

SOME STUDIES ON RAT-FLEAS AND THEIR BEARING ON HOST-SPECIFICITY

**THESIS SUBMITTED
UNIVERSITY OF BOMBAY**

**FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
ZOOLOGY**

**SUBMITTED BY
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BOMBAY**

1966

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1966

ACKNOWLEDGEMENT

I am grateful to Dr.P.J.Deoras, M.Sc.,Ph.D.,LL.B., Assistant Director, I/c Department of Entomology, Haffkine Institute, under whose guidance this work was carried out. His apt suggestions and timely advice have been of immense value in the completion of this work.

I must particularly mention and thank the Government of India, Ministry of Education for the financial assistance given during the period of this work.

I am grateful to Mr. H.I.Jhala, M.D.,F.C.P.S.,D.T.M., Director, Haffkine Institute, for providing me with all the facilities required to carry out this work.

My gratitude is due to the authorities of Bombay Municipal Corporation for rendering me help in the completion of the field survey studies.

I wish to express my thanks to Shri N.E.Vad, M.Sc.,J.S.O. Entomology Department for helping me with the photographs, Dr. A.K. Joshee and Shri K.P.C. Vasudevan for their help in giving the final touches to this thesis.

My thanks to Sarvashri V.P. Chodankar and P. Gangadharan for helping me in the statistical analyses.

I am all gratitude for Shri A.S. Narasimham, S.S.O. Department of Clinical Pathology, Haffkine Institute and Shri H.C. Chaturvedi, M.Sc.,J.S.O. Department of Entomology, Haffkine Institute and all other colleagues of mine who have lent a hand to me during these studies in the Department of Entomology.

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CHAPTER I

GENERAL INTRODUCTION

The Indian Plague Commission in 1905 established the relationship of fleas in the transmission of Bubonic plague. The realization of the role played by rats and fleas in the epidemiology of plague was a land-mark in the annals of the history of the disease. This revolutionized the whole approach to the epidemiology of plague and then it was evident that a proper understanding of the ecology of the rodents and their ectoparasites is a must to predict a possible outbreak and to check it from its further spreading. Thus the importance was shifted from man to rodents and their ectoparasites. This point has been outlined by Hirst (1953) when he wrote "Prevention of plague is an ecological problem which requires for its solution an intimate acquaintance with all the four-fold relationship of man, microbe, rat and flea".

A reviewer of this subject can never miss the observations of Ogata (1897) who incriminated fleas as vectors of plague, Simond (1898) who supported it with experimental evidence and Bacot and Martin (1914) who propounded the celebrated "Theory of regurgitation". Burnett Ham (1907) drew a parallel line between the abundance of fleas and the epidemic outbreaks. He suggested the possible effect climatic conditions could have on the seasonal variations exhibited by the fleas. Brooks (1917) propounded that plague did not establish itself when the temperature rose above 80°C (27°C) accompanied by a saturation

deficiency of over 0.3 inch (7.6 mm). This added colour to the plausible role of climate in guiding the course of epidemics. It was thus shown that climatic conditions may influence the course of epidemics not only through the influence on the "blocking phenomenon" in fleas, but also through their prevalence, abundance and mode of activities. However, it should be mentioned here, that other factors which influence the prevalence and abundance of the fleas were never ignored. Thus Liston (1904) who tried to correlate the breeding season of rats with the abundance of the fleas peculiar to them, mentioned that "the season at which the greatest number of young rats are present has a two fold influence on the spread of plague. In the first place the arrival of the young members among the community increases the number of individuals susceptible to the disease. In the second place the breeding season of rats, as a rule, is the period of increase of the fleas which are peculiar to the rat". (He added that the fleas were most abundant at a time when young rats were more numerous.) Though Burnett Ham (1907) did not observe any correlation between the breeding season of rats and the increase in the number of their fleas, later workers confirmed the observation of Liston (1904) by showing that young rats carried more fleas than the adults. In this connection it may be mentioned that Petrie and Todd (1923) found that the infestation of fleas on Rattus rattus increased, upto a maximum, as the rat increases in size and then declines as the adult becomes more adept at getting rid of fleas. Eskey (1930) maintains that the smallest

rats which spend their time in the nest harboured more fleas. As the rat grows in size the flea index becomes less until half grown stage is reached, when there is a rise in flea index again. Stark and Miles (1962) also established that the relative infestation of the rodents varied according to their ages.

These field observations have brought out the possible relation between the breeding of rats and that of fleas, and the relative infestation according to the ages of the hosts. But physiological studies to show the real relation between all these factors are meagre. To cite a few, Buxton (1948) showed that the breeding potential of the rat flea Xenopsylla cheopis was low when fed on a young mouse and he pointed out that to reach the normal rate of egg production, a flea must have at least one blood meal from the adult mouse. In comparative experiments he found that the fecundity increased as the ages of the host also increased. A possible hormonal relationship of the mouse (host) and the breeding of the flea was suggested. Allen (1956), working on the rabbit flea Spilopsyllus cuniculi, found that shortly before the breeding season of the rabbits commenced, the female rabbits carried an extra load of fleas. He thereby suggested that the breeding cycle of the rabbit flea was linked with the hormonal cycle of the host. His observations were confirmed by the carefully planned laboratory experiments of Mead Briggs and Rudge (1960); Miriam Rothschild and Bob Ford (1964-a and b) and others. Their conclusions point out that the maturation of the ovaries of the rabbit fleas could take

place only when fed on a pregnant rabbit and that immediately after parturition the fleas from the does were found in the nest containing young rabbits where they later fed on the young and laid eggs.

The field surveys conducted by plague workers revealed yet another important factor i.e. the distribution of flea species and the occurrence of plague. In 1914 when Rothschild described Xenopsylla astia , it was evident that what was thought to be a single species in India contained Xenopsylla cheopis , Xenopsylla astia and Xenopsylla brasiliensis. This initiated a series of studies on their distribution and vector efficiencies. The results indicated that while Xenopsylla cheopis is a very efficient vector of plague, the other species, Xenopsylla astia, is comparatively a poor vector. This coupled with the field studies on their distribution made it possible for Cragg (1921) to postulate that the comparative immunity against plague enjoyed by certain localities in India was due mainly to the absence of Xenopsylla cheopis.

The Parasitological Theory of plague given by Hirst (1927) runs as follows:

1. The incidence of bubonic rat plague is primarily governed by the magnitude of the respective populations of plague carrying flea species infesting the rats of any given district.

2. The population of any given species of rat-flea in any given locality varies according to the rat population, the nesting conditions, the suitability of the climatic conditions prevailing in that locality for breeding of the particular species and according to the number of fleas introduced in all stages of development from elsewhere.
3. The flea population requisite for the continuous propagation of the epizootic varies in magnitude according to the species of flea.
4. The vector efficiency of each individual flea of each sex and of each species, and therefore the flea population effective for transmission, varies according to the climatic conditions.
5. The degree of dependence of human epidemic on the rat epizootic varies directly with the power of the species of rat-fleas concerned in the spread of epizootic to parasitize man and the flea bearing rats inhabiting the locality under consideration.

But Goyle (1928) concluded from his study that "it appears that the proximity to infected areas and the facilities for communications with them, seem to have been more important than the flea species factor in determining the plague incidence". However, he never ignored the flea species factor in the spread of plague which is evident when he puts forward a view that "the flea species is a factor of considerable

importance though not the only factor involved in the spread of plague and that the presence of Xenopsylla astia to the exclusion of all other fleas over a large tract of country, may possibly account for its immunity from plague".

Hirst (1926), from his observations, postulated that "though the infection is introduced into a place there is no danger of an epidemic as long as the Xenopsylla cheopis-index remains below one". Meyer (1942) states "Epidemiologically of importance is not only the seasonal density but also the seasonal flea species composition".

Sharif (1948) postulated that the microclimate of the rodent burrows and the type of larval food present, influence the prevalence, abundance and even the host preference of fleas in a locality. Holland (1956) showed the importance of host distribution in relation to that of their ectoparasites. He showed that in many cases, the geographic distribution of a species of flea coincides approximately with that of a species of mammal on which the flea is regularly found. He explains that "the apparent host-flea relationship may be due to the fact that (a) the flea is physically and/or physiologically associated with one species of host, records from the others resulting from the accidental or temporary associations do not permit the breeding of the flea or (b) that flea and host depend on similar or associated ecological requirements so that ranges coincide although within those ranges some other hosts are satisfactory". Under the head of physical adaptation,

Holland (1956) included (1) skin thickness of host in relation to the mouth parts of the flea. (2) Pelage of the host from the standpoint of heat insulation. (3) Vestiture of the flea in relation to pilosity of the host. Under physiological adaptation he included (1) Reproductive potential of the species. (2) Specificity of blood meal requirements (not only of species of the host, but also ^{of} age and sex of the host). (3) Presence of the essential food ingredients for flea larvae in the nest of the host. (4) Ranges of temperature, humidity and light in the nest of the host, in relation to tolerances by flea eggs, larvae, and pupae. (5) Timing of life history stages of flea in relation to the occupancy and eventually to the abandoning of the nest by the host. (6) Longevity of the adult fleas in relation to the breeding intervals of host, especially hosts that do not repeatedly use the same sleeping site and lastly, (7) Host-finding capacity of adult fleas.

Considering these factors, it is highly essential to elucidate the importance of the host-flea relationship through the studies on the host preferences of the fleas, their morphological adaptations to the hosts and their breeding potentials on different hosts.

Bombay has been witnessing an interesting change in the rat population around human habitations during the last few years. It is stated in the annual report of Haffkine Institute 1940-41 that Bandicoota bengalensis formed about 40% of the entire rodent population in the city as compared to the 1%

in 1907. It is seen from table I given below that the percentage of Bandicota bengalensis increased upto 52% in the year 1947. The percentage of Rattus rattus that year was only 18.9%. These rats were collected from field and brought to Haffkine Institute by the Bombay Municipal Authorities for examination.

Table I
(From Haffkine Institute Report and Politz^ler-1954)

Year	R. refuscens	R. norvegicus	G. kok (B.benga- lensis)	B. malabari- cus	Incidence of rodent plague
1938	24.7	25.4	27.3	1.2	-
1939	23.8	24.4	35.5	1.2	-
1940	24.9	23.9	34.8	1.0	-
1941	24.2	23.0	37.1	0.8	-
1942	20.9	22.5	45.7	0.8	-
1943	20.3	22.7	46.3	1.1	-
1944	21.9	22.1	47.0	1.1	-
1945	21.0	21.6	49.2	1.8	-
1946	19.6	23.7	49.0	1.1	-
1947	18.9	23.0	52.0	0.6	-
1948	23.7	18.3	47.4	0.8	2 R.r.r. and 42 G.k. found infected
1949	23.3	20.0	42.2	0.4	4 R.r.r. and 2 G.k. found infected
1950	23.9	17.6	39.3	0.7	-
1951	21.1	16.5	36.7	0.8	-
1952	22.6	17.7	38.1	1.0	-

The percentage of different rats collected in Bombay recorded by Joshee (unpublished thesis-1961) is as follows:-

Table II
(Date from 1957 August to 1958 July)

Area	R.r.	R.n.	B.b.	B.i.	M.m.	S.m.
Greater Bombay	22.5	17.7	46.0	0.4	3.9	9.6
E/W ward	14.7	28.2	43.4	0.1	3.6	10.0
G/N ward	28.2	2.6	48.2	1.5	5.9	13.6
Entire suburbs alone	58.5	0.9	16.9	1.5	6.8	15.4

The remarkable rise in the percentage collection of Bandicota bengalensis brought to Haffkine Institute in Greater Bombay seems striking. Equally conspicuous is the higher percentage collection of Rattus rattus brought from suburbs over that of Bandicota bengalensis. These findings have been attributed to the urbanization of the adjoining rural areas of Bombay. Joshee (unpublished thesis-1961) states that "no cultivated fields are left for these wild rodents and consequently they have been forced to depend upon man for food and shelter". Deoras (1963) studied the frequency of rat population in Bombay city and one of its suburban areas.

Table III gives the summary of his results.

Table III

Locality	Total rats caught	Period of collection	No. of traps used	Percentage rat collection	
				R.r.	B.b.
Bhandup (field)	1010	12 months	100	17.0	20
Kamatipura (residences)	330	1 month	70	37.27	50.6
Butcher Island open area	31	3 weeks	100	91.7	2.7

Table IV

Percentage rat collection as per Municipal data for the same year(1960)

Locality	Total rats caught	Period of collection	No. of traps used	Percentage rat collection	
				R.r.	B.b.
Kamatipura	4552	122 days	70	21.5	45.4
Mahim	7372	do	120	28.3	43.2
Bhandup (huts)	94	28 days	50	91.5	5.3
Bhandup & Ghatkopar	2394	122 days	25	88.3	3.9
Port trust	1368	do	unspecified	54.9	28.2

Bandicoots are not without any blemishes as far as plague epidemiology is concerned. It is evident from table I that in Bombay in the years 1948 and 1949 42 Gunomys kok (B.bengalensis) and 2 Gunomys kok respectively were found infected in nature with plague. George and Webster (1934) working in Madras Presidency noted that dead bandicoots were occasionally found from plague-infested villages. The percentage mortality of Bandicoots to plague infection, from two localities in Bombay, has been worked out by Deoras (1960) who showed that they were 80% and 92%. Seal and Bhattacharji (1961) working in Calcutta proved that B.bengalensis collected from wards 8 and 10 gave 98.8% and 100% mortality respectively to standard dose of 5,000 virulent P.pestis. Their results are summarized in the table below.

Table V

Susceptibility of rats to plague infection in Calcutta and Bombay cities between the years 1949 and 1960 (Against standard dose of 5,000 virulent P.pestis).

Species of rat	Calcutta			Bombay			
	1949	1953 - 54		1953*	1965 ⁺	1960	
		Ward 8	Ward 10			Kamati-pura	Mahim
R.rattus	92.0	60.0	100.0	13.7	7.5	17.0	27.0
R.norwegian	100.0	62.8	100.0	5.8	2.2	"	"
B.bengalensis	100.0	98.8	100.0	82.5	75.2	80.0	92.0

* Annual Report. Haffkine Institute, Bombay

+ Deoras (Curr.Sci.Dec. 1960)

As regards the possible role of Bandicoots in spreading plague, Pollitzer (1954) remarks "while no convincing evidence has been so far brought forward that bandicoots in India play a role in the establishment and maintenance of sylvatic plague foci, the findings made in Bombay city are of importance since they prove that plague-susceptible rodents (other than commensal rats and mice) which are able to accommodate themselves to life near man may become dangerous not only because they are apt to act as intermediaries in the spread of the infection but also because they may take the place of less-susceptible rat strains in the houses".

Xenopsylla cheopis (Roths) and Xenopsylla astia (Roths) are the two common rat fleas found distributed among rats of Bombay. While the former, a noted vector of plague, shows more affinity to Rattus rattus, the house rat, the latter shows more affinity to field rodents. In India all the rat-flea surveys indicate this predominance of Xenopsylla cheopis on Rattus rattus and Xenopsylla astia on field rodents.

Sharif (1948) working at Dharwar and Barsi talukas, observed that Xenopsylla cheopis was predominantly found on domestic rats, R.r.rufescens of Barsi taluk Xenopsylla astia was present to the exclusion of other fleas on the Indian gerbille Tatera indica, found in the fields and predominantly on Bandicoota malabarica in houses all over the two taluks of Dharwar and Barsi.

A brief outline is given below to indicate the flea distribution on these two rats in the town of Bombay.

The work of Webster and Chitre in Bombay in the years of 1929-30 summarized in Table VI showed that Xenopsylla cheopis being 5 to 12 times more numerous on Rattus rattus while Xenopsylla astia index for Gunomys was 14 times greater than that for Rattus rattus.

Table VI

R.rattus by areas

Area	Rat examined	Fleas	X.cheopis	X.astia	X.brasiliensis
1	91	217	1.9	0.4	0.1
2	134	700	4.8	0.4	0.0
3	102	336	2.9	0.4	0.0
4	79	277	3.1	0.4	0.0
5	144	868	5.3	0.7	0.0
6	132	590	2.9	0.6	1.0

B.bengalensis by areas

1	10	45	1.0	3.3	0.0
2	8	111	3.25	10.6	0.0
3	22	170	2.4	5.3	0.0
4	33	313	2.6	6.9	0.0
5	12	177	1.1	13.25	0.4

The distribution of the two species of *Xenopsylla* collected on rats of Bombay given by Deoras and Gokhale (1958) shows their preferences in general way for the years 1954 and 1955.

Table VII

Rat Sp.	Year	X.cheopis	X.astia
R.rattus	1954	92.9	7.1
R.norvegicus	1954	68.5	31.5
B.bengalensis	1954	52.3	57.7
R.rattus	1955	84.1	15.9
R.norvegicus	1955	68.5	31.5
B.bengalensis	1955	35.4	64.6

Joshee (unpublished thesis-1961) shows that "from specific flea indices it can be seen that in both the wards (G/N and E/W) Rattus rattus carries more Xenopsylla cheopis than Xenopsylla astia while it is reverse for Bandicota bengalensis in G/N ward. In E/W wards B.bengalensis carried nearly equal number of Xenopsylla astia and Xenopsylla cheopis".

Table VIII

Joshee (unpublished thesis-1961)

Flea distribution on R.rattus and B.bengalensis
(Average data of 12 month collection)

	R.rattus		B.bengalensis	
	X.cheopis	X.astia	X.cheopis	X.astia
E/W ward	0.52	0.09	0.21	0.31
G/N ward	0.84	0.32	0.25	1.41

Table IX

Kamath (unpublished thesis-1961)

(Average data of 9 month collection)

		R.rattus			B.bengalensis		
		X.cheopis		X.astia	X.cheopis	X.astia	
		G/N ward	1.04	0.26	0.14	1.68	
Rat sp.	No. of rats	No.of fleas			Percentage		Total index
		X.cheopis	X.astia	Total	X.cheopis	X.astia	
R.rattus	794	771	154	925	97.1	19.4	1.30
B.bengalensis	895	127	1344	1475	14.1	150.2	1.84

The different rat populations of Bombay have been mingling as a result of which the probability of the exchange of flea population among them also increases. In spite of this mixing up of rats and fleas in Bombay, a striking factor that cannot escape notice is that Xenopsylla cheopis predominates on Rattus rattus while Xenopsylla astia on Bandicota bengalensis. But Kamath (unpublished thesis) in his collection of fauna from 126 burrows of Bandicota bengalensis in Bombay, showed that all 200 fleas caught from the burrows were Xenopsylla astia.

