 3.0 μl blood meal amount, and therefore, body weight becomes less important a factor for mosquitoes to take more blood to produce more eggs. In this study, I only weighed mosquitoes but did not measure their body size (length of wings), therefore maybe it is necessary to do in future studies to confirm whether or not body size plays a role in blood-feeding weight and rate.

Critical blood meal volume is the most important factor for mosquitoes producing eggs. In the view of

epidemiological implications, it was found that first, mosquitoes were interrupted often during a blood-feeding; second, the anemia, loss of red blood cells caused by blood-borne parasites, did not always cause death of the host. Therefore, both mosquitoes and parasites would benefit when mosquitoes feed on an anemic host. When mosquitoes feed on an anemic host, they could reach the threshold blood amount easily to produce eggs. As a consequence, they could reach the critical vector population to transmit parasites.

The role of anemia in the transmission of vector-borne blood parasites is one more important example of parasite manipulation of the vector-host relationships. The pervasive presence of anemia in blood-borne diseases certainly suggests an important role but, as with many other such interactions, its influence may be subtle and interact with many other parameters. Further work should concentrate on infected host behavior and other vectors; indeed a paradox may already exist because infected hosts are often lethargic (Day and Edman, 1983) and more likely to let a vector feed, thus leading a relationship outside the limits stated above.

Chapter 2: Influence of anemic blood on egg production of mosquitoes

Introduction

Mosquitoes are known for their fecundity. Recent work shows that protein is the primary nutrient requirement for egg development. The number of eggs produced by mosquitoes is correlated with the volume of blood ingested.

According to Daniel and Kingsolver's blood-sucking model, mosquitoes may feed at a faster rate on an anemic host. This has been confirmed in the previous chapter. If anemic blood has no influence on egg production of the mosquitoes, then the parasites may derive advantages. It is likely, however, that anemic blood does affect the fecundity of mosquitoes.

This experiment accordingly was conducted to investigate the influence of anemic blood on egg production of mosquitoes.

Material and Methods

Mosquito rearing:

All the mosquito rearing procedures are the same as in the previous experiment except that fewer eggs and food were put in the glass finger-bowls. About 100 eggs were hatched in each bowl, which provided enough developmental space for larvae. Food was put in the glass finger-bowl, which was the least amount possible but sufficient for larvae to eat. Both conditions provide sufficient space and food environment for larvae

development to minimize the difference of body weight which was believed to be a major factor that affect egg production.

Adult mosquitoes were kept following the same procedure as in the previous experiment. Mosquitoes were held at room temperature.

Mosquito weighing:

Mosquitoes were weighed in the same way as in the previous experiment (Chap. 1).

Hematocrit determination:

All the RBC treatments and procedures were the same as in the previous one except the method to make different RBC treatments.

In this experiment, rabbit blood was centrifuged at 4500 rpm for 15 min (in a hematocrit centrifuge). Then, serum was taken and added to rabbit blood to obtain different RBC levels.

Egg laying chamber:

After blood-feeding, each female mosquito was put individually in a rearing cage, 2" in radius and 4" in depth, covered by nylon netting. A piece of white paper, 5.5" in length and 1.6" in width, was placed in a small plastic cup, 1" in radius and 1.2" in depth. Then water was poured into the cup, which provide water for mosquito to drink. The moist paper was the substrate for mosquito to lay eggs. Water was filled daily.

Egg number counting:

The white paper was replaced and the eggs on the paper were counted every day, until the mosquito died. The total egg number produced per female and the mortality rate was calculated.

Egg size measurement:

After the eggs were counted, the papers were kept in moist conditions for five days. After that, 11 hatches of eggs were taken by using simple random sampling method in each treatment. Then 10 eggs were also selected by using simple random sampling in each hatch, and the size of each egg was measured by using microscope. The magnification of microscope used to measure the egg size was 25X.