While the microclimatic conditions of the burrow, nesting habits of the hosts and the larval food present in the burrows undoubtedly influence the establishment and continued breeding of a flea species in a locality, the effect of certain factors on the adult fleas such as its attraction to the host, its

longevity, and reproductive potentials may also influence the predominance of the flea species concerned. These latter aspects of the problem have been comparatively neglected by the previous workers. In this connection, Meyer (1942) states: "To emphasize further the state of ignorance, it may be said that relatively little is known of the ecology and activity of the hordes of fleas which loom as the most important parasite vector. The problem of host specificity has been explored quite superficially. The host-ectoparasite relationship deserves particular attention since it is one of the factors which guide the spread and the dispersion of the plague bacillus". The foregoing account will give sufficient emphasis to the significance and the possible role the flea-host relationship can play through its multiple influence on the spread and maintenance of flea-borne diseases.

The present work is an attempt to elucidate the delicate flea-host relationship through the studies on the natural host preference of the fleas, their comparative reproductive potentials with regard to the type of host offered, and certain biological factors relating to feeding mechanism which may influence ingestion of blood of the host.

CHAPTER II

FIELD SURVEY OF RATS AND FLEAS

Introduction

"The theoretical justification of vector counts rests primarily on the widely-held tenet that when a disease and its vectors are present in a community the size of the vector population largely determines the probability of transmission to man and therefore tends to be proportional" (Cole 1947).

The importance of flea count from the epidemiological point of view has been brought to light as early as 1926 by Hirst. Various techniques have hitherto been employed for flea census by the researchers in different parts of the world of which the index system is the one which is widely practised. Many objections have been raised from time to time to the method of calculating flea-index which is nothing but the arithmetic mean of the total flea-ectoparasites on the rats, and many solutions also have been suggested to overcome these objections. A few of them may be cited here. [The corporeal flea-index does not correspond to the strength of a flea population. [WHO expert Committee on plague (1950) suggested an "absolute flea-index" which when computed accurately, yields more or less the nearest possible value for the strength of a flea population. But the absolute flea-index does not account for the wandering fleas. The formula for calculation of absolute flea-index as given by the committee is as follows.

$$AFI = \frac{RF + NF}{TR}$$

where AFI is the absolute flea-index; RF is the fleas found on rats; NF stands for the fleas found loose in the burrows or other harbourages and TR, the total rats caught.]

Exposure of guinea-pigs on the floor of houses or guinea-pigs on "de-flead" rats in cages; use of flea traps and adhesive paper are a few techniques used in collecting the floating fleas from houses. In order to collect the burrow fleas Babenychov, Bykov et al (1937) and Stewart and Evans (1941) used cotton wad which was inserted into the mouth of the burrows. George and Webster (1934) used "de-flead" rats which were tethered by a piece of wire 4-5 feet long and left in the burrows overnight. Deoras (1965) worked out a modified technique for flea-indices where he kept a layer of sand under the trap which harboured the fleas falling loose from the body of the rat, or from the surrounding areas.

Variations in the flea indices may not be an indication of the corresponding variations in the flea population because many factors like the rat-density, sex and size of rats, climatic conditions, species, sex and the physiological state of the fleas concerned may influence and give a twist to the flea-index. Buxton (1938) remarked that one should not overlook the fact that changes in the rat population would tend to dilute or concentrate the population of fleas on the rats if their number remains unaltered. Another serious objection to the flea-index is that relatively few very high counts can distort the index so that it may not represent the parasite

prevalence and distribution. Some authors tend to exclude these exceptionally high counts when calculating the flea-index. But then, there should be a general agreement as to the upper limit of the normal index. Rumreich and Wynn (1945) hammered out a solution to this. They showed that "Index may be derived by mathematically fitting an appropriate curve to the frequency-distribution of parasite count in any host population and thus determining the normal upper limit". The use of geometric mean (by averaging logarithm) has been advocated to minimize the errors which may creep in due to the wide irregularities in the flea distribution on the hosts. The rats, free of fleas, also pose a problem. Some workers prefer to exclude the number of rats, free of fleas, for the final calculations while others argue that these rats also should be included when computing the index. The latter group base their arguments on the fact that in a host population, if the infested members carry a total number of vectors which, when distributed evenly among all the individuals of the populations, gives a vector index of one or more per host, it can still spread the disease. According to Hirst (1926), there is no danger of an epidemic as long as the cheopis index remains below one. Another index proposed by Cole (1945) for X.cheopis is the mean number of females per rat. He showed that the females are less variable than males in their association with the hosts under varying conditions of temperature. Carl Mohr (1958) worked out an "index-number" to correlate the percentage infestation of rats with their flea-indices. Miles,

Finney and Stark (1957) calculated the percentage occurrence from the total and specific flea-indices of a flea species.

A wealth of information can be obtained from the field observations of rats and their fleas. The usefulness of these studies, not only on the interpretations of the delicate interactions between the host and their parasite populations but also to predict any danger of a possible disease outbreak, has been emphasized by Hirst (1926). A close acquaintance with the changes that occur in nature between the ecto-parasites and their host populations may work out to be an ideal index for the public health workers from an epidemiological point of view where the vertebrates and their ectoparasites are involved in the spread of a disease.

Area of survey and methods

G/North Ward of Bombay Municipality was the area chosen for the present work of rat and flea survey. The two places selected in this area were Mahim and Dharavi. These are two adjoining places in the G/N ward with contrasting ecological conditions. Mahim proper has the environment of modern housing with underground sewer systems and well-tarred roads, but it has also small houses with compounds called "Jadis". Dharavi, on the contrary, is a low level slum area predominated by poor-class working people who live in huts. There is no sewer system. The place includes a number of tanneries with attached chawls for the workers.

These differences in ecological set-up offer ideal situation to study the changes in rat and flea populations of these places. This also will throw light on the effect various environmental conditions may have on the rat and flea populations.

Multiple-capture and single-capture traps were used to collect the rats from houses, for the one year survey. 60 multiple-capture traps were laid at a time and collection made on all days of the week except Sundays. All the traps were gathered at one place before brushing the rats for fleas. The traps were covered with canvas bags on the spot to prevent escape of fleas. Spot brushing was done by catching live rats from the traps with the help of tongs, and a wire brush was used

for brushing. The fleas thus disturbed were allowed to fall into a white enamelled basin from which they were collected by a suction tube. These fleas were later brought to the laboratory for identification.

For specific studies on flea distribution on the hosts, single-capture traps were used. The collection was made for 40 trap-days, using 30 traps a day. All these days the traps were set in one locality. The rats trapped were killed by strangulation and kept singly in plastic bags, and brought to the laboratory, where the rats and fleas were identified and the necessary data collected.

The data thus collected were statistically analyzed. Percentage occurrence of the two flea species was calculated using the formula of Miles; Kinney and Stark (1957). This helped to compare the host specificity and evaluate the occurrences of the species on various host species. This also helped to compensate for the disproportionate numbers of hosts. One method used here to compare the relative infestation of different species of rats and the different size groups of the same species of rat is, by calculating the density distribution of fleas on rats. This is calculated by dividing the total number of fleas found on one species of rat by the total body length of the same species of rat. This gives the flea-infestation per unit length of the body of the host. This has been developed in this work in order to achieve a

more accurate comparison of flea infestation, because the pulicoid-index may be inadequate for the purposes of comparison.

Cole (1947) showed that when the average (m) is approximately equal to the variance (σ^2) the distribution of the ectoparasite on rat is said to be at random and then it follows a "Poisson" distribution. If the average (m) is not equal to the variance (σ^2) then the distribution is contagious. These calculations are made in this study also to examine the type of distribution of fleas on rats.

Observation and results

The results of the field work indicated that both Rattus rattus (Linnaeus) the house rat and Bandicota bengalensis (Gray) the field rat are present in appreciable numbers (Table I) in and around the houses. As observed by the previous workers in Bombay, Bandicoots could be easily trapped from the ground floor of the houses. Of the 1631 rats examined during the whole year, Rattus rattus formed 42.2% (688) and Bandicota bengalensis 57.8% (943). Xenopsylla cheopis (Roths) and Xenopsylla astia (Roths) were the two common rat fleas encountered during these field surveys. Chenoccephalides felis (Bouche), the cat flea, was also collected but their number was so negligible that it was not taken into consideration.

Table I is a summary of the total collection of fleas and rats from Mahim and Dharavi during the one year survey. A total of 5961 fleas was collected during the one year trial, out of which 2389 (40.1%) were X.cheopis while the rest 3572 (59.9%) were X.astia. The fleas found loose in the bag formed 15.2% of the total fleas collected. In general, the analysis of the one year data showed that 60.3% of the total R.rattus collected were infested with fleas, the rest 39.7% were free of fleas. In the case of B.bengalensis 68.1% of the total were infested while the rest 31.9% were free of fleas (Table II).

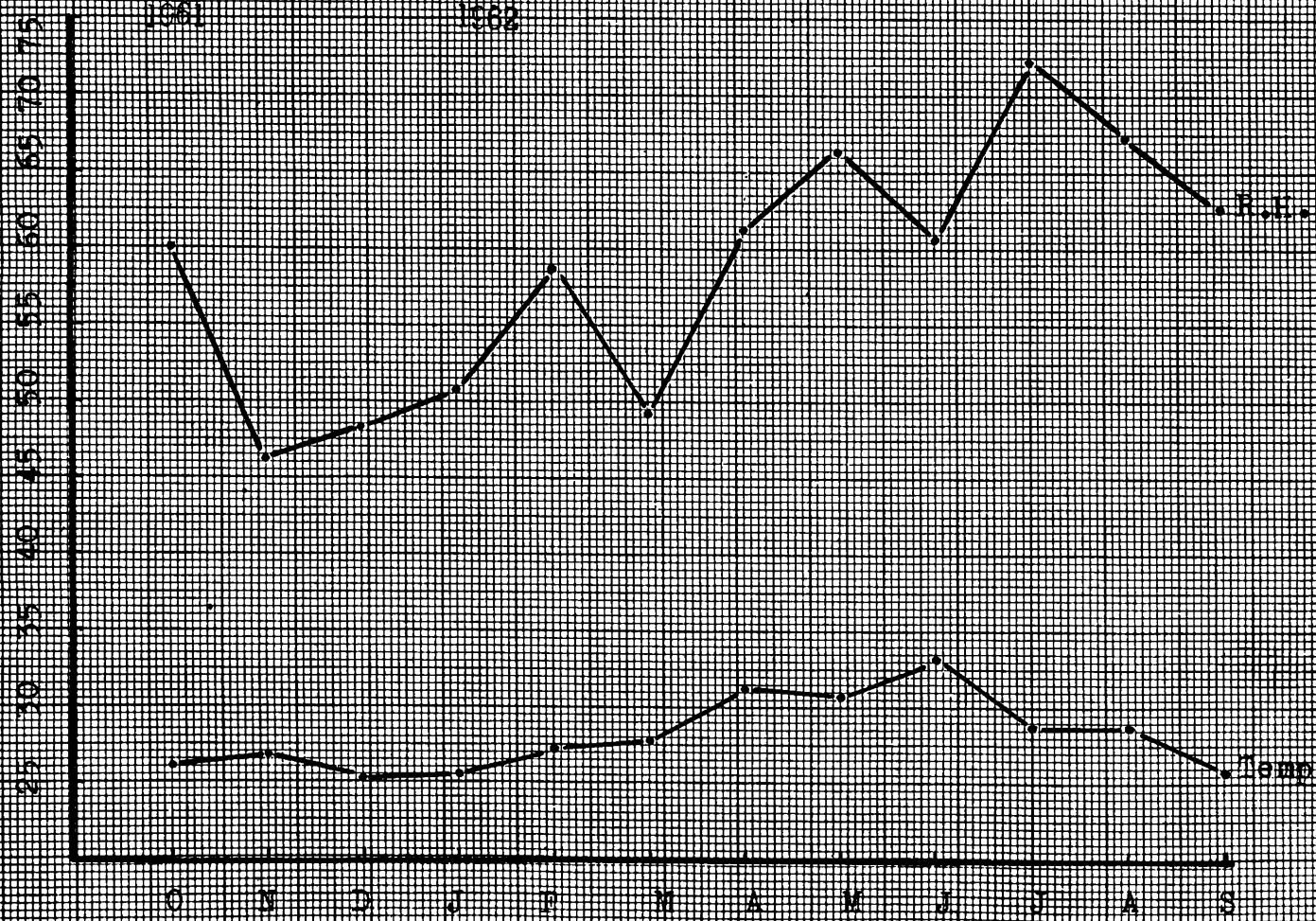
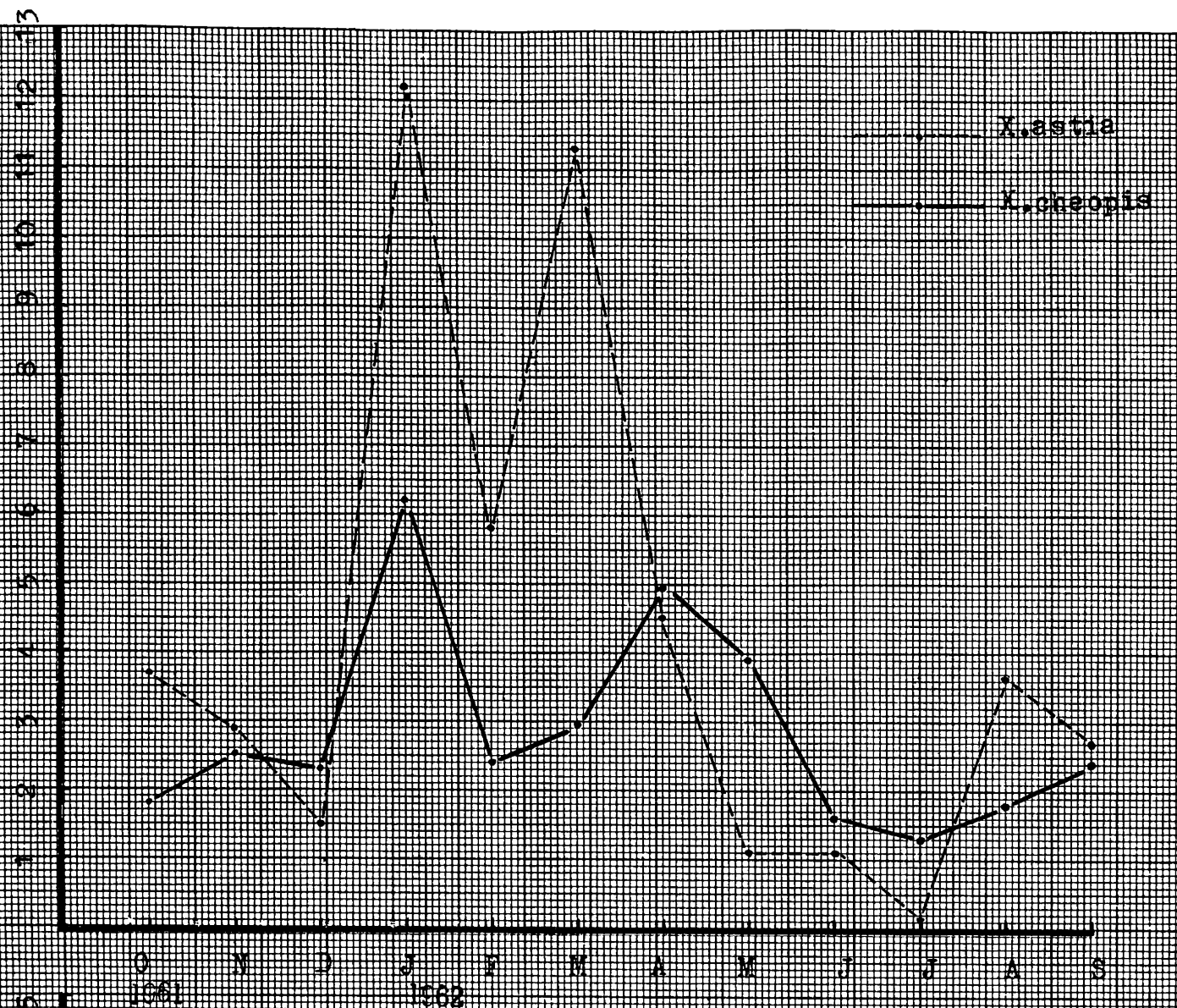
Table I

Grand total of Rats and Fleas collected

MONTH	R.rattus		B.benga- lensis		X.cheopis		X.astia		Ct.felis		Temperature	Humidity (R.H.)	Other rats
	M	F	M	F	M	F	M	F	M	F			
Jan.	43	51	16	25	198	237	169	295	0	1	25.51°C	50.9%	R.norvegicus 1M and IF B.gigantia 1
Feb.	29	24	36	55	113	103	255	402	0	1	27.03°C	58.9%	R.norvegicus 2M and IF B.gigantia 1M
March	10	24	29	49	90	74	383	525	0	3	27.95°C	49.26%	-
April	14	23	36	42	130	144	164	254	1	1	31.08°C	61.3%	M.musculus 1M
May	19	23	40	46	154	141	33	57	0	1	30.54°C	66.4%	R.norvegicus 1M and 1F M.musculus 1M
June	40	41	39	43	128	149	35	67	0	0	33.07°C	60.9%	-
July	31	44	19	12	82	96	4	8	0	0	28.58°C	72.15%	-
August	42	35	41	56	129	118	119	248	0	0	28.68°C	67.33%	-
Sept.	23	35	22	43	53	45	49	70	0	0	25.75°C	62.9%	-
Oct.	24	24	31	42	27	23	54	92	0	0	26°C	60%	-
Nov.	8	15	83	59	33	34	101	144	0	0	26.8°C	46.4%	-
Dec.	35	31	36	43	42	46	14	30	0	0	25.08°C	48.3%	R.norvegicus 1M
Total	318	370	428	515	1179	1210	1380	2192	1	7	-	-	

Table II
Corporeal fleas (See the Graph)

MONTH	Rat species examined	No. of rats infested	% infested	X. cheopis		X. astia		Plicoid-indices	
				M	F	M	F	X.o.	X.a.
Jan.	R.r.	67	79.8	194	224	7	6	6.2	- 0.19
	B.b.	37	90.24	4	13	162	289	0.46	- 12.19
Feb.	R.r.	42	79.23	58	44	37	55	2.4	- 2.2
	B.b.	83	91.2	40	44	181	297	1.0	- 5.8
March	R.R.	21	61.8	42	20	12	14	2.95	- 1.2
	B.b.	75	96.2	29	37	362	487	0.9	- 11.3
April	R.r.	29	78.4	66	64	36	52	4.95	-3.04
	B.b.	61	78.2	25	51	108	169	1.3	-4.5
May	R.r.	39	92.9	76	75	8	8	3.9	- 0.4
	B.b.	58	67.4	42	37	23	39	1.4	- 1.1
June	R.r.	38	46.9	33	29	3	6	1.6	- 0.24
	B.b.	65	79.3	39	51	29	44	1.4	- 1.1
July	R.r.	35	44.9	21	25	2	2	1.3	- 0.1
	B.b.	14	45.2	7	4	0	1	0.8	- 0.07
August	R.r.	46	59.7	46	36	19	31	1.8	- 1.1
	B.b.	70	72.2	10	15	77	178	0.36	- 3.64
Sept.	R.r.	22	37.9	28	25	11	11	2.4	- 1.0
	B.b.	31	47.7	4	8	34	50	0.39	- 2.7
Oct.	R.r.	20	41.7	21	14	1	5	1.8	- 0.3
	B.b.	38	53.3	7	9	53	87	0.42	- 3.7
Nov.	R.r.	19	82.6	22	25	3	4	2.5	- 0.4
	B.b.	82	57.8	11	9	98	140	0.24	- 2.9
Dec.	R.r.	37	56.1	41	45	0	1	2.3	- 0.03
	B.b.	28	35.5	1	1	14	29	0.71	- 1.5
Total	R.r.	415	60.3	648	626	139	195		
	B.b.	642	68.1	219	279	1142	1810		



One year data of the number of rats infested, number of corporeal fleas collected, their infestation percentages and their total monthly indices are summarized in table II. The total index for R.rattus reached its peak during the month of April when it was 7.5 and the minimum (1.4) in the month of July. During the other months there was a great fluctuation in the total flea indices for R.rattus but in general it may be stated that the index stayed high during the months of January, February, March, April and May. Then there was a sudden fall in the index and another high index occurred in the month of September. In the case of B.bengalensis, the peak was in the month of January, when the index was 12.7 and the minimum (0.86) was in the month of July. In general for B.bengalensis the index stayed high during the months of January, February, March and April. Then it gradually decreased until it rose again during the months of August, September, October and November. In December the index was only 1.6.

Table III

Rat sp. examined	No. examined	% infes- tation	<u>X.cheopis</u>		<u>X.astia</u>		Total index X.c.&X.a. combined
			M	F	M	F	
R.rattus	132	84.6%	244	172	42	56	4.6
Bandicoot group	92	92.4%	152	83	325	679	14.5
S.murinus	54	92.6%	141	129	21	21	6.2
M.musculus	5	0	0	0	0	0	0

Table III indicates the results of single-capture trap trials. During the 40 days trial a total of 132 R.rattus were captured out of which 20 were free of fleas making the percentage infestation 84.6%. 92 Bandicoots were caught and 7 of them were free of fleas making the percentage infestation 92.4% and of the 54 S.murinus trapped 4 were free of fleas, the infestation percentage was 92.6%. Thus the percentages ^{of} infestation of Bandicoots and S.murinus were almost similar whereas, that of R.rattus was lower than these values. All the 5 M.musculus captured were found to be totally free of fleas. As to their flea ectoparasites, a total of 941 X.cheopis and 1146 X.astia were collected.

Seasonal prevalence

Table IV

Season	Rat infested	% infested	X.c.		Index	X.a.		Index
			M	F		M	F	
Summer	Rr 77	62.6%	109	104	2.8	11	14	0.33
(May-June)								
Temp. 31.8°C	Bb 123	73.2	81	88	1.4	52	83	1.1
RH. 63.7%								
Rainy	Rr 103	48.8	95	86	1.8	32	44	0.74
(July-Sept.)								
Temp. 27.7°C	Bb 115	59.6	21	27	0.42	111	229	2.96
RH. 67.5%								
Autumn	Rr 39	54.9	44	39	2.1	4	9	0.33
(Oct.-Nov.)								
Temp. 26.4°C	Bb 120	55.8	18	18	0.3	151	227	3.2
RH. 53.2%								
Winter	Rr 146	68.6	293	313	4.2	44	62	0.73
(Dec.-Feb.)								
Temp. 25.9°C	Bb 148	70.2	45	58	0.7	357	615	6.6
RH. 52.7%								
Spring	Rr 50	70.4	108	84	3.8	48	66	2.3
(March-April)								
Temp. 29.5°C	Bb 136	86.6	54	88	1.04	470	657	8.3
RH. 55.3%								

The seasonal variations in the corporeal flea indices of rats and their percentage infestation are contained in Table IV. The results show that the maximum index for X.cheopis on R.rattus was in winter whereas the maximum index of X.astia on B.bengalensis was in Spring. Maximum percentage infestation of R.rattus was in winter (68.8%) and that for B.bengalensis in Spring (86.6%) . Needless to say that the influence of the

locality of collection has its effect on the corporeal flea indices. But here a seasonal trend, according to the differences in climatic factors such as temperature and humidity may be noticed.

Survey at Mahim

Table V indicates the total collection of infested rats from Mahim and the percentage of the two different species of fleas collected from them. On an average 136 trap days work was done at Mahim. Of the total 650 rats trapped 176 (27.1%) were R.rattus and 474 (72.9%) were B.bengalensis.

Table V

Rats and fleas collected from Mahim and Dharavi

Locality	Rat sp. trapped	Total No. of rats examined	X.cheopis		X.astia		% Catch of fleas	
			M	F	M	F	X.c.	X.a.
Mahim (136 trap days)	R.rattus	176	246	205	117	168	77.1%	22.9%
	B.bengalensis	474	129	167	917	1415	11.2%	88.8%
Dharavi (92 trap days)	R.rattus	239	402	421	22	27	94.4%	5.6%
	B.bengalensis	168	90	112	224	395	24.6%	75.4%

A total of 3364 corporeal fleas were collected at Mahim from R.rattus and B.bengalensis of which 2617 (77.8%) were X.astia, whereas only 747 (22.2%) were X.cheopis. Of the total 2617 X.astia, 89.2% (2332) were recovered from

B.bengalensis while the rest 10.8% (285) were from R.rattus of the 747 X.cheopis collected, 60.4%(451) were from R.rattus and 39.6% (293) were from B.bengalensis. 77.1% of the total flea ectoparasites of R.rattus were X.cheopis and 22.9% were X.astia whereas on B.bengalensis 88.8% of its total flea ectoparasites were X.astia while only 11.2% were X.cheopis.

Survey at Dharavi

407 rats were trapped from Dharavi during the 92 trap days of which 239 (58.7%) were R.rattus and 168 (41.3%) were B.bengalensis.

Corporeal fleas collected from rats 1025 (60.5%) were X.cheopis and 668 (39.5%) were X.astia. 80.3% (823) of the total X.cheopis collected (1025) were from R.rattus while the rest (202) 19.7% were from B.bengalensis, of the 668 X.astia collected 92.7%(619) were from B.bengalensis, while only 73% (49) were collected from R.rattus.

Relative infestation rates among the hosts

Multiple-capture traps gave the percentage infestation rates of R.rattus and B.bengalensis as 60.3% and 68.1% respectively. In the case of single-capture traps as has been mentioned above 84.6%, 92.4% and 92.6% were for R.rattus, Bandicoots and S.murinus respectively. Relative flea-indices and fleas-density distribution of the rats may be summarized as follows.

Table VI

Single-capture trap	Rat species	Total flea index	Density distribution
	R.rattus	4.6	0.28
	Bandicoots	14.5	0.65
	S.murinus	6.2	0.45
Multiple-capture trap	R.rattus	3.9	
	B.bengalensis	5.4	

Both these, the flea-indices and density distribution, in both the cases of single-capture and multiple-capture trap trials indicated a higher infestation rate for Bandicoots and shrews. R.rattus showed a general tendency to be less heavily infested than the others. Five out of 8 R.norvegicus and one out of two M.musculus trapped in the multiple-capture traps were free of fleas.

Sex of rat and infestation rate

Table VII

(Single-capture trap results)

Rat sp.	Sex	Total index	Specific index	
			X.cheopis	X.astia
R.rattus	M	5.2	4.2	0.96
	F	4.2	3.4	0.85
B.bengalensis	M	13.9	3.7	10.2
	F	14.95	2.4	12.5
S.murinus	M	6.7	6.1	0.67

Total flea indices for the sexes of R.rattus, B.bengalensis and S.murinus did not show much of a difference. However, in all the three cases the males showed a tendency to have a slightly higher index than females. As for the specific indices X.cheopsis gave slightly higher indices for the males of all species studied here while X.astia showed slightly higher indices for females of B.bengalensis and S.murinus. R.rattus did not give much of a difference.

Size of the rat and infestation rates

Table VIII

Size in cm.	Density distribution	
	<u>R.rattus</u>	<u>B.bengalensis</u>
Less than 12 cm	0.4	-
12 cm to 14.5 cm	0.2	1.93
15 cm to 17.5 cm	0.24	1.21
18 cm to 20.5 cm	0.22	0.40
21 cm to 23.5 cm	-	0.38
24 cm to 26.5 cm	-	0.74

The rats collected in single-capture traps were classified into 6 classes ranging from less than 12 cm to 26.5 cm of these 6 classes. R.rattus represented four and B.bengalensis had members in 5 classes. The total length was added up for every class and the total number of fleas collected from each class was divided by the total length of the rats of that class to get the density distribution per rat centimetre. Table VIII

indicates the relative density distribution per rat centimetre. The number of individuals examined classwise, in each class of R.rattus were 7 for the first class 14,48 and 63 for the remaining 3 classes respectively. In the case of B.bengalensis the first class had 7 individuals while for the other four the numbers were 5; 11; 25 and 23 respectively. In the case of R.rattus the individuals below 12 cm showed the highest flea density and for the other three classes the density distribution is almost the same. In the case of B.bengalensis the highest values were for the first two classes i.e. 12 cm to 14.5 cm and 15 cm to 17.5 cm . For the rest of the 3 classes density distribution is almost similar. In all these cases non-infested rats were taken into consideration. This was done because it may be generally assumed that failure to infest an individual may largely depend on its cleaning habits and hence this factor when taken into consideration, may show the state of activity of the age group of the rats under consideration. However, it should not be ignored altogether that the non-infested condition may result due to other factors also. S.murinus was not taken into account in these calculations as they formed only two classes and the variations in the sizes were not striking.

Distribution pattern of X.cheopis and X.astia on their hosts
X.cheopis (Roths)

X.cheopis has been collected from R.rattus, R.norvegicus, M.musculus, B.bengalensis and S.murinus. The number of R.norvegicus and M.musculus trapped is so negligible that it

does not seem suitable to draw any conclusions. Here the attention will be focussed on the distribution of X.cheopis on R.rattus, B.bengalensis and S.murinus. When a comparison is made of the data from Mahim and Dharavi, it sheds light on another interesting point. The percentage of X.cheopis on R.rattus and B.bengalensis of Mahim were 77.1% and 11.2% while those of Dharavi were 94.4% and 24.6%. Table IX shows the percentage distribution of X.cheopis and X.astia in Mahim and Dharavi.

Table IX

Locality	<u>R.rattus</u>		<u>B.bengalensis</u>	
	X.c.	X.a.	X.c.	X.a.
Mahim	77.1%	22.9%	11.2%	88.8%
Dharavi	94.4%	5.6%	24.6%	75.4%

X.cheopis percentages on the two types of rodents from Dharavi were higher than the X.cheopis percentages of rodents from Mahim, ^uWhereas the X.astia percentages on the rats from Mahim were higher than those from Dharavi.

The results of single-capture trap trials indicated that 80.7% of the total flea count of R.rattus were X.cheopis, S.murinus and X.cheopis to the tune of 86.6% of its total flea ectoparasites; Bandicoots had only 17.8% cheopis in its total flea count. The cheopis indices for R.rattus, Bandicoots and

S.murinus were 3.7; 2.8 and 5.4 respectively. 103 out of 112 R.rattus (91.96%) carried X.cheopis, 48 out of 50 S.murinus (95.98%) carried X.cheopis and 56 out of 85 (65.89%) Bandicoots carried X.cheopis.

Summing up the results of the corporeal flea counts, it may be said that R.rattus carried a great bulk of the X.cheopis collected during the field trials.

Table X

Percentage occurrence of X.cheopis and X.astia on the hosts

(a) Multiple-capture trap studies:

Rat sp. examined	No. exa- mined	Infes- tation %	<u>X.cheopis</u>				<u>X.astia</u>			
			M	F	Index	% occu- rence	M	F	Index	% occu- rence
R.rattus	86	68.6%	116	123	4.1	80.3%	17	5	0.54	8.4%
B.bengalensis	203	82.3%	66	103	1.01	19.7%	388	591	5.9	91.6%

(b) Single-capture trap studies:

R.rattus	132	84.6%	244	172	3.7	31.1%	42	58	0.89	6.6%
Bandicoots	92	92.4%	152	83	2.8	23.54%	325	679	12.8	86.98%
S.murinus	54	92.6%	141	129	5.4	45.2%	21	21	0.88	6.5%

Percentage occurrence of X.cheopis on the hosts is contained in Table X. Rats collected with the help of multiple-capture trap showed a concentration (and consequently a higher

% occurrence) of X.cheopsis on R.rattus. In single-capture trap results, S.murinus have been included which also carried a very high proportion of X.cheopsis. Here the results were a little different. It may be clearly seen that X.cheopsis showed a tendency to form a diffused distribution pattern among these hosts. However, the percentage occurrence of X.cheopsis on R.rattus was higher than that of B.bengalensis. But S.murinus showed a percentage which was still higher than that of R.rattus.

Frequency distribution of X.cheopsis (Ref.Tables XV;XVI and XVII)

It is assumed that if variance (σ^2) is approximately equal to the mean, (m) then, the distribution may be random where-as if they are not equal, then the distribution is said to be contagious and hence do not correspond to a poisson distribution (Cole 1949).

Table XI

Variance and mean for X.cheopsis on the hosts

Host	Variance	Mean
R.rattus	13.13	3.1
Bandicoot	22.24	2.5
S.murinus	30.52	5

The differences between the mean (m) and variance clearly indicate that the distribution is contagious. This was checked up by calculating Poisson distribution and chi-square test as a goodness of fit. All these clearly indicate a contagious distribution of X.cheopis on their hosts.

"Contagious distribution refers to the fact that this type of frequency distribution will be given by an event which, having once occurred, has by virtue of this occurrence an increased probability of recurrence just as one case of a communicable disease in a community increases the mathematical expectation of a second case occurring in the same community. Similarly, one interpretation of the contagious distribution of ectoparasites could be that there is less chance of a noninfested rat becoming infested than there is of an infested rat acquiring additional ectoparasites" Cole and Koepke(1947).

Sex of the rat and sex of X.cheopis

Chi-square was tested for a possible association between the sex of the rat and that of X.cheopis. But in all the cases the results were negative and hence no such association is evident.

Sex ratio of X.cheopis

X.cheopis collected from R.rattus trapped in multiple-capture traps showed that its sex distribution on the rat did not seem to follow a rigid pattern. During the months of January, July, November and December, females were found to be

more than males. Whereas during the other months males were more than females. Collection of X.cheopis from B.bengalensis showed that during the months of January, February, March, April, June, August, September and October females were more than males and during May, July and November males were more than females. In December, the proportion of females to males was equal. Studies with the help of single-capture traps indicate that sex ratio of cheopis on R.rattus, Bandicoots and S.murinus were 1.4M:1F; 1.8M:1F and 1.1M:1F respectively.

Table XII

Month	Rr		Bb	
	M	F	M	F
J	0.87	1	0.31	1
F	1.3	1	0.9	1
M	2.1	1	0.8	1
A	1.03	1	0.5	1
M	1.01	1	1.1	1
J	1.1	1	0.8	1
J	0.84	1	1.8	1
A	1.3	1	0.67	1
S	1.1	1	0.5	1
O	1.5	1	0.78	1
N	0.88	1	1.2	1
D	0.9	1	1	1

X.astia (Roths)

X.astia has been collected from Bandicoots, R.rattus, R.norvegicus and in small number from S.murinus. Here the attention shall be focussed on X.astia collected from Bandicoots, R.rattus and S.murinus, as the others do not seem to warrant a proper conclusion.

Taking into account the flea collection from Dharavi and Mahim, of the total 668 X.astia collected from Dharavi, 92.7% were from B.bengalensis while of the 2590 X.astia collected from Mahim 89% were recovered from B.bengalensis. Percentage of X.astia in the total flea ectoparasite count of B.bengalensis at Dharavi was 75.4% and that of Mahim 88.8%. Results of single-capture trap trials indicate that the X.astia index for B.bengalensis was nearly 14 times bigger than those of R.rattus and S.murinus. R.rattus had 19.3% X.astia in its total flea parasites; B.bengalensis had 82.2% X.astia and S.murinus had only 13.4% X.astia in its total flea-parasite count.

R.rattus collected from Mahim had X.astia to the tune of 22.9% of its total flea-parasite count while B.bengalensis had 88.8% X.astia of its total flea-parasites. In the case of rats from Dharavi the X.astia percentage was lower than this on both the rats. For R.rattus it was 5.6% and for B.bengalensis it was only 75.4%.

Percentage occurrence of X.astia on the hosts

This was calculated for X.astia also as it had been done for X.cheopis. Table X indicate the results of multiple-capture and single-capture trap trials. In both the cases it is evident that X.astia exhibited a tendency to concentrate on Bandicoots.

Frequency distribution of *X.astia* (Refer Tables XV, XVI and XVII)

X.astia

Frequency distribution calculated for *X.astia* as explained before for *X.cheopsis* indicate a contagious distribution on *R.rattus* and *B.bengalensis* . Table XIII gives the differences between mean and variance.

Table XIII

Host	Variance	Mean
<i>R.rattus</i>	2.7	0.8
<i>B.bandicoot</i>	348.1	10.91
<i>S.murinus</i>	1.8	0.8

According to the calculation it is evident that *X.astia* does not show a contagious distribution on *S.murinus*, but it is a random distribution. This fact has been clarified through calculations of Poisson distribution and ^{the} application of Chi-square test.

Sex of the rats and sex of *X.astia*

χ^2 tested for the association of the sex of rat and that of *X.astia* gave negative results showing ^a lack of any such association.

Sex ratio of X.astia

Sex ratio of X.astia showed a marked constant feature in the collection from B.bengalensis (Table XIV). That is, throughout the year females predominated. But the sex ratio of X.astia recovered from R.rattus did not show any such trend.

Table XIV

Month	R.r.		X.astia	B.b.	
	M	F		M	F
January	1.2	1		0.6	1
February	0.67	1		0.6	1
March	0.86	1		0.74	1
April	0.69	1		0.64	1
May	1	1		0.59	1
June	0.5	1		0.66	1
July	1	1		0	1
August	0.61	1		0.43	1
September	1	1		0.68	1
October	0.2	1		0.62	1
November	0.75	1		0.7	1
December	1	1		0.5	1

During the months of June, August, October and November X.astia collected from R.rattus showed that females were more in the collection. But during January males were more than females whereas during May, July, September and December the ratio of female to male was of same proportion. Results of single-capture trap trials gave a higher female ratio on the two hosts, R.rattus and B.bengalensis. The sex ratios for R.rattus and B.bengalensis were 0.72; 1 and 0.43:1, respectively, while S.murinus carried equal number of males and females making the ratio 1:1.