Results

First, I compared the egg numbers that were produced by mosquitoes after blood-feeding on different hematocrit levels in blood-feeding chamber. The regression line (Fig. 3) suggests that there is a correlation between egg number and RBC level fed to mosquitoes. The slope of this linear model is 0.40, R-square is 93%. Table 10 shows that the number of eggs produced by mosquitoes after blood-feeding on different RBC treatments were divided into three groups. The first group is 100% RBC treatment, mosquitoes laid the largest number of eggs.

Fig 3 Regression line of the number of eggs on different RBC levels

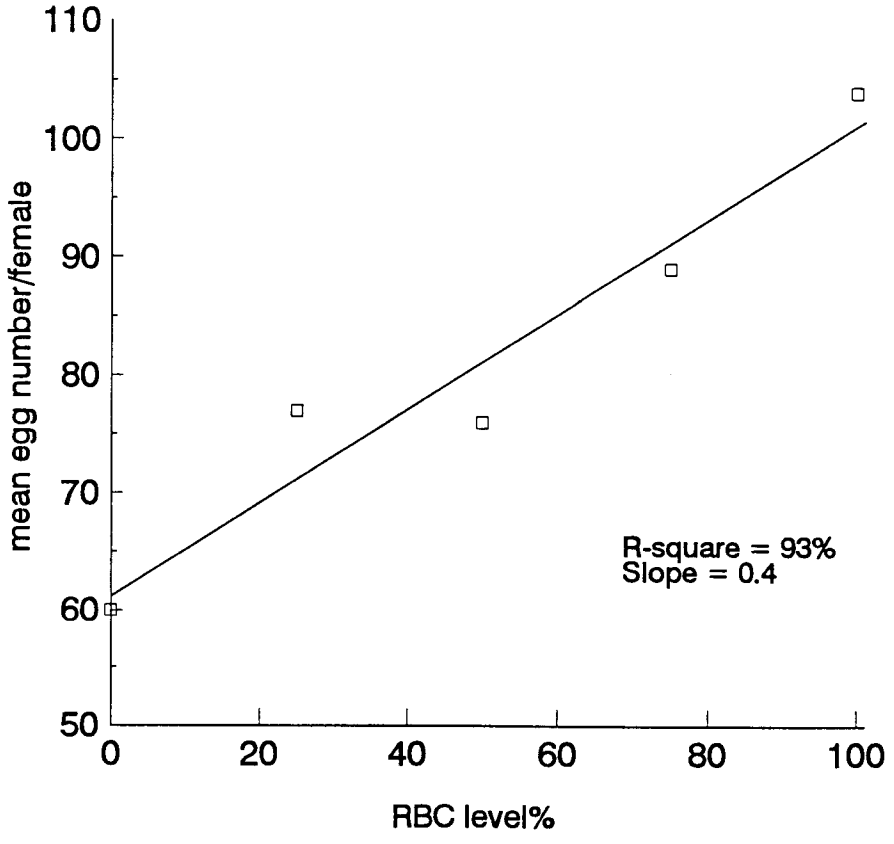


Table 10 Comparison of egg numbers after blood-feeding on different RBC treatments

Treatment	Egg number (mean)	Homogeneous groups
Artificial system		
0% RBC (H.V. = 0%)	60.23 ± 4.24	a
50% RBC (H.V. = 20%)	76.18 ± 3.98	a b
25% RBC (H.V. = 10%)	76.71 ± 3.98	b
75% RBC (H.V. = 30%)	88.68 ± 3.98	b
100% RBC (H.V. = 40%)	103.83 ± 3.69	c
Live rabbit		
Normal (H.V. = 38%)	94.26 ± 4.73	
Anemic (H.V. = 31%)	90.53 ± 4.38	
	P = 0.58	

Note: H.V. represents Hematocrit value.

The second group includes the treatments of 75%, 50% and 25% RBC. The third group is serum (0% RBC) treatment, mosquitoes laid the least number of eggs.

When the number of eggs after blood-feeding on live animals with different hematocrit value, that of normal rabbit is 38%, that of anemic rabbit is 31%, was compared, it was found that there was no significant difference between them (Table 10). Possibly the difference of hematocrit value between them was not large enough.