Frequency distribution of 2 species of fleas on

R.rattus

Table XV

No. per rat	No. of rats with			
	X.cheopis		X.astia	
	Observed	Expected	Observed	Expected
0	29	5.8	79	59
1	22	17.28	33	47.2
2	23	27.65	12	18.88
3	18	29.5	5	5.04
4	8	23.6	1	1.01
5	10	15.1	0	0.59
6	7	8.05	0	-
7	1	3.7	0	-
8	3	1.4	0	-
9	3	0.497	0	-
10	1	-	0	-
11	0	-	1	-
12	1	-	0	-
13	1	-	1	-
14	2	-	0	-
15	1	-	0	-
16	1	-	0	-
18	1	-	0	-

S.murinus

Table XVI

No. per rat	No. of rats with			
	X.cheopis		X.astia	
	Observed	Expected	Observed	Expected
0	6	0.36	31	24.26
1	5	1.82	15	19.41
2	12	4.55	4	4.76
3	5	7.58	1	2.07
4	5	9.48	1	0.41
5	4	9.48	0	0.066
6	2	7.899	2	-
7	2	5.64	0	-
8	3	3.53	0	-
9	1	1.96	0	-
10	5	0.98	0	-
13	1	-	0	-
14	1	-	0	-
16	1	-	0	-
34	1	-	0	-

Table XVII

Frequency distribution of 2 species of fleas on
Bandicoot group

No. per rat	No. of rats with			
	X. cheopis		X. astia	
	Observed	Expected	Observed	Expected
0	35	7.55	10	0.0017
1	15	18.88	7	0.018
2	15	23.6	14	0.098
3	5	19.66	3	0.357
4	7	12.28	7	0.974
5	3	6.14	5	2.13
6	4	2.56	10	3.86
7	2	0.914	4	6.02
8	1	0.29	3	8.02
9	1	-	3	9.73
10	1	-	4	10.6
11	0	-	3	10.5
12	0	-	0	9.56
13	1	-	1	8.03
14	0	-	0	6.26
15	0	-	0	4.55
16	0	-	0	3.1
17	1	-	1	1.99
18	0	-	3	1.21
19	0	-	2	0.6923
20	0	-	1	0.377
22	0	-	1	-
27	0	-	1	-
30	0	-	1	-
31	0	-	2	-
33	0	-	1	-
37	1	-	0	-
51	0	-	1	-
52	0	-	1	-
63	0	-	1	-
84	0	-	1	-
129	0	-	1	-

Discussion

Two types of traps used to collect rats were the multiple-capture traps and single-capture traps. While the multiple-capture traps give a large sample of rats at a time and are hence better suited to evaluate the rat density, the single-capture traps are used for specific studies of rats and their ectoparasites. Multiple-capture traps used for studies on the host preference of fleas may give misleading results, because the possibility of flea transfer from one rat to the other, (while in the trap) can never be avoided and disturbances caused in the trap may also force the fleas to leave the body of rats. But looking at it from another angle a true host-specific parasite may never be affected much by the proximity of another host animal. Though in the collection majority of X.cheopis occurred on R.rattus and X.astia on B.bengalensis,^{the} presence of certain percentage of X.cheopis on B.bengalensis and that of X.astia on R.rattus have been noted. This mixed population of fleas on their rats, and their notorious polyhaemophagous nature are clear-out evidences of the possibilities of host transfers while in the traps and in nature. Hence conclusions drawn from the results of multiple-capture trap trials tend to be inaccurate.

Rat collections at Dharavi yielded more R.rattus while that of Mahim had more B.bengalensis. This difference in the type of rats caught reflected on their flea yield also. X.cheopis distribution on both the rats collected from Dharavi

was higher than those of Mahim and the reverse was the case of X.astia collected from the rats of Mahim.

Dharavi and Mahim are two adjoining areas in the same ward (G/North ward) of Bombay Municipality but with contrasting ecological set-up. As has already been explained Dharavi mainly a slum type area, affords a rural type of surroundings in the housing conditions and drainage arrangements. This stands out in contrast to the urbanized area of Mahim with its "Pakka" houses and other modern facilities. In Dharavi one would find tanneries and shops where waste paper, rags, iron filings etc. are sold. Probably this along with the hut type houses, affords better nesting conditions for the house rats and may thereby encourage their breeding. From the corresponding differences observed in flea collections of these two places, it may not be preposterous if one concludes that Dharavi is a place where ecological conditions and host prevalence are better suited for X.cheopis breeding rather than X.astia. Thus the combined effects of the prevalence of proper ecological conditions and the proper access to the preferred host are important from the point of view of the survival and abundance of a flea species.

Inspite of all these factors of a mixed rat population and the promiscuous feeding habits of these two fleas, a fact which has been repeatedly observed and also proved in the present attempt is an obvious one that still a preference is shown by the two fleas to their respective host species.

X.cheopis was found in good numbers on R.rattus and S.murinus.

Bandicoots did not harbour them to this extent. Over 60% and above of X.cheopis collected in these field trials came from R.rattus. S.murinus also had equally high cheopis count. Similarly a large proportion of the total X.astia collected was from Bandicoot groups.

Many workers have clearly indicated a positive seasonal abundance in the flea ectoparasites of rats. As has been shown, X.cheopis index for R.rattus was in its peak in the month of January. The months of March, April and May also gave fairly high indices for X.cheopis. Almost similar finding resulted in the case of X.astia on R.bengalensis where the peak incidence in January and the indices for February, March and April were high. Needless to mention here that this higher index in no way represents a corresponding higher population density of the fleas concerned. Seasonal variations of the corporeal flea indices worked out in various parts of India show that the higher index falls roughly between the months of January and June. Advisory Committee (1906) showed the highest index between the months of March and April. Cragg (1921) found that cheopis index rises from May to September. Strickland and Roy (1930) found that at Calcutta higher index falls during the summer months. King and Pandit (1931) showed that the higher index was between September and January - the cooler months of the year. Rao (1941) at Calcutta found a higher index during the winter months of January and February. In Bombay Deoras and Tonpi (1956) and Deoras and Gokhale (1958) indicate that higher

flea index falls during summer months. Joshee's (unpublished thesis- 1961) results are also in agreement with this.

It is known for a long time that there is some difference in the feeding frequency of the two sexes of fleas. Males having lesser stomach capacity have to feed more frequently than females which can engorge well at a time and hence feeding frequency is lesser than that of males. It has also been suggested by many that the females spend much of their time away from the host for egg laying and hence visit the host only at a lesser frequency than males. These contentions were amply supported by field surveys which indicated a preponderance of male fleas in the collection. Single-capture trap trials of the present work indicated a higher proportion of X.cheopis males on all the three hosts. But in the case of X.astia the results have been just the reverse, where females were collected more than males. Deoras and Tonpi (1956); Deoras and Gokhale (1958) also found similar results. But Joshee (unpublished thesis-1961) found that "X.cheopis female exceeded over the male population". Regarding X.astia Joshee's (unpublished thesis-1961) results are in agreement with the data of the present work as he also collected more female X.astia than male..

All the above mentioned possibilities and the physiological necessities must undoubtedly affect X.astia also and yet this difference. Under these circumstances it may be a

good conjecture to suggest that there may be other factors yet unknown which govern the differential occurrence of the two sexes of these fleas on their hosts.

Relative infestation rates of R.rattus and Bandicoot group vary considerably. R.rattus carry less number of fleas than Bandicoots. This point had been brought to light by the previous workers also. Relative infestation calculated in terms of "index" and compared, may have objections as the body sizes of the different rats compared are not the same. According to the index R.rattus carried the ectoparasites at the rate of 4.6 per rat, while Bandicoots had an index of 14.5 which is nearly 3.5 times more than that of R.rattus. S.murinus also had an index of 6.2 which is slightly higher than that of R.rattus. This difference in infestation rates may be attributed to various reasons. The body size available for parasitism may be one. Cleaning habits and the type of habitat of the rodents may be another. The choice and degree of attraction of the fleas to a particular type of host combined with the difference in their abundance in the area of study may be yet another and so on. If this difference is due to the body size, then the question will naturally arise that if R.rattus and S.murinus had the same body size as the Bandicoots, would they acquire an equal proportion of flea ectoparasites? The best way to compare the infestation is to have a unit measurement for all the three hosts compared and with this view in mind the technique to calculate density

distribution of fleas per "rat-centimetre" was developed for the first time. This method of calculating density distribution per rat centimeter is of course not the accurate method for purposes of comparison. One objection may be that when one centimeter is taken in a linear way, then the surface area covered by this one centimeter will be different with rats of different body sizes. But this method has been used here because, it gives a better approximation than the index where the whole body of the rat is taken into account. One suitable method for the purpose of comparison is to take a unit measurement of the surface area of the body, and calculate the flea distribution over that area. This density distribution also indicates a higher density of fleas in the case of Bandicoots. The next claim goes to the shrew, and R. rattus comes last. At such an occasion, it may not be out of place to conclude that this difference in infestation may be due to the differences in the habits (such as cleaning habits and wandering tendency of the hosts concerned) and the habitats (such as type of locality where the rat lives etc., the difference in the number of fleas breeding in the nest of these mammals) of the rats.

Mohr (1961) has given a comprehensive idea of the physical factors which may affect infestation. He suggested that "what generally passes for host preference" among certain parasites is suggested to be partly the result of (1) a difference in amount of area of the host available for infestation or exposure to infestation, (2) a difference in

amount of area covered per individual expressed as size of standard home range. The remaining differences in infestation of the several hosts are, then, a measure of more purely biological factors such as differences in devotion of time to subhabitats in a given habitat such as bushes or trees, unshaded areas as compared with shaded areas, or to physiological conditions, cleaning ability intensity of activity and observational error. Though Mohr (1961) laid stress mainly on ticks in these analyses, many of the above mentioned factors may be conveniently extended to the infestation studies of flea ectoparasites as well. One factor which may be added here is that the differences in the time spent on the body of the host by the different species of fleas may reflect on their occurrence on the host.

circ. refer
A general conclusion reached by workers is that the young rats are more susceptible to the attacks of fleas than the adult ones. The present work indicates that in both R. rattus and R. bengalensis, a higher infestation rate is evident for the smaller members of the species. Eskey (1930) concluded that this difference may be due to the fact that the young rats spend more time in the burrows or nests than the adults. Another factor which may come into play is the ability of the rats to get rid of fleas. Young rats may not be so active in their cleaning habits as compared to the adults.

Worth (1951) noted that greater number of ectoparasites were found on male cotton rats. Tanaka and associates (1958)

reported that the rate of parasitism seems to depend on the range of movement of the host. Adult male mammals are known to travel more extensively than females (Mohr 1961). However, Krishnaswami et al (1963) suggested that "The high proportion of females among the trapped rats seemed to show that more females went out of their burrows". The results of the present work showed that males of R.rattus carry more fleas than females while it is the reverse with bandicoots.

Distribution pattern of the two species of fleas on the rats presents an interesting problem. Distribution pattern was studied in two ways : first by calculating the percentage occurrence using the formula of Kinney, Miles and Stark (1957) and second by finding out the frequency distribution. The results of percentage occurrence indicate that when single-capture traps were used; 31.1% of the total X.cheopis collected were from R.rattus while for Bandicoots and S.murinus, the percentage occurrences were 23.54% and 45.2%. These indicate two important points in the distribution pattern of X.cheopis. The percentage of X.cheopis collected from Bandicoots is lesser than that of the other two and the percentage of X.cheopis collected from S.murinus is more than that of R.rattus. In the case of X.astia, 86.98% of the total were collected from Bandicoots and only 6.6% and 6.5% occurred on R.rattus and S.murinus respectively. The percentage occurrence calculated from multiple-capture trap results also showed that 91.6% of the total X.astia collected were from B.bengalensis while 80.3% of the total X.cheopis collected were from R.rattus.

results gives a hint that the X.astia population shows a tendency to concentrate on the Bandicoots while the distribution of X.cheopis though more diffuse than that of X.astia may be said to predominate on R.rattus and S.murinus. This type of distribution may be explained in two ways. First, preference of fur type of the host to the flea. Thus Deoras and Gokhale (1958) stated "X.cheopis is found more on R.rattus which has a ^ufur of thick density and smooth texture, and this may be favourable for X.cheopis. On the contrary, X.astia might be requiring ^afur of thick density and coarse texture which it gets on B.bengalensis". It may be noticed that R.rattus and S.murinus have some-what similar type of fur arrangement. The second explanation may be that the chances of B.bengalensis coming to houses and contacting the X.cheopis breeding places are more than either R.rattus or S.murinus wandering into the X.astia breeding places which may ^{be}presumed to be the burrows and other harbourages of Bandicoots. Then the small percentage of X.astia which occur on R.rattus and S.murinus possibly represents the small number of X.astia which jumped off the body of the Bandicoots during their forages into the houses. These fleas then possibly exist as floating fleas in houses and neighbouring areas from where R.rattus and S.murinus may carry them off.

Frequency distribution shows a contagious nature in all the cases except the distribution of X.astia on S.murinus. Needless to say, that this type of distribution will considerably affect the flea index, as a good proportion of rats is

totally free of fleas or carry^{ies} only very few fleas. So while computing the index, a proper understanding of the nature of distribution of fleas on their host is a prerequisite, so that proper allowances may be made while drawing conclusions.

Summary and Conclusions

Rattus rattus (Linnaeus) and Bandicota bengalensis (Gray) could be collected from houses at Mahim and Dharavi areas of G/N ward of Bombay Municipality. Housing conditions and sanitary facilities of Mahim and Dharavi may be considerably influencing the rat populations of these two places. B.bengalensis were collected more from Mahim than R.rattus, while Dharavi yielded more R.rattus than B.bengalensis. The hut type houses, the open drainage and the associated ecological conditions of Dharavi may be compared with those of a rural environment and may possibly encourage the breeding of R.rattus than Mahim with its urban facilities, like 'pakka' houses, underground drainage system etc. There was a corresponding change in the flea populations also. At Mahim, X.astia predominated in the collection while at Dharavi, X.cheopis. Suitable ecological conditions and the proper access to the preferred host may be factors which help X.cheopis to flourish in the conditions of Dharavi.

Seasonal indices indicated that for R.rattus the maximum X.cheopis-index was during winter (consisting of December, January and February) while that for B.bengalensis was during

spring (March and April). Monthwise total flea-indices gave highest index for R.rattus in the month of April (7.5) and the lowest (1.4) in the month of July. For B.bengalensis the peak was in the month of January (12.19) and the minimum (0.86) occurred in the month of July.

Both multiple-capture and single-capture trap trials, showed that Bandicoots were more heavily infested than R.rattus. Infestation rate was high in the case of S.murinus also. Infestation rates may be influenced by factors like body size open to parasitism; sex and wandering tendencies of the rat species; its cleaning habits, time spent in the burrows or nests etc. Since the density distribution of fleas per rat centimeter also gave a higher value for Bandicoots, it is possible that body size alone may not be the main factor in acquiring more flea-parasites by these rats. Such being the case, the movements of Bandicoots; their cleaning habits and such other activities may give a clue to the higher infestation rate. From the calculations it was clear that the size variations in the same species of rat also influenced infestation. Thus it was found that smaller rat (presumably of younger age) carried more fleas than the larger ones in both the cases of R.rattus and B.bengalensis.

The distribution pattern of the two flea species indicated that X.cheopis was found to preponderate on R.rattus and S.murinus, while X.astia on the Bandicoots. The pattern of distribution of the fleas among these hosts indicated that

X.cheopis tend to form a type of diffused distribution on the rats while in contrast to this, X.astia showed a tendency to concentrate on Bandicoots. R.rattus and S.murinus carried only a very small percentage of X.astia. This situation may be explained in two ways (1) the preference of fleas to the type of fur of the host and (2) the chances of Bandicoots wandering into the houses and coming in contact with breeding places of X.cheopis may possibly be more than the chances of the other two hosts wandering into X.astia breeding areas.

Within the same species of hosts also X.cheopis and X.astia showed a contagious distribution. This type of distribution may influence the calculation of flea-indices. The ideal type of distribution for a well balanced flea-index would be a random one. When contagious, it indicates that the fleas tend to concentrate on one individual or a small group of individuals in a collection. This can affect the flea-index.

Sex ratio of occurrence of X.cheopis did not follow any rigid pattern. It was found to vary considerably. Females were collected more on R.rattus during the months of February , March; April, May , June, August, September and October whereas in the other months males were more. Male proportion was highest during March. In the case of X.astia on B.bengalensis during all the months of the year females predominated.

CHAPTER III

REPRODUCTIVE POTENTIALS OF X.CHEOPIS
AND X.ASTIA

Introduction

The conclusions of the last chapter give sufficient indication to the nature of distribution of X.cheopis and X.astia on their mammalian hosts of the locality surveyed. The polyhaemophagous nature of fleas sometimes brings about a diffused pattern of distribution and hence this factor when taken as a criterion to judge the host-parasite relationship, can be quite inconclusive. A distinction should be always made between the maintaining host (which can support and permit continued breeding of a flea species) and the accidental one. Hirst (1927) suggested that "It would appear that ability of a flea to maintain its population on a given host under conditions favourable to its development on its definite host constitutes a better indication of the natural affinities of the particular species of flea than the results of biting experiments which are liable to be affected by so many uncontrollable or difficultly control^lable factors".

Fleas may be physically and/or physiologically adapted to a particular host. Studies done with a view to elucidate the role of blood meal in reproduction and longevity have shown that though fleas may be kept alive for ^aconsiderable period of time on blood other than that of its natural host, there is a marked fall in the reproductive potential of the flea species concerned. Bacot (1914) showed that X.cheopis can be kept alive for long periods of time on a diet of human blood

but they did not lay fertile eggs under the conditions of his observations, unless allowed to feed more than once a day. Even with frequent feeds he found that the number of eggs laid by X.cheopsis was insufficient to maintain the flea population.

when Strickland (1914) proved that "Ceratophyllus fasciatus (Nosopsyllus fasciatus) which fed only on man did not lay eggs. On the other hand, a number of the same lot of fleas that were fed on a rat at the same time laid eggs shortly after the meal. On rabbit also the flea was found to feed well but did not lay eggs. The same case with the fleas fed on mouse". Hirst (1927) tried to breed X.astia on different hosts and found that they could be successfully bred on the guinea pig, the Indian house mouse, Ceylon house rat and the Sewer rat. Hudson and Prince (1958) while trying to find out the reasons for the difficulties in rearing different strains of P.irritans found that the urban and sylvan strains of P.irritans showed apparent biological differences in their breeding capacities on hosts offered.

While these experiments shed light on the specific blood requirements of fleas, Buxton (1948); Allen (1956) Miriam Rothschild (1960) and others tried to bring out the relation between the breeding of fleas and the physiological states of their host. Buxton (1948) showed that the age of the mouse(host) had considerable effect on the number of eggs laid by the flea, X.cheopsis. Allen (1956) and Miriam Rothschild (1960) showed that the maturation of ovaries of rabbit flea occurredⁿ only when fed on a pregnant rabbit.

Certain amount of work had also been done on the difference in the degree of attraction of the fleas to their hosts.

Shulov and Noar (1964) studied the olfactory responses of X.cheopis to its hosts. They designed an apparatus specially for this purpose. Olfactory responses of starved X.cheopis to white rat (R.norvegicus var.albino) and three murids-Microtus guentheri, Meriones tristrami and Acomys dimidiatus were studied.

Haas (1964) worked out the suitability of host to fleas. He found that "feeding of X.cheopis and X.vexabilis was low on R.norvegicus and M.musculus; mortality was high on R.rattus retention was high on R.exulans and low on M.musculus fertility was high on R.norvegicus and R.rattus and low on M.musculus.

Characteristic changes in the metabolism and reproductive potential may be expected if a flea species evolutionally adapted to one type of host, comes across another. These changes when studied along with the factors mentioned above form a better criterion to judge the relationship of flea to its host. Fleas, which are physiologically adapted to a particular species of host, might have evolved certain basic requirements from the blood of the host which permit proper breeding and normal longevity to the adults. If these basic requirements are inadequately met, then it may tend to affect the breeding activities of the flea and its longevity.

In this chapter, the comparative reproductive potentials of X.cheopsis and X.astia have been worked out using different rats as their hosts. The influence of the age of the host on the reproductive potentials of X.cheopsis and the relationship between the feeding frequency and fecundity also have been worked out.

Materials and Methods

X.cheopis and X.astia were collected from R.rattus and B.bengalensis respectively. These rats were trapped using single-capture traps and were killed and brought to ^{the} laboratory singly in plastic bags. X.cheopis thus collected from R.rattus were left in a breeding jar in the laboratory with a live R.rattus. X.astia were left with B.bengalensis. These rats were confined in wire cages 7" x 4" x 4" in size, and were regularly provided with food and water. As for the other factors as food and substrate for the larvae Leeson's (1932) method was followed. A Blotting paper piece sufficient to cover ~~in~~ ^{the} bottom was kept below the cages to prevent the urine of the rat from coming in contact with the larval medium. Since the blotting paper absorbed the urine, the larval medium was left dry. The cage was kept on an wooden plank so that it did not rest on the sand directly. When the colony was well established, large number of pupae were removed from the colony of the species of fleas to be studied and kept for emergence. The adults which emerged during a period of 24 hrs, were used for the experiments. This was done with the view of using fleas of same age for all the experiments. These fleas which emerged were kept with the three rats used in these experiments; R.rattus, B.bengalensis and Mouse (M.musculus Var.albino) in the same way described before. The second set of colony ^{was} thus contained one species of fleas of known age but with different hosts.

These fleas were thus kept in these three rearing jars for 7-10 days. By this time their ovaries were well developed and egg laying became regular. To start the experiment, a known number of fleas (males and gravid females) were taken from the three jars and left with another set of rats. Care was taken to make sure that fleas taken from one host were kept with the same species of host in the experimental set also. These fleas were observed for a period of 5 days to study their egg laying. In these jars the substrate used was sand sieved through a (80 mesh Fisher Scientific) strainer. Every day the sand from each colony was sifted for eggs of fleas. The 80 mesh gauze held the eggs back while allowing the sand to pass through. These eggs were counted under a binocular microscope and records were made. Eggs laid on the blotting paper and the wooden plank were carefully counted and added to the eggs collected from sand, to make the total. The eggs thus collected from each colony were kept separately in three jars to follow the developmental stages like hatching, pupation and emergence.

Throughout the experiment the number of fleas used had to be kept constant. For this the rats were caught every day from the cages and the number of fleas examined. Since these daily disturbances may possibly cause some ill effects on the hosts as well as on the fleas, another method also was followed where the rat and fleas were left undisturbed during the period of the experiment. But every day the eggs were collected as described above. The number of eggs per female was calculated

in this case, after ascertaining the total number of females left (alive) in the jar at the end of the experiment (Both the methods gave similar results). Since the experiments were of short duration, the discrepancy in the number of eggs per female, which may creep in due to loss of fleas, was minimized. The same techniques were repeated for X. astia.

For the feeding frequency studies, fleas of known age were taken and divided into three lots. One was left with a mouse (host), the second was given opportunity to feed on a mouse twice a day (25 min. each) and the third was given opportunity to feed only once. Daily egg count was done in these cases using 80 mesh strainer as described above.

Experiments with age of the host and reproductive potentials were carried out with mice of different ages. 9 day old mice were replaced daily in the jar so that the ages were same every day. 3 week old mice were kept in the breeding jar for one week at the end of which it was replaced with another individual aged 3 week. This kept the age of host between 3 and 4 weeks throughout the experiments. Experiments were carried out in the following way (a) Larval count: One freshly emerged female and three males were left in the jar of each host and the larvae counted every third day throughout the period of the experiment. The presence of the female in the jars was checked up every day and readings taken only if the female lived throughout the experiment. The jars were discarded if the

female was found missing. In the next lot 5 females and 10 males were used. As in the previous case the number of females was kept constant. Here, along with the experimental jars, a 'stock' jar for each host also was set up and this provided fleas of the same age with that of experimental jars. When a female was found missing from the experimental jar, it was immediately replaced by a female from the corresponding stock jar. The conclusions based on the larval count alone may raise certain objections because the egg number is not taken into consideration. To avoid this, egg count was done in another set of experiments. The technique used to count the eggs was ^{the} same as in the previous case.

The yolk granules were stained using the triple stain of Himmes and Moriber (1956). Yolk deposition may be also followed by dissecting out the ovaries and examining under reflected light with a light microscope. It may be also confirmed from the vigorous pulsations of the wall of the ovarial chamber while the yolk deposition is in progress.

Observations and results

Reproductive organs of female fleas

The female genitalia consist of a pair of ovaries, oviducts which unite into a common median oviduct before opening into the vagina. Vagina opens at the base of the eighth sternite. The entrance into the female genital chamber (vagina) is guarded by long setae of the lateral expansions of the large tergal plates of the eighth abdominal segment. Ovaries consist of ovarioles, the number of which may vary even in the same species. Generally 5 ovarioles may be seen on one side and 5 on the other. But in X.cheopis some had only 4+4 while in X.astia some had 6+5 and others 4+5.

The bursa copulatrix open on the dorsal aspect of the vaginal wall. The bursal duct which is broad and short of arise from a small aperture in a shallow pocket of the vaginal wall which is bordered by a chitinous sheath (Fig.44). This pocket is not seen in the case of X.astia (Fig.45) but as in the other case the opening is bordered by a chitinous sheath. From the sac like bursa copulatrix a fine duct leads into the spermatheca. Spermatheca helps in storing the sperms after mating. The spermathecal duct which is long and fine, gets thickened and widened before entering the bursa copulatrix. In both X.cheopis and X.astia only one spermatheca is seen. But a vestige of a second spermathecal duct remains as a blind second duct (Fig.44B) called ductus obturatus originating

from the main duct before it opens into the bursa copulatrix. The spermatheca differs in shape in different species and is taken as an important taxonomic character in identifying the species. Spermatheca is a chitinous sac having a tail portion and a head portion. The main spermathecal duct arises from the head of the spermatheca. There is a bunch of muscles extending from the tip of the spermathecal tail and gets attached to the head.

Development of eggs

Fleas have panoistic type of ovarioles i.e. the nurse cells are absent and yolk deposition is mainly carried out by the follicular cells and the oocyte nucleus. Fleas require blood meal (in some cases of a specific type) for the maturation of the oocytes. It has been seen that the first feeding of a freshly emerged female alone is not sufficient for yolk deposition and subsequent feedings are important. Under the laboratory conditions (temp. 29.25 °C) yolk deposition commences within 24 hrs. after leaving freshly emerged fleas with the host. By the second day egg laying starts. This period is undoubtedly influenced by temperature, as during cooler months a considerable delay may be observed in all these processes.

Table I indicates the measurements of the developing ova of X.cheopis and X.astia at different stages at 29.25 °C temp. When the fleas were collected and their ovaries examined it was

evident that in all the three cases yolk deposition had started by 24 hrs. after leaving with the host.

Table I
Measurements of ovarial growth (in μ) of X.cheopsis and
X.astia

Freshly emerged	<u>X.cheopsis</u>		<u>X.astia</u>	
	L	B	L	B
	125.13	96.4	115.5	96.3
24 hrs. after leaving with the hosts	R.r.367.5	267.8	420.0	280.0
	B.b.347.4	238	355.3	245
	M 308.9	189.9	285.3	178.5
Mature egg after it is laid	494.4	325.9	525.0	332.5

Yolk may be clearly seen as whitish granules in reflected light. The pulsation of the ovarial chamber while yolk deposition may be taken as an added evidence to confirm yolk deposition. Yolk granules could be differentially stained with the help of the triple stain of Himmes and Moriber for polysacchrides, proteins and DNA. Yolk gave a positive staining reaction to protein^{or}/polysacchrides. Presence of fat has not been tested. When the mature egg has been released from the ovariole, the ovarial pellicle shrinks bringing the

next developing oocyte near the oviduct. This thin shrunken membrane could be stretched out with the help of needles. A corpus luteum is formed in the empty chamber after the release of the mature egg. This could be seen as a white cluster of cells. Some Russian students of Siphonaptera (Darskaya et al 1962) feel that this may be used as a criterion to judge the age of the female flea. But further work is needed before a proper conclusion may be arrived at.

Another set of experiments was kept to study in detail, the rate of feeding, ovary development and host visitation of freshly emerged X.cheopis and X.astia. These fleas were starved for 24 hrs. before releasing. This was just to make sure that they were ready for feeding. The results were recorded at the end of 24 hrs. after leaving with the host. The findings are summarised in tables II and III. Percentage feeding and ovary development were not considerably affected by the difference in the host offered within the ^{first} 24 hrs. period, but a high percentage of X.cheopis were collected from the body of R.rattus while in the other two cases only negligible proportion of the total X.cheopis released was found on the hosts at the time of examination. This may possibly indicate a higher rate of attraction to R.rattus.

X.astia results were not very different from those of X.cheopis. Percentage of fed and ovary development were not considerably affected by the host within the 24 hrs. period.

Table II

X.cheopis

Host	No.released		No.recovered		No.fed		% fed	Condition of ovary of fed famels		% developed	Distribution of fleas in the colony at the time of examination	
	M	F	M	F	M	F		No	Y.D. Y.D.*		On the body	in sand
1	2	3	4	5	6	7	8	9	10	11	12	13

R.rattus

(a)	20	20	20	12	7	8	46.9	2	6	75	20	12
(b)	20	20	17	17	10	15	73.5	4	11	73.3	22	12
(c)	15	15	15	15	15	14	96.7	4	10	71.4	24	6

R.benga-
lensis

(a)	20	20	20	18	3	17	52.6	7	10	58.8	2	36
(b)	20	20	20	18	17	18	92.1	4	14	77.7	5	33
(c)	15	15	15	15	10	15	83.3	2	13	86.7	8	22

M.mus-
culus

(a)	20	20	20	13	12	11	69.7	3	8	72.7	0	33
(b)	20	20	13	9	6	8	63.6	2	6	75	2	20
(c)	15	15	15	15	2	7	30	1	6	85.7	1	29

* Y.D. = Yolk deposited.

Table III

X.astia

Host	No.released		No.recovered		No. fed		% fed	Condition of ovary of fed females		% developed	Distribution of fleas in the colony at the time of examination.	
	M	F	M	F	M	F		No Y.D.	Y.D.*		On the body.	in sand
1	2	3	4	5	6	7	8	9	10	11	12	13
R.rattus												
(a)	20	20	20	19	20	19	100	3	16	84.2	25	14
(b)	20	20	20	20	19	18	92.5	0	18	100	26	14
(c)	15	15	14	13	14	12	96.3	0	12	100	18	9
R.benga- lensis												
(a)	20	20	20	20	20	20	100	0	20	100	27	13
(b)	20	20	20	19	19	18	93.6	0	18	100	29	10
(c)	15	15	14	15	14	12	89.7	0	12	100	18	11
Mouse												
(a)	20	20	19	15	19	15	100	7	8	53.3	1	33
(b)	20	20	19	15	14	12	76.5	3	9	75	1	33
(c)	15	15	15	15	9	11	66.7	10	10	90.9	4	26

* Y.D. = Yolk deposited

It was found that in both the cases of R.rattus and B.bengalensis larger proportion of fleas was collected from the body of the host. Mouse gave a typical reaction for both X.cheopis and X.astia , that is, only a very negligible number of fleas were found on the body when the colony was examined at the end of 24 hrs. This is an apparent result of the active grooming habits of the mouse.

However one factor must be mentioned here. When gravid females of both the species were left with the host it was observed that in the colonies of R.rattus and B.bengalensis only a very small number of females was found away from the body of the host, while in the case of M.musculus only a very small number was found on the host's body when the colony was examined at the end of 24 hrs.

These findings establish the fact that when the flea procures regular blood meal, yolk deposition commences within 24 hrs. after leaving with the host.

Influence of hosts blood

X.cheopis

The experiments done with R.rattus, B.bengalensis and mouse (Mus musculus) as hosts for X.cheopis indicated in general that X.cheopis laid more eggs with R.rattus and Mus musculus as hosts. There was a considerable decrease in the total number of eggs laid by X.cheopis when B.bengalensis was offered. Table IV summarizes the average number of eggs laid per female during the period of the experiment.

Table IV

Average egg per female

Hosts	1	2	3	4	Average
<u>R.rattus</u>	53.4	50.1	55.5	52.6	52.9
<u>B.bengalensis</u>	33.1	31.3	34.2	34.6	33.25
Mouse	59.0	47.3	44.3	51.8	50.6

The results point out the fact that as far as the host value is concerned (as can be judged from the egg production) X.cheopsis does not show much of a difference between R.rattus and mouse (Mus musculus). But when it comes to the field rat B.bengalensis it shows that the host value of this rat is much lower than the other two. The results of Table IV were tested statistically to find out if these differences were significant. For this purpose F and t tests were done. The results are summarized in table V and VI. The F/Ratio gave a significant result.

Table V

Source of variance	Degrees of freedom	Sum of squares	Mean sum of squares	F/Ratio	Remark
Between hosts	2	923.3	461.7	28.7	Significant
Error	9	144.8	16.1		
Total	11				

Table VI

S.No.	Hosts compared	Difference in mean egg productions	Standard deviation of difference	Column (3) ----- Column (4)	Remarks
(1)	<u>R.rattus</u> B.bengalensis	19.65	2.767	7.036	Significant
(2)	<u>R.rattus</u> Mouse	2.3	6.79896	0.3383	Not significant
(3)	<u>B.bengalensis</u> Mouse	17.35	6.60939	2.636	Significant

Table VI shows the results of hosts compared in pairs. Hosts when compared in pairs gave significant results for R.rattus B.bengalensis and B.bengalensis/mouse. Where as the difference between R.rattus/mouse was not significant.

Table VII

No. of female used	10	10	10	15	15	15	10	10	10	10	10	10
No. of females alive at the end of exp.	10	10	10	15	12	10	10	6	9	10	10	10
Hosts	Rr	Bb	M	Rr	Bb	M	Rr	Bb	M	Rr	Bb	M
Total No. of eggs	534	331	590	751	373	473	555	205	399	526	346	518
Total No. of larvae	480	253	538	600	286	331	458	33	259	489	303	473
% hatching	89.9	76.4	91.2	79.9	76.7	69.97	82.5	16.1	64.9	92.96	87.87	91.33

The percentage hatching represented in Table VII indicate that a slightly higher percentage hatching for eggs from X.cheopis reared on R.rattus is evident. Eggs from X.cheopis reared on mouse did not show much of a difference in hatching from that of R.rattus. It may be possibly assumed that various other factors also influence the percentage hatching.

Table VIII
Percentage Pupation

Hosts	Rr	Bb	M	Rr	Bb	M	Rr	Bb	M	Rr	Bb	M
Total No. of larva	480	253	538	600	286	331	458	33	259	489	303	473
Total No. of pupae	465	250	513	543	225	305	393	30	226	470	300	468
% pupa- tion	96.9	98.8	95.4	90	78.7	92.2	85.8	90.93	87.3	98.4	99.02	98.2

It is evident from table VIII that in the case of pupation the stock reared on R.rattus did not show any higher values over that of others.

It may be stated in general, that at least for the first generation of X.cheopis reared on these different rodents, hatching and pupation did not show any marked or constant differences between these hosts. Table IX indicates the emergence and sex ratio. These also indicate that there is no marked or constant differences between groups of X.cheopis reared on different hosts. Survival values calculated for X.cheopis on all the three rodents indicate that the values for R.rattus and mouse are decidedly higher than those of B.bengalensis.

Most of the gravid females were found on the body of host (in the case of R.rattus and B.bengalensis only) when colony was examined. But in the case of mouse the number of fleas found on the body was negligible

Table IX

Percentage emergence sex ratio survival value of X.cheopis

Hosts	No.of Pupae	No.of adults	Percentage emergence	M	F	Sex ratio
(1)Rr	465	457	98.3	231	226	1:0.98
Bb	250	239	95.6	127	112	1:0.88
M	513	492	95.9	243	249	1:1.02
(2)Rr	543	540	99.5	269	271	1:1.007
Bb	225	216	96	108	108	1:1
M	305	300	98.4	147	153	1:1.041
(3)Rr	393	307	78.11	161	146	1:0.91
Bb	30	27	90	11	16	1:1.5
M	226	226	100	102	124	1:1.2
(4)Rr	470	464	98.72	233	231	1:0.99
Bb	300	292	97.33	146	146	1:1
M	468	465	99.4	238	227	1:0.95

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Table X

Survival value

Host	(1)	(2)	(3)	(4)	Average
R.r	0.86	0.72	0.55	0.88	0.75
B.b	0.72	0.57	0.13	0.84	0.57
M	0.83	0.63	0.57	0.898	0.73

X.astia

Table XI indicate the average egg production of X.astia female during the period of the experiment. It is seen that X.astia breeding on R.rattus and B.bengalensis did not show much difference in the average egg production, but the differences between the mouse (M.musculus-white) and the other two amounted to nearly 20. Though t-test did not give significant results; F test done between hosts yielded significant result at 5% level. The

Table XI

Host	(1)	(2)	(3)	(4)	Average
R.r	20.7	29.2	52.1	46.1	37.0
B.b	25.6	38.0	49.7	40.9	38.6
M	14.3	20.3	18.5	22.1	18.8

Table XII

Source of variance	Degrees of freedom	Serum of squares	Mean sum of squares	F/Ratio	Remark
Bet.Hosts	2	963.5	481.8	4.5	Significant
Error	9	967.2	107.5		
Total	11				

Table XIII T-test

S. No.	Hosts compared	Difference in mean egg production	Standard deviation of differences	Column (3) Column (4)	Remark
1	<u>R.rattus</u> <u>B.bengalensis</u>	1.6	17.73	0.09	Not significant
2	<u>R.rattus</u> <u>Mouse</u>	18.2	15.01	1.213	Not significant
3	<u>B.bengalensis</u> <u>Mouse</u>	19.8	10.6	1.868	Not significant

Table XIV

Percentage hatching

No. of O used +	10	10	10	15	15	15	10	10	10	15	15	15
No. alive	10	10	10	15	11	12	10	10	10	14	14	7
Hosts	R.r	R.b	M	R.r	B.b	M	R.r	B.b	M	R.r	B.b	M
Total No. of eggs	207	256	143	438	419	244	521	497	185	645	573	155
Total No. of larva	168	155	138	393	331	219	218	172	35	516	399	108
% hat- ching	81.2	60.5	96.5	89.7	79	89.7	41.9	34.03	18.9	79.9	69.6	69.7

Average survival value (Table XVII) seems to be higher for X.astia reared on R.rattus and mouse rather than B.bengalensis. But a proper scrutiny of the tables XIV, XV and XVI (representing percentages hatching, pupation and emergence) shows that the life cycle and its different stages are not very much affected by the difference in the ^{type of} host on which the adult feeds. It may be surmised that other factors like the climate, food and the environment influence the hatching, pupation and emergence more than the type of host offered on which the adult feeds.