I then compared egg numbers between the "normal" hematocrit values of the animal and artificial system, 38% and 40%, respectively. I found that there was no significant difference between them (Table 11). These results suggest that the egg production of mosquitoes was not affected by the feeding apparatus, either a live host or a feeding chamber.

To circumvent the possible covariance, namely that different blood weight ingestion might affect egg number, I compared the means of ratio (whole blood-feeding weight divided by egg number, which is blood meal weight required to produce an egg,) among these different RBC treatments. The regression line (Fig. 4) shows that there is a correlation between RBC level and ratio, (the slope is -0.019, R-squared is 87%). Table 12 shows that the ratio of these treatments is divided into three groups again. The first group is the ratio at 100% RBC

Table 11 Comparison of egg production after feeding to repletion on an artificial system and an animal

	Artificial system	Animal	P-value
Hematocrit value	40%	38	
Sample size	41%	39	
Egg number	103.83 ± 3.69	94.26 ± 4.73	0.11

Fig 4 Regression line of ratio of blood meal weight to egg on different RBC levels

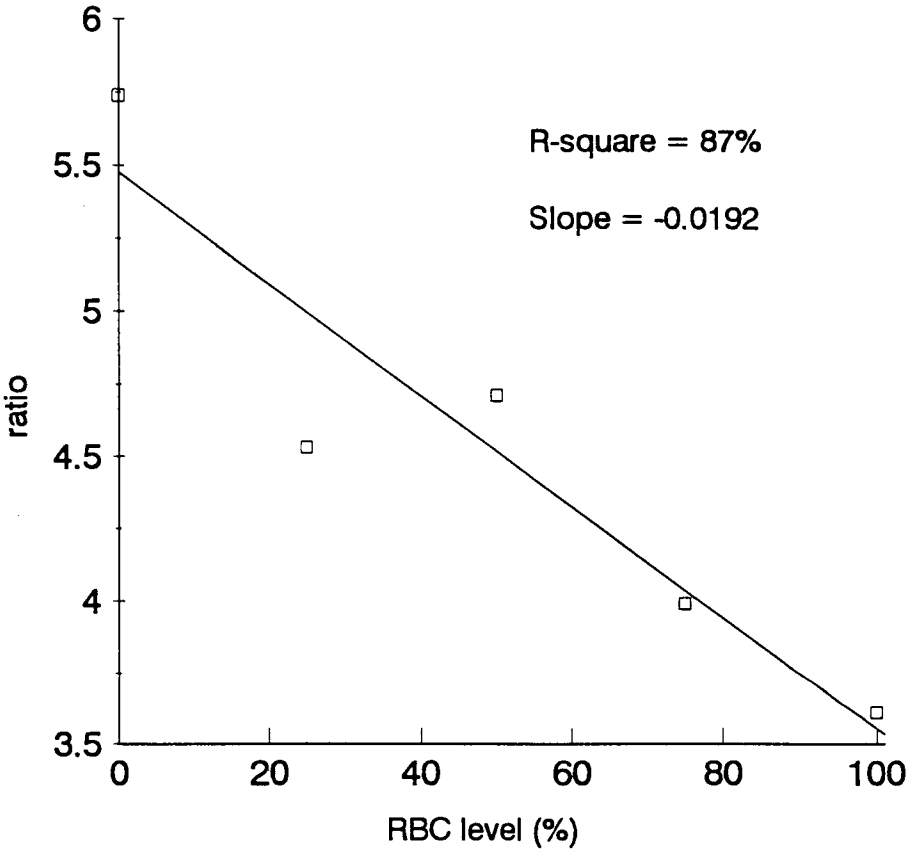


Table 12 Comparison of ratio, blood meal weight/egg number, after blood-feeding on different RBC treatments on an artificial system

Treatment (% RBC)	Ratio	Homogeneous groups
0	5.74 ± 0.21	a
50	4.71 ± 0.20	b
25	4.53 ± 0.21	b
75	3.99 ± 0.19	b c
100	3.61 ± 0.18	c

treatment, which is the smallest one. Only the ratio at 75% RBC has no significant difference from it. The second group includes the ratios at 75, 50 and 25% RBC treatments. The third group is the ratio at serum treatment, which is the largest one (Table 12). Mosquitoes thus need more blood (higher ratio) to produce eggs when they feed on lower RBC level blood.