Table XV.

% Pupation

Hosts	R.r	B.b	M	R.r	B.b	M	R.r	B.b	M	R.r	B.b	M
Total No. of larvae	168	155	138	393	331	219	218	172	35	516	399	108
Total No. of Pupae	168	154	136	300	329	212	203	160	25	492	383	105
% Pupation	100	99.4	98.5	76.3	99.4	96.8	93.1	93.03	71.4	95.4	95.98	97.23

Most of the gravid females were found on the body of the host (only in the case of R.rattus and B.bengalensis) when the colony was examined whereas in the case of mouse, the number of fleas found on the body was very negligible.

Table XVI
Percentage emergence and sex ratio of X.astia

Hosts	R.r	B.b	M	R.r.	B.b	M	R.r	B.b	M	R.r	B.b	M
Total No.of pupae	168	154	136	300	324	212	203	160	25	492	383	105
Total emergence	165	153	136	283	329	212	203	145	25	400	380	95
% emergence	98.22	99.4	100	94.3	100	100	100	90.6	100	81.3	99.2	90.5
sex ratio	1:0.81	1:0.98	1:1.2	1:0.95	1:0.98	1:0.83	1:0.07	1:0.99	1:2.6	1:2.007	1:1.24	1:0.98

Table XVII
Survival value

Host	(1)	(2)	(3)	(4)	Average
R.r	0.65	0.8	0.39	0.62	0.62
B.b	0.79	0.6	0.29	0.66	0.59
M	0.87	0.9	0.14	0.61	0.63

Age of the host and fecundity of X. cheopis

This was worked out in two ways (1) By taking into account the eggs produced per female for a period of 15 days, when left with hosts of different ages (Table XVIII). Here 5 females and 5 males were used with each host (2) Larval count per female (only one female along with three males were used) for a period of 30 days.

The total number of eggs and larvae produced, when allowed to feed on hosts (mouse) of different ages ranging from 9 day old to pregnant, are given in tables XVIII and XIX.

Table XVIII

Egg count

Duration of the experiment: 15 days

No. of fleas used : 5 females and 5 males

Hosts	(1)	(2)	(3)	Average	Average number of eggs per female for 15 days
3 week old female	761	772	466	666.3	133.3
Adult female	633	641	419	564.3	112.9
Pregnant female	634	652	405	563.7	112.7

Table XIX

Larval count

Duration of the experiment: 30 days

Number of fleas used: 1 female and 3 males

Hosts	(1)	(2)	(3)	(4)	(5)	(6)	Average
9 day old	361	264	283	232	272	242	275.7
4 week old female	454	422	414	250	396	326	377
Adult female	404	259	251	284	248	312	293
Pregnant	311	299	333	316	392	240	315.2

It is evident from these results that X.cheopis does not show any positive relation between adult blood and higher fecundity. On the contrary there is a slightly higher fecundity when fed on 3 to 4 week old mouse. The minimum fecundity among these observations resulted when fed on 9 day old mice. Improper hair growth and differences in body temperature with that of adult may be some of the reasons for this reduced fecundity.

Feeding frequency and fecundity

Fleas of same age (freshly emerged) were divided into three lots of five each. 1st set was kept with host (mouse) for the whole duration of the experiment. Second

set was allowed to feed on the host twice a day (15 minutes each. The third set was allowed to feed only once a day (15 minutes). After feeding they were left in sand from which the eggs were sieved out. Total number of eggs produced by the 1st, 2nd and 3rd sets during the 15 days experiment was 540, 64 and 11 respectively. Two eggs were collected from the 1st set on the 2nd day after leaving with the host and the maximum number of eggs produced in one day by the females was 46. Whereas in the 2nd and 3rd sets eggs were observed for the first time on the 8th and 11th day, after commencing feeding, respectively. The maximum number of eggs laid in one day by the females in 2nd and 3rd sets were 16 and 6 respectively. This experiment was repeated thrice and the results obtained were similar to these. This certainly indicates that feeding frequency affects not only the average egg production but delays maturation of ovaries as well, in a freshly emerged flea.

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Disoussion

Fleas, in nature may often get involved with hosts which are not natural to them. Such accidental occurrences may be explained through the movements and activities of their hosts. Mere presence of one flea species on a host does not in any way indicate its ability to breed and colonize on the host. For such parasites as fleas, the difference between the maintaining host and the accidental one should be always realized. Such information is not only important for evaluating the relation between the host and the parasite communities, but it is important from epidemiological point of view as well. It is not improbable that an accidental occurrence may lead eventually into a more or less permanent association, which may form an important epidemiological background.

Fecundity is one of the criteria with which one could judge the degree of association of the ectoparasite to its host. Fleas require regular blood meal from their host, if they are to oviposit normally. In order to obtain their regular blood meal, certain conditions should be satisfied. Hosts they feed on, should be their elected variety. Fleas should have proper hold on the host while feeding and hence the body coat of the host (like fur or feather) should be one with which the fleas are accustomed. Host's body temperature and the degree of attraction of the flea to the host are also important factors. It is quite possible that one of the accidental transfers, a flea species might find a host which satisfies many of the above mentioned

points. But still it may fail to colonize on that host because the habitat of the host, should be congenial to the development of the immature stages of the flea. When thus accidentally transferred to a new host, the flea certainly must react to the new environment. These reactions are then reflected on their metabolism and reproductive potential. Physiologically adapted flea parasites will demand certain specific requirement from the blood of the host failing which, delay in maturation of ovaries or irregular oviposition may result. It is evident from the present work that frequency of feeding is equally important for normal and regular oviposition. X.cheopis and X.astia used, in the present work, to compare the compatability of different hosts indicate that X.cheopis shows some differences as far as R.rattus (which is supposed to be the autochthonous host of this species) and B.bengalensis are concerned. Whereas when fed on mouse (M.musculus var, albino) the fecundity rate remained almost the same as that of R.rattus. The reaction of X.astia seems to be a little different. It gave no statistically significant values for the three hosts compared. Regarding R.rattus and B.bengalensis, one may as well see that there is not much difference. But X.astia gave a poor response to the mouse (M.musculus var.albino). On the whole the average

egg output per individual of X.astia when compared to X.cheopis seems to be low. The attempt made to show the differences between the feeding, ovary development and host visitation of X.cheopis and X.astia supports the fact that between these three hosts, freshly emerged X.cheopis and X.astia do not show very significant differences in feeding and ovary development within the 24 hrs. period. These factors may lend additional support to show that the blood preferences of the fleas are not too narrow. But one common factor is that the number of fleas found on mouse at the time of examination in both X.cheopis and X.astia was negligible. It clearly indicates that mouse is less tolerant to the presence of fleas on its body. Again, in the case of X.cheopis only a very small number was found on the body of B.bengalensis. But this was not the case with R.rattus and X.cheopis. This may be an apparent difference in the degree of attraction to Bandicoot. It

It may be possible that X.cheopis is a much more prolific species than X.astia. One factor which should not be ignored here is that environmental conditions seem to influence the fecundity of X.astia at a rate higher than that of X.cheopis. The experiments which gave very varying results for the same host are indications of this factor. The nutritive value of blood to the flea is very important. From certain blood samples the flea may not be able to derive enough nourishment to utilize for their day-to-day metabolism on ^{the} one hand and to the production of ova on the other. This may result in an abnormal gonotrophic cycle. The other factor which may be equally

important is the feeding frequency. Experiment done in this line has revealed how feeding frequency could influence fecundity and the time taken to mature ^{the} ovaries. It is natural for a flea species, which is conditioned to the smell of one type of host, to hesitate to feed readily on another unrelated type. But when starved they do feed on a host which is not natural to them. This difference in the degree of attraction undoubtedly affect the fecundity because for the normal rate of fecundity a regular blood supply is needed. The differences in fecundity observed in X.cheopis and X.astia may be a combined effect of these two factors. It may be possible that, if ample opportunity is given to the flea to acquaint itself with the new host, it may adjust and level up these differences, in the association with the host. Liston (1906) had shown a rise in the flea collection at a time when the young rats were caught more. This may either be due to the availability of more number of animals for the fleas to feed or to some adjustment, in the relation between the hormonal cycle of the host and the breeding of the flea. Buxton (1948) showed that for a normal rate of fecundity the flea, X.cheopis, must have at least one blood feed from the adult mouse. He concluded that there is a relation between the fecundity of X.cheopis and some adult hormones of the mouse. This point was taken up in the present work and the results are contrary to the expectation. X.cheopis did not show any considerable change in its fecundity when offered adult mouse or pregnant one. Young mice (3 week to

4 week) gave a higher fecundity for X.cheopis. This may be one of the factors associated with the skin thickness of the host and feeding of flea. Young animals having comparatively thin skin may make feeding easy for the fleas. Kartman (1954) found that when feeding the fleas artificially through an animal membrane, best results were observed when a young animals skin was used. However, in the present work, when a mouse of 9 days old was used as a host, the fecundity was reduced. This may be explained by the fact that a proper vestiture (physical security) is essential for the flea while feeding. Probably the poor hair growth on the 9 day old mouse does not afford proper security for the flea. The fleas may have to make several attempts to complete their normal meal because when the young mouse shakes its body the flea is easily dislodged, and this may reduce the fecundity. Since X.cheopis gave no indication of any hormonal relation to the host, the breeding season of the host may influence the flea only through the increased number of individuals in the host community available for parasitism. These experiments (age of host and fecundity of flea) were not repeated with X.astia because of poor host value of the mouse and the inavailability of a ready supply of B.bengalensis of known ages. This could be done with X.cheopis because mouse gave almost same host value as R.rattus for X.cheopis. Studies on the electrophoretic pattern of the serum protein of laboratory rats and certain other animals have revealed that the pattern changes according to the different strains of rats. Moore (1945) concluded that

"It is evident that many species under the conditions here studied present serum protein patterns which are so characteristic that it is possible to name the species and in some cases even the strain (rat) and sex (chicken) from a glance at the pattern". He found that young rat serum (rats between 21 to 29 days old) contrary to that of the adult contained a definite α globulins and a hardly measurable amount of γ globulins. Young rats have been shown to have a high percentage of albumin fraction of the blood than adults. The influence of such differences in the blood of various hosts on the flea ectoparasite needs further attention.

One other factor^{that} may be noticed is that, the change of host has not affected the immature stages of the flea very much and whatever differences observed may be very negligible. So it may be stated that probably the influence the host has on the immature stages of the flea species is an indirect one, i.e. only through the influence of the microclimatic conditions prevailing in the host's nest. From the results it may be seen that maximum mortality happened during egg and larval stages. After pupation the mortality was very low.

It may be thus clearly understood that the host influences the adults directly and the immature stages of the flea indirectly. Larvae do not survive to pupate and to form adults unless the host's habitat affords conditions which are congenial to their survival and development. Thus when an association of host and parasite is studied, it

necessitates one to study completely the effect of the host on the different stages of the life cycle of the parasite.

Conclusion and Summary

The female genitalia of X.cheopis and X.astia are basically same though certain structural changes may be observed. The shape of spermatheca is taken as a taxonomic criterion to identify the species. Both possess the vestige of the second spermatheca which remains as a blind duct (Ductus obturatus). Bursacopulatrix is a chitinous chamber from which a broad tube opens into the oviduct. In X.cheopis this opening is situated in a shallow pocket which is absent in X.astia. Ovaries consist of panoistic ovarioles. Blood feed is essential for maturation of oocyte. Yolk gives protein/polysaccharide positive reactions. Within 24 hrs. after releasing the freshly emerged flea with the host, yolk deposition commences. Measurements made of the developing oocytes of X.cheopis and X.astia releasing 24 hrs after with different hosts, showed that fleas from R.rattus colony showed maximum growth. Parallel experiments set to study percentage feeding, ovary development and host visitation indicate no measurable difference in the processes of feeding and ovary development of fleas with different hosts, but the number of fleas collected from the body of the host varied.

Blood compatibility and suitability of the hosts were tested and found that R.rattus and mouse gave almost same host value for X.cheopis, but B.bengalensis gave comparatively low

value. The fleas seem to spend more time on the body of the rat in the case of R.rattus and B.bengalensis whereas with mouse, they were found mainly in the sand. The same host reactions were found for X.astia also. X.astia gave minimum fecundity when offered mouse as the host. Host value of R.rattus and B.bengalensis seem to be ^{the} same for this species. The immature stages of both these species of fleas do not seem to be affected by the difference in the host species. But the development of X.astia seem to be influenced by environmental condition at a rate higher than that of X.cheopis. Feeding frequency has a considerable effect on the fecundity and the time taken for maturation of ovaries of X.cheopis. Relation between the age of the mouse and the fecundity of X.cheopis was worked out. It shows ^{ed} that with young mouse (3 to 4 weeks old) the fecundity was high. This may be due to the fact that the thin skin of the young mouse permit easy engorgement of the flea. The possible relation (if any) of the difference in the blood of young and adult mice, and the fecundity of flea has to be established by further research. These experiments were not repeated with X.astia mainly because of the poor host value of mouse, and the unavailability ^{of} the required ages of B.bengalensis (its preferred host in nature). It was possible with X.cheopis because mouse showed the same host value as R.rattus (the preferred host of X.cheopis in nature). Vestiture of the flea while feeding is an important factor which may considerably influence the engorgement of the flea on a given host.

CHAPTER IV

MORPHOLOGY OF MOUTH-PARTS AND
MECHANISM OF FEEDING

Introduction

The differences in the fecundity observed in the last chapter may be an apparent result of a physiological adaptation of the flea species concerned to the blood of a particular host, or may be due to the morphological and/or ethological (behaviour of the parasite in relation to the type of host present) adaptation. In the last chapter conclusions were also drawn which indicate the close relation between the feeding frequency and fecundity. A proper screening of the morphological peculiarities (if any) of the feeding behaviour of the two species of fleas corresponding to the type of host they infest is a prerequisite before a final conclusion is drawn from these observations.

The importance of morphology as an indication of the habits of the flea had been pointed out by Rothschild (1914) who stated "The species with the broadest and most heavily serrate mandibles are stationary parasites fixing themselves firmly in the skin of the host by means of piercing organ. The strength and increased width of the mandibles of Pulex irritans is explained by the fact that when the flea adopted man as its host, the bare skin and the garment covering it rendering the claws of the legs insufficient for keeping the insect steady when sucking blood, the strengthening of the mandible moreover, preventing them from being easily injured". In these words of Rothschild, two major points are brought to light. One is the relationship between the morphology of mouth

parts and feeding habits of flea and the other is the modification in the mouth parts according to the host chosen by the flea. Holland (1964) stated that "Most fleas feed intermittently while others like the stickfast (e.g. Echidnophaga sp.) and vermipsyllids and tunga become attached much like ticks. Such fleas have a suitably modified mouth parts with heavily denticulate maxillary laciniae".

Gordon and Lumsden (1939) were the first to introduce direct studies on the biting mechanism of a blood sucking insect, until then the observations done on the biting mechanism mainly centred round the indirect evidence gathered from the lesions produced on the skin of the host where the insect has bitten. Blacklock (1926) concluded, from his observations on the lesions produced on the skin by the gnat Simulium damnosum, that the superficial rasping nature of the bite of this fly caused it to pick up the skin inhabiting microfilariae of Onchocerca volu^ulens. Shortt and Swaminath (1928) successfully attempted to cut sections of the fly, Phlebotomus argentipes killed while feeding, along with the part of the skin where the fly was biting.

Gordon and Lumsden (1939) in their attempt to study the biting mechanism of mosquitoes, devised an apparatus which enabled them to observe the movements of the mouth-parts of mosquito, in a living tissue. They used frogs web as the host tissue on which the mosquito was made to bite and observations were made under a microscope. Leeson and Buxton (1949) raised some objections to using the frogs web saying that the movements

of the proboscis may be different in a mammalian tissue as the histological structure of the two are different. To tide over the situation, Griffiths and Gordon (1952) designed another apparatus in which they used the ear of an anaesthetized mouse on which the starved insect was made to bite. But there was one defect in this apparatus, in that the shadow of the insect falling on the ear hindered a clear observation. Gordon, Crewe and Willett (1956) made some improvements in the apparatus designed by Griffiths and Gordon (1952) for their studies on the biting mechanism of Tsetse-fly. In this case they allowed the insect to bite in such a way that the shadow of the insect did not hinder the observations. Lavoipierre, Dickerson and Gordon (1959) used the same apparatus to study the biting mechanisms of Rhodnius prolixus, Triatoma protracta and T. infestans. Deoras and Joshee (1961) used the apparatus designed by Griffiths and Gordon (1952) to study the biting mechanism of X. cheopis Roths. Lavoipierre and Hamachi (1961) studied the mechanism of feeding in different species of fleas using a modified apparatus where the flea was made to bite in the same plane as the ear of the mouse.

In the present study a new apparatus was designed to get a better picture of the mechanism of feeding in fleas X. cheopis (Roths) and X. astia (Roths). A comparative study of the morphology of mouth parts and feeding mechanism of these two flea species was done with a view to understand any factor in these which may influence the feeding and engorgement which in turn may influence the fecundity and thereby affecting the efficiency in getting themselves adapted to the change of hosts.

In addition to these factors, a proper understanding of the feeding mechanism of the flea may offer valuable solutions to the problem of differential vector efficiencies. A comparative study of this type may throw light on the probable influence the mechanisms of feeding may have on the vector efficiency of the flea species concerned. X.cheopis has been established as an efficient vector of plague whereas X.astia is a comparatively poor vector.

Materials and techniques

A simple apparatus was designed with which the biting mechanism of the flea could be observed without the shadow of the insect hindering the view.

The apparatus (photograph No.33) consists of two micro-slides A and B. One placed over the other. On B (which is the lower one) a thin chamber is made with the help of coverslip pieces (two on each side). These coverslip pieces will cover the three sides of the chamber. When slide A is placed on slide B a complete feeding chamber is formed with the lower end open for the insertion of the mouse ear. In this chamber the flea could move about on its lateral side. Slide A is kept in position over slide B with the help of two pieces of adhesive tape which will also work as a sort of hinge to open and close the apparatus. The ear of an anaesthetized mouse could be introduced through the open lower portion of the feeding chamber of the apparatus and the flea anaesthetized with carbon dioxide could be released into the feeding chamber, after which the whole apparatus along with the mouse could be placed on the stage of a microscope where observations could be made with the low power. To anaesthetize the mouse, Nembutal (Pentobarbital sodium) was used. A dose between 0.2 and 0.4 cc of a 2% solution varying with the size of the mouse was found suitable. A depilatory was used to remove the hair on the ear of the mouse. Male fleas were starved for 24 hours and the females for 48 hours. To kill the flea while feeding, for preparation of whole

mount as well as for serial sections, boiling water was mainly used. However, other methods like a fine jet of carbon dioxide or snipping off the fleas' head with a fine blade also were tried but none was found to be so effective and convenient as the boiling water. A drop of boiling water placed exactly on the flea killed it immediately giving it no time to withdraw its proboscis.

Preparation of salivary gland emulsion for coagulation studies.

The salivary glands were dissected out of female fleas of a known age and emulsion made in normal saline. This emulsion was mixed with whole blood taken from a fresh puncture on a human finger. The mixture was kept in small tubes which were incubated in a water bath at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ and clotting time recorded. The precise clotting time was calculated when a solid clot was found in the tube. Cases where a solid clot was not formed were rejected.

Morphology of mouth-parts

Mouth-parts of the flies consist of a minute labrum (Fig.1) a long, slender and unpaired epipharynx (Fig.2), a pair of maxillae with paired median stylets (Figs.3 + 4); a small hypopharynx (Fig.5) and a simple median labium (Fig.6). A comparative morphological study was made of the mouth-parts of X.astia and X.cheopis. The table No.I shows the measurements of the various parts. These studies were extended to both the sexes.

Labrum (Fig.1)

The labrum is a small triangular double walled piece hidden below the inflected margin of the clypeus. The dorsal side of it is connected to the lower margin of the clypeus by a membrane. A similar membranous connexion persists on the ventral side with the dorsal piece of the epipharynx. The two arms of the labrum are attached to the sides of the hypopharynx. The enclosed space between the labrum on top and the hypopharynx below forms the prestomum which forms the entrance to the cibarial chamber. The labrum is generally bigger in females (of both the species) than the males. In the male and female of X.cheopis the sizes are 169 μ and 208 μ respectively while in those of X.astia they are 156 μ and 197.6 μ respectively.

Epipharynx (Fig.2)

The epipharynx is an unpaired rod like structure made of a dorsal piece and a ventral piece. The dorsal piece is attached to the labrum by a membranous connexion. The ventral half is continued back to the cibarial loop (Fig.2C). Epipharynx is hollow with an unpaired nerve running through its cavity and innervates the sensory bristles at the tip of the epipharynx. Wenk 1953 calls these bristles as taste sensillae. On the dorsal half of the epipharynx are regularly distributed nodules which are 14 in number. The measurements of the epipharynx in male and female of X.cheopsis are 364 μ and 465 μ respectively while those of the male and female of X.astia are 390 μ and 499.2 μ respectively. The cibarial loop (Fig.2C) forms the cibarial chamber, the dorsal wall of which is formed by a tough membrane to which are attached the cibarial dilator muscles (Fig.28A) which extend between the tergum of the head and the dorsal wall of the cibarial chamber. The floor of the cibarial chamber is formed by the sitophore (Snodgrass 1946) of the underlying hypopharynx (Fig.27H). A membrane extends between the cibarial loop and hypopharynx (Fig.27) which covers the sides of the chamber. This chamber works as an efficient pump with the help of the dilator muscles.

The pharynx of the flea (Fig.2B) is an 'U' shaped structure and forms a second pump lying between the cibarial pump and the brain and hence is also called as the precerebral

pump. The dorsal dilator muscles (Fig.28C) of the precerebral pump arises from the dorsal wall of the head and are attached to the dorsal wall of the pharynx. The dilators of the precerebral pump arise from the genal region of the head and are attached to the ventrolateral aspect of the pump. A third pump is formed by the pharynx, called the post-pharyngeal pump (Fig.2A). By virtue of its position posterior to the brain it is also called the post-cerebral pump. This is immediately in front of the tentorial bridge. Dilator muscles of this pump (Fig.28H) are of a radiating type. The pharynx passes on to the oesophagus. There is no apparent difference in the arrangements of the muscles between the species and the sexes of the same species.

Maxilla (Fig.3)

The maxilla in fleas are formed of a broad triangular maxillary lobe (Fig.3B) a four segmented palp (Fig.3A) and the lacinia (Fig.4). The cutting blades of the proboscis of the flea are the maxillary (Fig.4) laciniae. The lacinial blade (Fig.4B) is convex on its outer surface and concave on its inner surface. On the outer convex surface there are four rows of denticles which are directed upwards (Fig.4D). The number of these denticles as shown in table I is 59 and 70 for X.cheopis male and female respectively. While 60 and 69 for X.astia male and female respectively. These denticles are broad plate-like at their bases. At the tip of the lacinial blade (in the species examined in this work) there is a single

curved spine-like process. The first few denticles at the tip of the lacinia are longer than the rest; they usually project out. There is a strong midrib (Fig.4C) running along the whole length of the lacinial blade. This depression houses a tendon from one of the muscles of the lever arm. Along the lower margin of the lacinial blade runs the salivary groove. The curved tip of hypopharynx fits into the groove at the base of the lacinial blade. At their proximal end the laciniae are bent back below the head and become thickened to form the basal arm that serves as lever for movements of the blades. The blades are joined to the lever in such a way that their posterior parts are turned mesally and horizontally beneath the hypopharynx. The lever arms (Fig.4A) of the laciniae are articulated on the bases of the respective maxillary lobes. Each lever is movable by opposing levator and depressor muscles which communicate movements of protraction and retraction of the lacinial blades. Musculature of the lacinia consists of a ventral muscle (Fig.25D) extending from the lacinial lever and the maxillary lobe (stipital protractor of the lacinial) and four dorsal muscles.

1. Extending between the head of the lacinial lever and posterior to the genal bridge (Fig.25C) (the retractor of the lacinia).
2. Extending between the sides of the cranium (below the eyes) and the head of the lacinial lever (Fig.26A).
3. Extending between the base of the lacinial lever and the dorsal wall of the cranium (Fig.26B).

4. Extending between the base and the head of the lacinial lever (Fig 26F). A branch is attached to the gena of the head. This muscle sends a tendon into the lacinial blade which traverses the whole length of it and gets attached at the tip of the blade. The lacinia in male and female of X.cheopsis measure 429 μ and 468 μ respectively. While those of X.astia are 452.4 μ and 504 .4 μ respectively.

The palpi of the maxilla are four segmented and arise from the base of the maxillary lobe. To this is attached one set of fan shaped muscle arising from the maxillary lobe which helps in the movements of the palps (Fig 26E). Two types of sensory bristles could be observed in the palps which are innervated by a nerve running along the palp. The palpi in male and female X.cheopsis measure 293.8 μ and 343.2 μ respectively while those of X.astia measure 345.8 μ and 351 μ respectively. The bases of the maxillary lobes are attached directly on the lateral margin of the peristome. No muscles are attached directly to the maxillary lobes. The measurements of the maxillary lobes in the two sexes of X.cheopsis and X.astia are as follows.

Length of the lobe in X.cheopsis male and female is 221 μ , and 249.6 μ respectively, while of those in of X.astia, measure 260 μ and 273 μ respectively. Breadth of the lobe in X.cheopsis male and female are 78 μ and 91 μ respectively while of those in of X.astia is 65 μ and 78 μ respectively.

Hypopharynx (Fig.5)

The hypopharynx is a strongly sclerotized, sharply tapering, decurved projection from the lower lip of the prestomum. This projection contains the salivary canal (Fig.5D) from the salivary pump which is situated at the base of the projection. From the base of this projection continued into the head is a horizontal plate the anterior part of which forms the Sitophore (Fig.27H). The posterior part of the plate forms a decurved apodemal lamella with inflected margins and accommodate the dilator muscles of the salivary pump. (Fig.27F). The salivary pump is a thick walled ampulla (Fig.5A) some what semicircular in shape. The dilator muscles arise from the ventral pocket of the hypopharyngeal plate. The anterior angles of the hypopharyngeal plate is prolonged into a pair of arms giving attachment to muscles extending between the latero-ventral aspect of the head and the hypopharyngeal arms. The common salivary duct enters through the ventral portion of the ampulla.

Labium (Fig.6)

The labium is formed of an immovable postmentum (Fig.6A) a median prementum (Fig.6B) and paired four segmented palpi (Fig.6D). The postmentum is a rod-like sclerite lying in the prestomal membrane and its posterior end is fixed on the post-genal bridge of the cranium. Its distal end supports prementum and gives attachment to the median muscles of the prementum, the only set of muscles of the labium. The prementum is a

median structure with a wide channel on its anterior surface and its distal end is notched at the base of the palp. The palpi are laterally flattened structures and have four segments. The tip of the palpi is pointed. The labium ensheaths epipharyngeal and maxillary stylets, which are held in the channel of the prementum and between closely appressed palpi. The postmentum in X.cheopis male and female measures 65 μ and 78 μ respectively while in of X.astia it measures 65 μ and 91 μ respectively. Those of prementum are 83.2 μ , 104 μ , 91 μ and 111.8 μ respectively. The palp measure 286 μ , 312 μ , 299 μ and 330.2 μ respectively.

Table I

Measurements in micro mm (average of 12 slides)

	<u>X.cheopis</u>		<u>X.astia</u>	
	Male	Female	Male	Female
<u>Labrum</u> : length	169	208	156	197.6
<u>Epipharynx</u> : length	364	465.4	390	499.2
<u>Maxilla</u> : <u>Lobe</u> : length	221	249.6	260	273
Breadth	78	91	65	78
Palp: length	293.8	343.2	345.8	351
<u>Lacinia</u> : length	429	468	452.4	504.4
No.of denticles	59	70	60	69
Hypopharynx length:	137.5	165.6	150	193.75
Breadth	50	57.5	56.25	59.4
Labium: Postmortem	65	78	65	91
Prementum	83.2	104	91	111.8
Palp	286	312	299	330.2

Mechanism of feeding

Formation of food channel:- Food channel (Fig. 32C) of the flea is formed by the embrasure of the lateral flanges of the epipharyngeal stylets between the concave inner surfaces of the maxillary laciniae. The lacinia possesses two grooves in its inner aspect. The epipharynx fits into the upper one while the lower one forms the sides of the feeding channel (Fig. 32). The ventral half of the epipharynx forms the roof of the feeding channel. This as the upper limit and the lacinial walls flanking the sides, a perfect tubular canal is formed through which the blood could be drawn up. The functional mouth is at the tip of the epipharyngeolacinial channel. This feeding channel is not connected with the salivary groove which runs through the lower margin of the lacinial blade (Fig. 32D). At the base of the lacinial blade the drawn-out pointed tip of the hypopharynx fits in well with the salivary groove of the lacinial blade. The saliva which is pumped into the groove by the salivary pump of the hypopharynx does not mix with the blood in the feeding channel. Mixing of saliva and blood occurs only outside the proboscis, whence the mixture is drawn up through the food channel. The food channel from the proboscis opens into the cibarial chamber^{which} is formed by the epipharyngeal loop, the sitophore of the hypopharynx and the tough membrane covering the loop to which are attached the cibarial dilators. True mouth is situated at the base of the cibarial chamber. Food channel from the cibarial chamber opens

into the pharyngeal chamber which is a 'U' shaped structure which then leads into the bulbous postpharyngeal pump and then to the oesophagus.

Feeding

When a starved flea is left on the host, it moves around in an agitated state moving the maxillary palps vigorously. When a suitable place is chosen the proboscis which is usually held a little back below the head is brought at right angles to the head (Fig.36). Piercing starts as soon as the tip of the labium touches the skin of the host. While piercing the flea ejects saliva. The proboscis, as it proceeds into the deeper layers of the skin, is bathed in saliva. The movements of the proboscis are very fast as it enters the tissue of the host. The labium does not enter the wound, made by the laciniae, but stands back as the proboscis advances deep into the skin of the host (Fig. 37). The labium bends at the joint of the prementum and the segment of the palp immediately next to the prementum and at both the joints of the labial palps.

A marked degree of flexibility is exhibited by the proboscis in the tissue making it possible to probe for a suitable blood vessel (Fig.41). The restless movements of the proboscis continues until it finds a suitable vessel and then the movements stop. At this time the cibarial and pharyngeal pumps go into action in sucking up the blood. When the proboscis punctures the blood capillary blood may be seen rushing into the proboscis. The synchronized movements of the

three pumps (the cibarial, the pharyngeal and the post-pharyngeal pumps) keep a steady flow of blood into the gut. First the cibarial pump dilates by contraction of the cibarial dilator muscles attached to the dorsal wall of the chamber, while contraction of the muscles attached to the arms of the hypopharynx drags down the sitophore (floor of the cibarial chamber). This dilates the chamber and allows the blood to flow into it. Now the pharyngeal pump dilates followed by the closure of the cibarial chamber and as a result the blood is pushed into the dilated pharyngeal pump. These movements go on in such rapid succession that a continuous flow of blood is maintained towards the gut. The upward thrust of the blood due to the pressure in the blood capillaries and the capillary action of the proboscis also help in the suction of blood.

However, it was observed, that feeding is not always continuous, that is, the flea stops for short intervals while feeding. At such times the flow of blood from the proboscis back into the blood vessel could be seen causing a reverse flow in the vessel.

The explanation given above holds good for both X.cheopsis and X.astia, because no difference was observed during this study in their feeding mechanisms.

Fleas (both X.cheopsis and X.astia) have been found to resort to pool (Pool of blood formed from the extravasation of blood oozing from capillaries punctured by the fleas) as well as capillary feeding (Sucking the blood directly from the

capillaries). But the latter is preferred by the fleas and the former is very rarely observed.

Functions of the various components of the mouth parts in feeding

Labrum (Fig. 1)

As has been mentioned before labrum lines the upper limit of the prestomum and has no muscles attached to it. It does not take any part in the processes of feeding.

Epipharynx (Fig. 2)

The median epipharynx flanked by the two laciniae form the proboscis proper. These are the only components of the flea's mouth parts which enter the wound. It has already been explained that the epipharynx has no muscles attached to it and evidently cannot initiate any movements on its own. It moves in unison with the two laciniae and enters the wound along with them. The epipharynx is a blunt rod and devoid of any cutting edges. So it is proper to presume that it plays no important role in the act of piercing. But the epipharynx is supplied with sensillae (Fig. 2E). There are nodular sense organs distributed along the dorsal side of the epipharynx and there is a group of sensory bristles at the tip of it (Fig. 2F). These sensory bristles are innervated by an unpaired nerve which originates from the labral ganglion. As the laciniae are devoid of any sense organs it is evident that the epipharynx is the only part in the proboscis proper which bear sense organs.

It is proper to think that the epipharynx helps the flea in "tasting" the blood before it is drawn up into the proboscis

Laciniae (Fig. 4)

These modified maxillary laciniae take up the function of the mandibles of certain other blood sucking insects. These are the cutting blades of the flea's proboscis. But unlike the mandibles of mosquitoes where the serrated cutting part is confined to their tips, in fleas 2/3rd portion of the laciniae are armed with four rows of denticles. The laciniae along with the epipharynx form the food channel. The laciniae work in alternate jerks. One of the lacinial blades first moves forward and gets anchored (by the virtue of the uncurved denticles it forms an excellent anchoring device) in the extended position when the other lacinia along with the epipharynx is thrust down into the new position. This is continued in rapid succession and thus the puncture is made.

The flexibility exhibited (Fig.41) by the proboscis while inside the tissue is also associated with the lacinial movements. From the morphological studies made on the mouth parts of the fleas it is possible to give an idea of the probable mechanism underlined in this phenomenon of flexibility. The tendon (described first by Work 1954) attached to the tip of the lacinial blade make it possible to bend. Since the attachment is at the tip of the blade, that part of it becomes the actively bending portion while the rest of the blade passively follows the tip. This tendon, as has already, been explained is attached to the muscle of the lacinial lever, and when this muscle contracts the lacinial tip also curves. Since

there is only one tendon in each lacinia, it can probably bend only in one direction. Probably each of them can bend in the opposite direction i.e. the right lacinia to the left and the left one to the right.

If, for example, the proboscis is to be bent towards the right, the flea will have to withdraw the proboscis a little. Then the left lacinia is pushed into the space made by the withdrawal of the proboscis and bends to the right side, makes a cut and holds the position while the other lacinia and the epipharynx could slide down into the new position.

This flexible movement, coupled with the ability of the proboscis to rotate (observed by Joshee 1961 an unpublished thesis) make it possible for the proboscis to probe in any direction it wants. However, this rotating motion has not been observed during the present study.

Flexibility of the proboscis is a feature seen in both males and females; but it is not necessary that the flea should exhibit it whenever it feeds. Sometimes when the flea gets a suitable capillary in the first attempt itself, it has not to do much of probing and as such marked flexibility may not be observed. On the contrary, when it fails to strike a suitable capillary, it probes all over till it finds one.

The role of laciniae in thrusting the proboscis into the tissue cannot be ignored. The thrust of the laciniae is effected by the contraction of the lacinial protractors, and

a push with the head and the body is not necessary. When the laciniae are anchored in the new position, the cranial flexors of the laciniae (attached to the lever head) work against the lacinial lever and their contraction pulls the head down to the new position. Thus the laciniae take the active part in driving the proboscis into the tissue and the head does not play any active part.

Thus the laciniae have many roles to play in feeding, such as forming the feeding channel, making the puncture, driving the proboscis deep into the tissue, functioning as an anchoring mechanism and helping in probing activities which enable the flea to search for the suitable blood capillary.

Labium (Fig.6)

Labium probably helps the flea to bring the proboscis to the feeding posture. Because when the labium was removed from the base (prementum and palps), the flea failed to bring the proboscis to the feeding posture. But when only the palps were removed leaving the prementum intact, the flea had no difficulty in bringing the proboscis to the feeding position. This probable role was hinted by Snodgrass (1946) in his paper; but he gave prime importance to the posterior revolution of the maxillary lobes.