When I compared the mean egg size produced by mosquitoes fed on these different RBC levels, it was found that there was no significant difference among them (Table 13).

I then compared duration after blood-feeding to the laying of the first egg among these treatments (Table 14). There was no significant difference among the means of duration. The earliest day to lay egg is day 4 after the blood meal in each treatment. Ranges are similar and vary from 4-9 to 4-13 days. The modes of the duration in all treatments were the same, day 5 after a blood meal. When comparing the egg laying patterns of mosquitoes after blood-feeding on normal and anemia rabbits, the egg-laying patterns are almost the same, more than 90% of eggs were laid during day 4 to day 8 after a blood meal (the first 5 oviposition day) on both normal and anemic rabbits (Fig. 5).

Mortality of mosquitoes after blood-feeding on normal and anemic rabbit was identical (Fig. 6). About 80% of mosquitoes survived in both treatments 3 weeks

Table 13 Mean egg size after blood-feeding on different RBC treatments on an artificial system

Treatment groups (% RBC)	Egg size (x 0.365 mm)	Homogeneous
0	1.589 ± 0.025	a
25	1.602 ± 0.040	a
75	1.626 ± 0.039	a
100	1.685 ± 0.035	a

Table 14 Comparison of duration, days after blood meal to lay the first egg, in different RBC treatments

Treatment groups (% RBC)	Range (days)	Mode (days)	Mean (days)	Homogeneous
100	4-10	5	5.40 ± 0.24	a
0	4-9	5	5.70 ± 0.28	a
75	4-10	5	5.71 ± 0.26	a
50	4-11	5	6.11 ± 0.26	a
25	4-13	5	6.21 ± 0.25	a

Fig 5 Egg-laying pattern of female adult Aedes aegypti after blood-feeding on normal and anemic rabbits

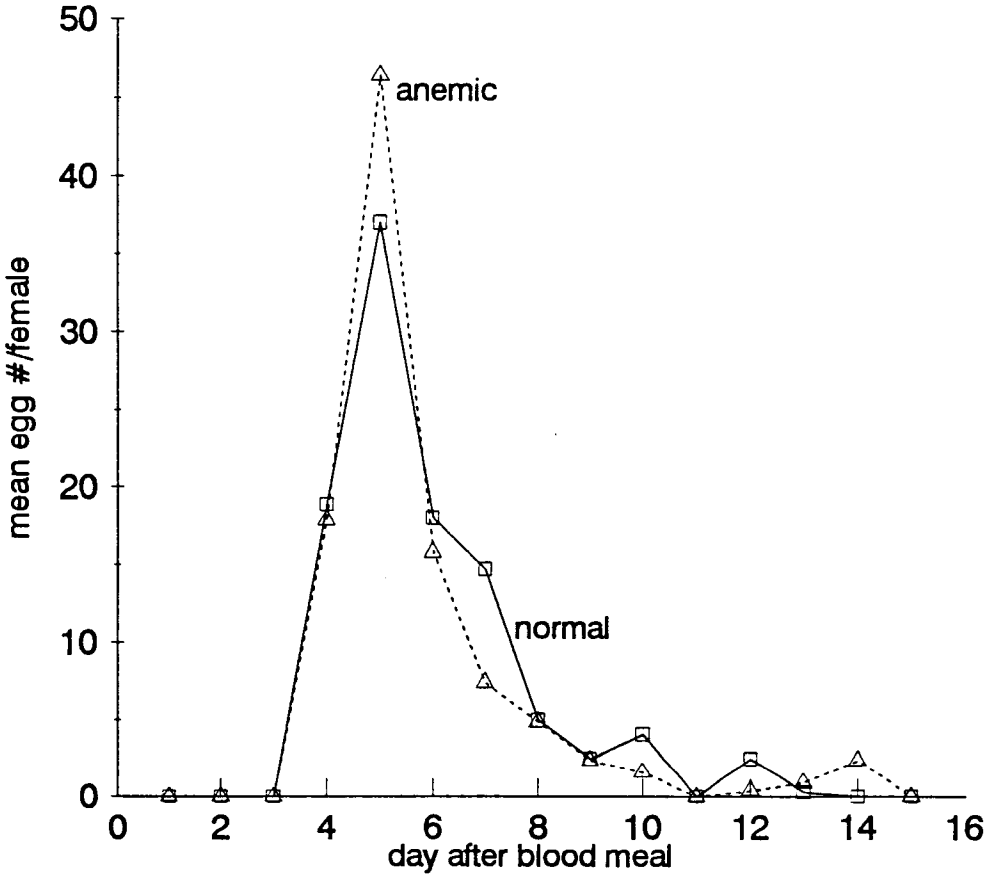
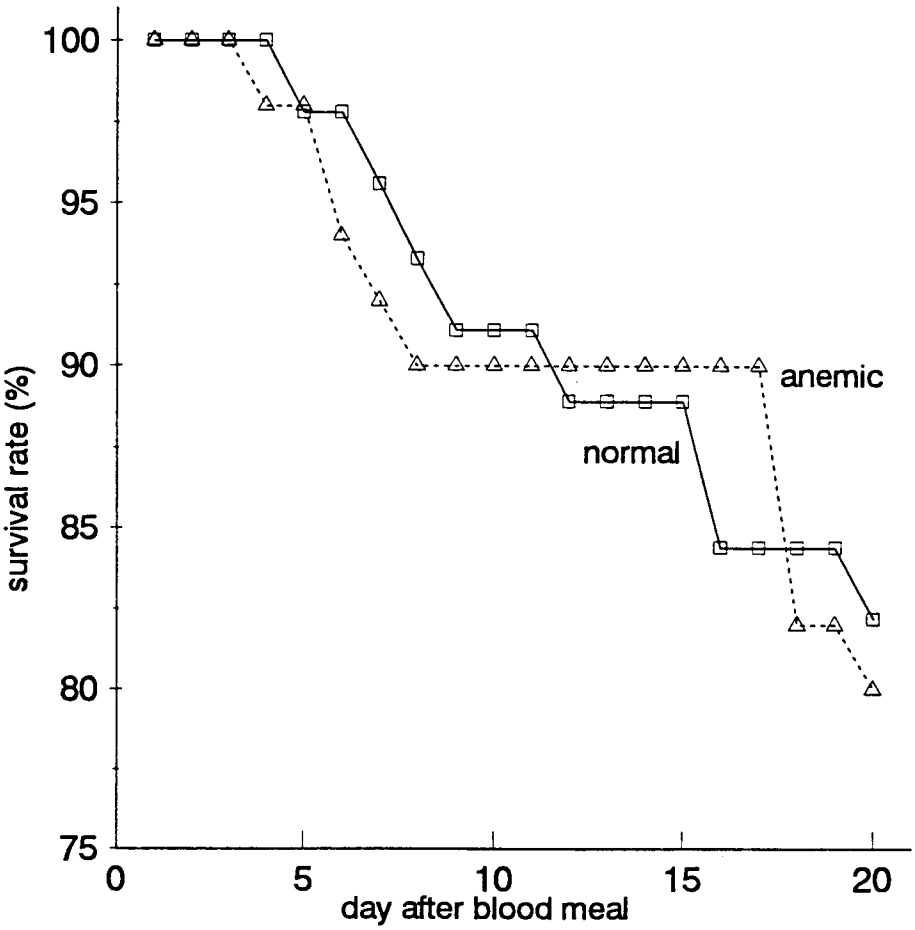


Fig 6 Mortality of female adult Aedes aegypti after blood-feeding on anemic and normal rabbits



later after a blood meal. Furthermore, no mosquito (in all feeding-chamber and live rabbit treatments) died within 3 days after a blood meal.

Finally, I checked the viability of those eggs produced by mosquitoes after blood-feeding on different RBC treatments (observation only). The eggs from each treatment hatched and developed into adult mosquitoes. Moreover, these adult mosquitoes appeared to have normal behavior, and take a blood meal and produce eggs.

Discussion

A number of conclusions arise from this study. First, RBC level affects the number of eggs. Second, egg size is not affected by blood quality. Third, mortality of mosquitoes after blood-feeding is not affected by RBC level. Fourth, there is no difference in egg production and egg-laying between mosquitoes blood-feeding on an artificial membrane and a live animal.

Klein *et al* (1982) reported that there is a possible post-blood feeding response in Anopheles dirus, (i.e., the mortality of mosquitoes is higher a few days after a blood meal). In this study, including both artificial system and live animal, I noted that no mosquito died during the first three days after a blood meal. It seems that there is no post-blood feeding response, at least in Aedes aegypti in the laboratory. Therefore, anemia, at

least experimentally induced anemia, did not affect the mortality and post-blood feeding response of mosquitoes. However, if anemia is caused by blood-borne parasites, then parasites might influence their host in some yet determined way. Further studies should focus on the influence of host anemia caused by parasites on mosquitoes.

Steinwascher (1982) reported that the number of eggs laid by an individual female mosquito in the first ovarian cycle depends on body mass. Many authors also have reported that blood-feeding amount is the main factor affecting the egg production of mosquitoes (Woke *et al.*, 1956; Nayar and Sauerman, 1975; Feinsod and Spielman, 1980). Previous results indicate that body mass might not play a role on the feeding volume. To avoid the unnecessary influence of body size on blood-feeding amount, I reared mosquitoes in nearly identical fashion to closely match body size. Because different blood meal volumes might result in different egg number production, I compared the ratio, weight of blood meal per egg number, among these different RBC treatments. This analysis suggests that RBC level did affect egg production. There is a correlation between the ratio and RBC level, the higher RBC level at which the mosquitoes fed, the lower ratios mosquitoes needed to produce an egg.

Bunner and others (1989) reported that a significantly lower percentage (47-70%) of Aedes aegypti engorged during membrane blood-feeding than during live host blood-feeding resulting in significantly lower egg production and egg eclosion. They suggested that even though egg production decreased with membrane blood-feeding, Aedes aegypti colonies could be maintained successfully on a variety of preserved mammalian bloods fed through a parafilm membrane. I compared egg production of mosquitoes after blood-feeding in an artificial apparatus and a live animal system, and found that there was no difference between them. As previously shown (Experiment 1), there is no significant difference in feeding rate, weight and time, between blood-feeding on the artificial membrane or a live rabbit. The artificial membrane I used influenced the blood-feeding behavior of mosquitoes, since the proportion of mosquitoes feeding in the artificial membrane apparatus is lower than that on a live host. Once mosquitoes feed to repletion, there is no significant difference on egg production between the artificial membrane and a live host. Therefore, if stimulating and increasing the proportion of mosquitoes feeding on an artificial membrane were to be improved, it would be a convenient way to maintain mosquito populations in the laboratory.

Steinwascher (1982) suggested that if females hatched from smaller eggs, their development was slower

than that from larger eggs, and as a consequence, they attained smaller adult size, took less blood-meal volume, and laid fewer and smaller eggs than those from larger eggs. But there was no optimal egg size within these observed eggs (Steinwascher, 1984). Wiklund and Karlsson (1988) also reported that offspring fitness might not be correlated with egg size, because the correlation is low. Begon and Parker (1986) also suggested that the critical minimum value below which eggs do not survive might not exist. In this study, I found that RBC level did affect the egg number produced but did not affect egg size.

Summary

First, RBC level does affect blood-feeding rate and egg number production of mosquitoes. A mosquito can feed at a faster rate on lower RBC level, but its egg number production is decreased (Fig 7). Second, body mass does not play a role in blood-feeding rate and weight.

Mosquitoes need to feed above a threshold blood meal volume to stimulate ovary development (Gwadz and Spielman, 1973). The acceleration of blood-feeding rate becomes more important for mosquitoes, because they are often interrupted during a blood-feeding session. In this study, the influence of host anemia on blood-feeding rate and egg production of mosquitoes are opposite. Mosquitoes feed at a faster rate on anemic blood than on normal blood, while egg number is decreased when they feed on an anemic host.

If mosquitoes could feed a little longer or to repletion, they would incur a disadvantage from an anemic host, because egg production would be decreased (Fig 8). However, if mosquitoes are interrupted often during a blood-feeding session, they could gain from blood-feeding on an anemic host, because they could then reach the threshold blood meal volume more easily.

The probability of mosquitoes finding blood is affected by the blood-feeding site (Ribeiro *et al.*, 1985). If mosquitoes were to feed on a site in which it is difficult to find blood, then faster blood-feeding

Fig 7 Influence of anemia on blood-feeding rate and egg production

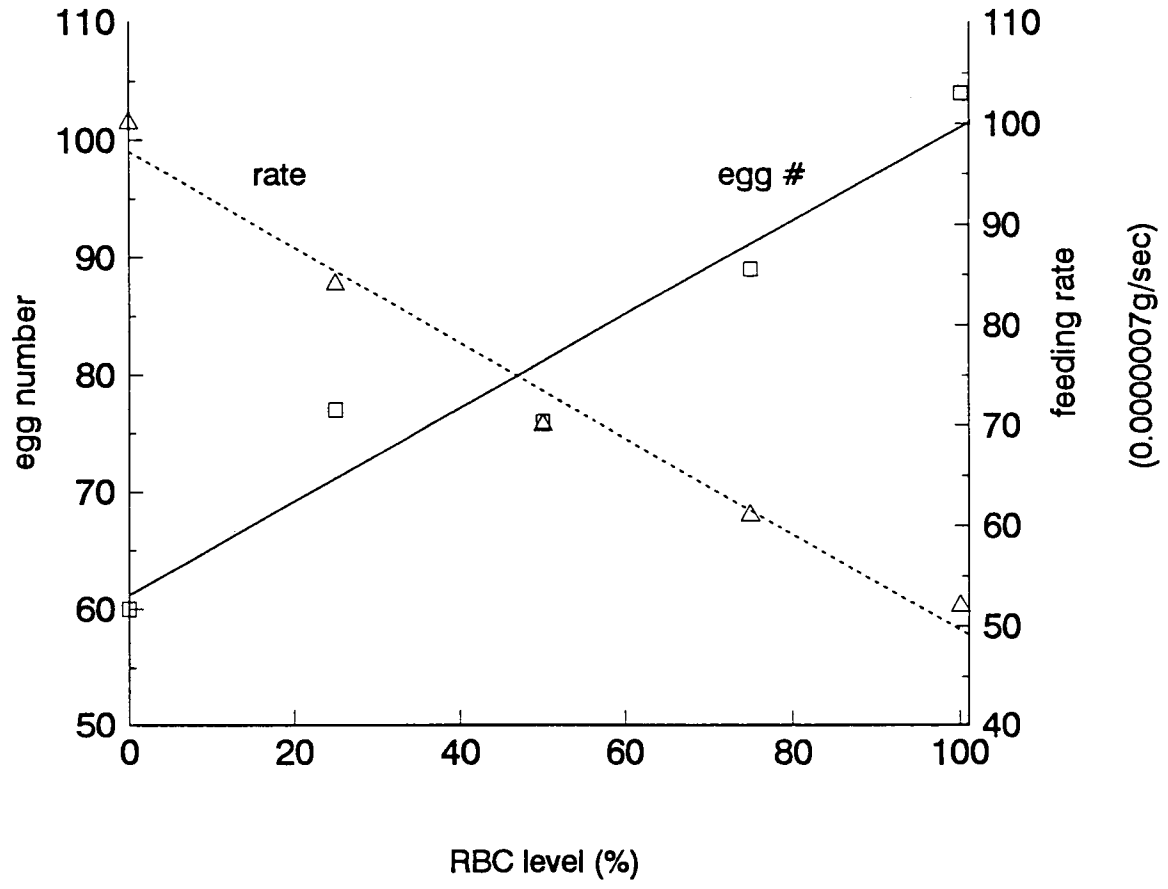
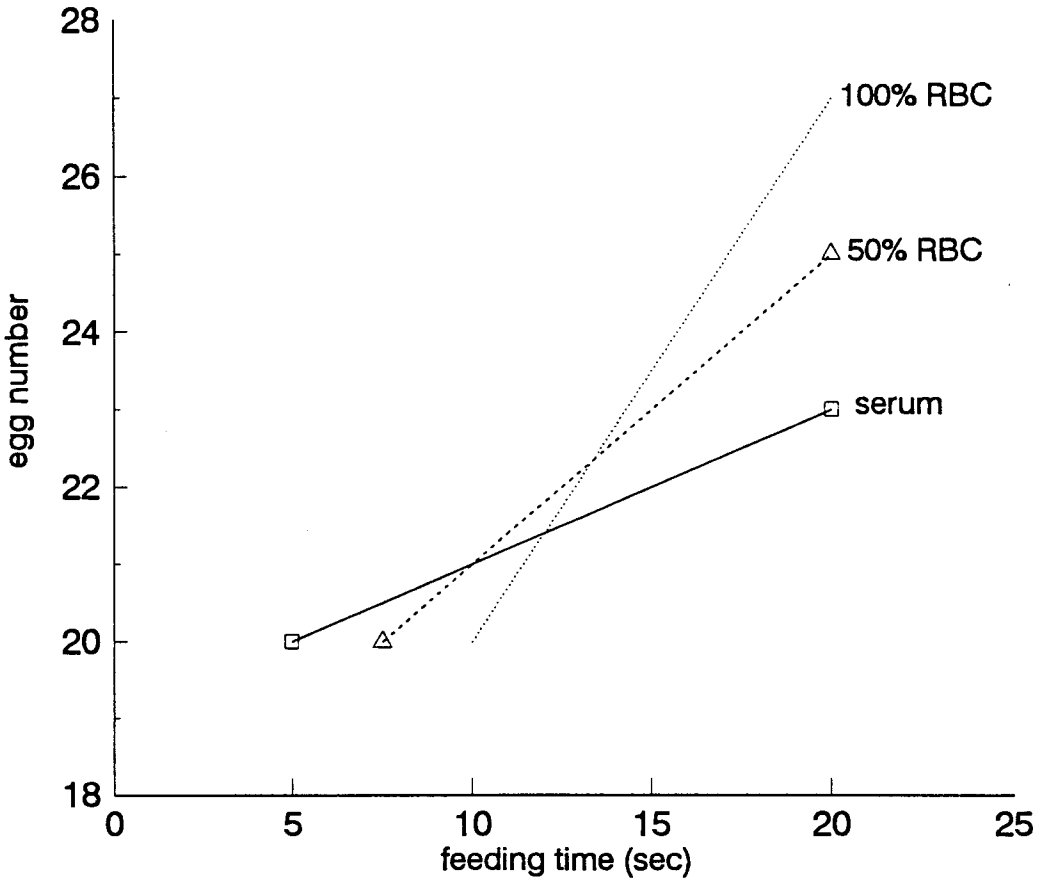


Fig 8 Influence of blood-feeding time on egg production in different RBC levels



rate becomes more important to them. Furthermore, anemic hosts are often lethargic, and mosquitoes could gain an advantage from faster and longer feeding on an anemic host.

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