Labium does not enter the cut made by the fasciole but it stands back bending on the joints of segments of the palps and at the joint of prementum and palp, as the fascioles

penetrated deep into the skin of the host. In the cases where the labium was removed, yet another interesting point was noticed. When the labium was removed the flea was unable to hold the proboscis stiff while piercing. In these cases the flea had to struggle for a long time with a "weak" proboscis to gain entry into the skin. However, once it manages to cut the tissue with the upcurved denticles, it could continue piercing. Thus it is evident that the labium helps to hold the proboscis stiff until the first puncture is made and helps to prevent warping of the stylets.

The maxillary palps and antennae

Maxillary palps and antennae ^{are} ~~are~~ profusely supplied with sensory organs. These structures no doubt help the flea in locating the host and in selecting a suitable place on the host to feed. When a starved flea is left on the host it is seen to move around in an agitated state, moving the maxillary palps vigorously. When a suitable place is chosen the movements of the maxillary palps stop and the proboscis is brought to the feeding position. This indicates the possible function of the maxillary palp in detecting a suitable place.

The function of claws

The main anchorage of the flea while feeding is effected by the lacinial blades. Hence the purpose of the claws is limited in the sense that it may only help to prevent the flea from being dislodged from the body of the host while it

scratches or shakes its body. The hold of the claws on the hairs is not a prerequisite for piercing the skin. This is evidenced by the fact that fleas could feed to engorgement on a part from where the hairs have been completely removed.

Role of saliva in feeding

Salivary glands (Fig.42) :- The salivary glands of fleas consist of 4 lobes, two on each side. The salivary glands lie well within the abdomen, a little posterior to the proventriculus. A duct proceeds from each lobe. The ducts of one side unite together and this single duct meets its fellow from the other side, a little before opening into the salivary pump in the hypopharynx, to form a common salivary duct. The lobes are saccular and contain one layer of flat cells with large nuclei (Fig.43). These cells surround a space in the centre into which the secretion is poured. These cells and the secretion contained in the space, failed to give a positive staining reaction with PAS; but showed positive reactions to protein stains like Naphthol yellow. The ducts are lined with chitinous rings and covering these rings is a photoplasmic sheath. These ducts before opening into the glands get folded many times. This may probably act as a sort of valve. There does not seem to be any other mechanism to regulate the flow of saliva. Under these circumstances one may be tempted to conclude that the main regulatory mechanism is the salivary pump itself.

The saliva

The saliva of fleas is a colourless fluid with a pH between 5.1 and 5.2(acidic) for X.cheopis (tested with Merck and BDH, pH paper) . It exhibits remarkable anti-coagulant properties. Experiments with whole blood clotting time, showed^{that} in the maximum cases the coagulation time may be delayed upto 1 hr and 5 min. in the case of X.cheopis; 34 min. in the case of X.astia and 4 min. for control (containing normal saline and blood). But addition of M/40 calcium chloride to the mixture of salivary gland emulsion and blood made it clot in the normal clotting time (3 to 4 min). To test whether the free calcium ions ^{were} are bound in this case, viper venom was used. Viper venom acts as a thromboplastin in the presence of calcium ions and thereby hastens clotting. Addition of viper venom (1/40,000) to the mixture did not make any effect in the prolonged clotting time. This may give an indication that in the initial stages of the delay in clotting, the free calcium ions are bound. However, there may be other factors also involved in its intricate anticoagulant action. This point requires further study.

The anticoagulant principle is resistant to heat (thermostable) and keeping the emulsion in boiling water for 5 min. does not reduce or destroy the anticoagulant action. When triple stain for polysaccharides protein and DNA was used, the secretion and the gland gave only protein positive staining and ^{did} does not contain polysacchar^aides. Since the flea

gut, after feeding, will contain a mixture of blood and its saliva, it may be worthwhile to study the influence of salivary secretion on the growth of P.pestis . Certain preliminary observations carried out, indicate that growth of P.pestis may be affected by the presence of the saliva of X.cheopis. However, no conclusion should be drawn from this observation because of its limited nature.

Discussion

Comparative observations made, in the present study, of the mouth-parts of X.cheopis and X.astia indicate clearly that morphologically they bear no dissimilarities. This is true for the sexes of these species also. But a glance at the measurements will indicate that males possess smaller mouth-parts than females and that X.astia possesses larger ones than X.cheopis. Considering the sizes of these fleas, these differences may be easily explained. Females of these two species are bigger than males and X.cheopis is smaller in size than X.astia. So the size differences of the mouth-parts only correspond to the size differences of the whole body of the fleas.

The feeding responses of the flea may be classified into three stages (recognised by Datheir 1953- in the feeding of an insect (1) Orientation to the food (2) Biting response and (3) Continued feeding response. The first stage ends at the arrival of the insect at a suitable site of feeding. Fleas are known to use the smell, warmth and sight of the host in locating a suitable one. Maxillary palps and antennae which are rich in sensillae, undoubtedly play a role in locating a host and a suitable place on its body to feed. There have been indications that in the case of fleas with eyes, they also part-take in finding a host.

Though there does not seem to be any apparent difference in the first phase of feeding as far as X.cheopis and X.astia are concerned, one point may be mentioned here which may be cited as a difference in the feeding behaviour. X.astia settles down to feed as soon as it touches the skin of the host, where-as X.cheopis moves around for some time and may even make several attempts to probe before settling to feed. However, this cannot be said as a rigid rule and it varies with the degree of starvation of these fleas. Hirst (1927) pointed out this difference in the feeding behaviour of X.cheopis and X.astia.

The second phase of feeding involves the application of the insects mouth-parts to the food, their movements and the initiation of entry of food into the gut. As has already mentioned the labium plays a considerable role in bringing the proboscis to the feeding position, and it also guides and holds the proboscis stiff while piercing. It is, however, surprising that Snodgrass (1946) has given only a secondary importance to the role of the labial movement in bringing the proboscis to feeding position. He laid stress on the posterior revolution of the maxillary lobe. It may be, no doubt, a combined effect of these two movements that brings the proboscis to the feeding posture, but the role of labium cannot be ignored.

Between the other three components (laciniae and epipharynx) there seems to be a division of labour. Laciniae form the cutting blades and the main anchoring mechanism for

the progression of the fasciole into the skin, whereas the epipharynx looks after the sensory functions. Laciniae undoubtedly work by alternate thrusts. If they work in unison, a jerky movement of the head should result. One who is observing the flea piercing will be surprised to watch the ease with which the flea guides the smooth progression of the mouth-parts in the skin. The contraction of the lacinial protractors brings about the effective thrust of the laciniae and a push with the head and body is not necessary. When the laciniae are anchored in the new position the cranial flexors of the laciniae (attached to the lever head) work against the lacinial lever and their contraction pulls the head down to the new position. This being the case, the use of the claws in the fleas may be only to prevent it from being dislodged from the body of the host while the host scratches or shakes its body. This hold with the claws is not a prerequisite for piercing the skin. This is supported by the fact that the flea could obtain its normal blood feed from an area on the host's body from where the hairs have been completely removed. Movements of the epipharyngeal stylet are strictly limited to those of the lacinial blades, because as has already^{been} mentioned, no muscles are attached to the epipharynx. This being the case, there does not seem to be any justification in the statement made by Lavoipierre and Hamachi (1961) who mentioned "Having reached a suitable vessel, usually of a small calibre, the tip of the epipharynx enters the lumen, and the maxillae (which from the salivary canal) remaining outside".

The third phase is the continued feeding response. If the flea has reached a proper capillary, it leaves only after full engorgement. While feeding, they are not easily disturbed; and slight mechanical disturbances do not affect them. Once proper feeding is commenced, the flea gets engorged within about 3 to 5 minutes. When it withdraws the proboscis, it causes an after puncture haemorrhage, at the site of the puncture. It has been mentioned that the flea while it sucks the blood, stops at certain intervals and then proceeds to feed. It has also been pointed out that at such times a reverse flow of blood from the proboscis to the capillary may be observed. This has been thought to be of some importance in the transmission of plague by fleas and has been shown by certain observers that an incomplete closure of the proventricular valve when the reverse flow takes place may cause the organisms lodged in the proventricu^{ricu}lus to be carried back into the blood stream of the host. If this is possible, then a block formation is not always necessary for a successful transmission. But from these superficial observations it is difficult to say whether a part of the blood from the proventricular region also flows back into the capillary. Blood sucking insects exhibit pool feeding as well as capillary feeding to obtain their blood meal. Shortt and Swaminath (1928) showed that P. argentipes resort to pool feeding. They also found that for successful transmission of Kala azar, the parasite should be released into the tissues of the host and not directly into the capillaries. Gordon and Lumsden (1939) demonstrated pool and capillary feeding in mosquitoes.

Gordon and Crewe (1948); and Gordon, Crewe and Willet (1956) described type of feeding in glossina as capillary feeding. Lavoipierre, Dickerson and Gordon (1959) found that T.infestans, T.protracta and R.prolixus adopt capillary feeding as the main source of tapping their blood meal. Lavoipierre and Hamachi (1961) and Deoras and Joshee (1961) showed that fleas are mainly capillary feeders, but at times they adopt pool feeding. ^{TR} A comparative observations of the feeding habits of X.cheopis and X.astia made in the present work confirms the observations of the previous workers.

Flexibility of the proboscis in insects with piercing and sucking type of mouth-parts was first noticed by Smith (1926). But it was then thought to be a passive action. Gordon and Lumsden (19³⁹~~29~~) who observed it for the first time in a blood sucking insect (mosquito), showed that flexibility is an active process and that the distal 5th of the proboscis is the actively bending part where-as the rest of it passively follows the cut made by the tip. Thus the belief that the insect with piercing and sucking mouth-parts should have a stiff proboscis in order to procure the food was proved erroneous. They tried to explain this phenomenon as something due to the action of the elevator and depressor muscles of the labrum. Flexibility of the proboscis was later on demonstrated in Glossina and Cimex (Gordon and Crewe 1948); R.prolixus, T.infestans and T.protracta (Lavoipierre, Dickerson and Gordon 1959). This phenomenon was demonstrated in plant bugs also (Smith, 19²⁶~~25~~; Flemion, 1958; Miles, 1958; and others).

Deoras and Joshee (1961) working on X.cheopsis came to the conclusion that in this flea also the proboscis exhibit flexibility. In the comparative study of the present work, on the feeding mechanism of X.cheopsis and X.astia, it was clear that while confirming the observation of the previous workers on X.cheopsis, it also showed that X.astia exhibited flexibility of proboscis. The flexibility involves mainly the distal portion of the proboscis where-as the rest of the proboscis passively follows the cut made. In one case of extreme flexibility it was observed that it even produced a sort of 'S' shaped bend, bending thrice. From the morphological studies made in the present work as well as by the previous workers, it is possible to give an idea of the probable mechanism underlined in this phenomenon of flexibility.

Wenk (1954) described for the first time the tendon running along the whole length of the lacinia. This tendon is seen in both the species of *Xenopsylla* included in this study. This tendon which is attached to the tip of the lacinia may be responsible for the flexibility. The attachment of this tendon at the tip of the lacinial blade makes that part of the lacinia the actively bending one. The contraction of the muscle (from which the tendon arises) at the lacinial lever curves the lacinial tip. Since there is only one tendon in each lacinia, it can probably bend only in one direction. Probably each of them can bend in the opposite direction i.e. the right lacinia to the left and the left to the right.

This coupled with the ability of the proboscis to rotate (observed by Joshee 1961 unpublished thesis) makes the proboscis bend in any direction it wants. For example, if the proboscis has to be bent towards the right, the flea will have to withdraw the proboscis a little. Then the left lacinia is pushed into the space made by the withdrawal of the proboscis and bends to the right side, makes a cut and holds the position while the other lacinia and epipharynx could slide down to the new position.

In the present work, however, no clue has been found which may indicate the capacity of the proboscis to rotate in the tissue.

Flexibility of the proboscis is a feature found in both males and females; but it is not necessary that the flea should exhibit it whenever it feeds. Some-times, when the flea gets a suitable capillary in the first attempt itself it has not to do much of probing and as such marked flexibility may not be observed. On the contrary, when it fails to strike a suitable capillary, it probes all over till it finds one and in such cases this phenomenon could be seen.

It is evident from the foregoing account that neither morphology nor the feeding mechanism shows any apparent differences in X. cheopis and X. astia. Since such differences, which may influence the blood feed, are not evident, it may be concluded that the differences observed in the fecundity may be mainly due to other reasons as incompatibility of blood of the host or the differences in the feeding frequency.

Conclusions and summary

Mouth-parts of the flea consist of a minute labrum, a long, slender and unpaired epipharynx, a pair of maxilla with paired median stylets (laciniae) and palps, a small hypopharynx and a simple median labium. Of these structures only the epipharynx and the two laciniae take active part in feeding. The laciniae help as cutting blades while the epipharynx act as sensory organ. The labium helps in holding the fascicles stiff and guiding them while piercing and does not enter the wound made by the lacinial blades. Food channel is formed by the embrasure of these three components. Salivary channel runs along the lower margin of the lacinial blades. The saliva which is pumped into this salivary groove by the salivary pump of the hypopharynx mixes with blood only outside the proboscis. The saliva by virtue of its anticoagulant property helps to prevent clotting of blood until it reaches the gut of the flea. Binding the free calcium ions of the blood seems to be one of the steps in delaying coagulation of blood. Further work is needed to elaborate these findings. The saliva is acidic in nature and has been shown to inhibit growth of P.pestis. This may be an important factor from the point of view of block information, as a mixture of saliva and the hosts blood is sucked in by the flea. It needs more elaborate studies to show the relation.

Mouth-parts have been seen to exhibit extreme flexibility while in the tissue. This factor undoubtedly helps the flea to

search for a suitable capillary in the host's tissue.

Evidently the flexibility can be associated with the tendon running along the whole length of the lacinial blade, getting attached to its tip.

Observations on the feeding mechanism confirms the previous observations that the flea species studied adopt both capillary and pool feeding to procure their blood meal. They seem to prefer capillary feeding to pool feeding, as the former is very frequently adopted by the flea.

Morphological studies and studies on the feeding mechanism of X.cheopis and X.astia worked out with a view to bring out any possible physical adaptation to the host, showed that there is no difference in the morphology and feeding mechanism in these two species which may suggest any possible influence on the blood intake and fecundity. However, certain differences which may be observed in these species are, that the size of the mouth-parts varies with the species and the sex of the same species. Thus males have a smaller sized proboscis than the females and X.astia possesses mouth-parts little bigger in size than X.cheopis. These differences may be explained in terms of the corresponding size differences between the sexes of the species and between the two species taken into consideration. One other difference may also be mentioned here. X.cheopis takes longer time to settle and start feeding. They generally

wander about and may even make a few punctures without sucking blood. Where-as X.astia immediately settles down to feed. However, this factor may vary according to the condition of the insect.

It may be thus concluded that the differences in the fecundity observed in the last chapter may be due to reasons other than morphological adaptations or differences in the mechanism of feeding, which could have influenced the blood feed and thus the fecundity.

CHAPTER V

GENERAL DISCUSSION

AND CONCLUSIONS

The two species of *Xenopsylla* treated in the present work are sympatric species but coexisting with different species of mammalian host occupying different ecological niches. Field survey carried out, indicates that *X.cheopis* is more closely associated with the house rats and shrews which inhabit human habitations. *X.astia*, on the contrary, preponderate more on the field rodents like the Bandicoot groups. Previous workers (King et al, 1929; Seetharama Iyer, 1933; Sharif, 1948 and others) have reached some-what similar conclusions after the study of the distribution pattern of *X.cheopis* and *X.astia*. However, Hirst (1927-a) found that in Colombo the domestic rats in residential areas are exclusively parasitized by *X.astia*, *X.cheopis* is found on rats in mercantile premises. *X.astia* is supposed to be an indigenous flea of India, its original home being the Oriental region. Originally this species must have occupied both the ranges of house and field rats. This typical condition may be seen in certain parts of the Ceylonese subregion (which includes South India and Ceylon) . *X.cheopis*, unlike *X.astia* was introduced into India through ships and other human agencies mainly from Egypt which is supposed to be the original home of *X.cheopis*. Wherever this species has been introduced one may find that it colonizes on house-rats and may tend to replace *X.astia* in these environments.

B.bengalensis, which is a very common rat in the houses in Bombay city, should not be considered as a "field rat" when used in the strict sense of the term. But the fact still remains

that these rats make burrows in the open areas around the houses as against the house rats which nest^s in houses and hence offer their fleas, environmental conditions, which are different ~~different~~ from those of the nest^s of house-rats. As has already been explained Bandicoots which visit houses frequently in the city of Bombay carry off a proportion of X.cheopis breeding in the houses. But these accidental transfers do not seem to affect much the general pattern of flea distribution. Kamath (1961-unpublished thesis) who studied the burrow fauna of B.bengalensis showed the total absence of X.cheopis in his collections. All the fleas collected from B.bengalensis burrows were X.astia.

In explaining this patchy type of distribution, various factors should be taken into consideration. Though the larvae are free-living feeders on organic matters and hence are influenced by changes in the environment (food and climate), the adults are parasitic and are thus influenced by an additional factor in the biocenosis, that is the host, and even the changes affecting only the host populations (without changes in other factors in the niche which are congenial to the life of adults and larvae) tend to affect the flea ectoparasites. The larvae are affected only indirectly by the type of host present in a locality because the conditions of the habitat of the host are of great importance for the survival and development of the flea larvae. Hence Rogers (1962) states that "Explanation for specificity must be sought over the whole range of factors which concern

the relationship of the parasite to the host throughout its life cycle".

Various attempts have been made by previous researchers to explain the differential distribution of X.cheopis and X.astia on their hosts. Sharif (1948) showed that the nutritional requirements of the larvae of X.astia are much more than those of X.cheopis and that the larvae of X.astia require higher temperature and humidity when compared to those of X.cheopis. Sharif (1948) made an attempt to explain away the differences in the distribution of X.cheopis and X.astia through his experimental results. It is quite evident that the nutritional requirements alone will not afford a satisfactory explanation to the question that if the larval food requirements of X.cheopis is lower than those of X.astia, what prevents the larvae of X.cheopis from thriving well in areas where X.astia flourish. The patchy distribution or preponderance of X.cheopis and X.astia in two niches is evidently the result of ecological isolation. This may be explained as isolation due to differential ecological preferences of these sympatric species. Microclimatic conditions are important factors to which the larvae are always susceptible. With a poor water regulatory mechanism (larvae are called as water-spenders) the changes in the atmospheric humidity will considerably affect the larvae. Ecological isolation may result from the fleas adaptation to the host which is then extended to the conditions of the niche in which the host lives or due to narrow preferences of ecological conditions of any stages in the development of flea which may eventually force the fleas to adapt themselves to the host which lives in those niches.

The common hosts available for X.cheopis and X.astia in Bombay are R.rattus, R.norvegicus; Bandicoots, the shrew, S.murinus and the house mouse, M.musculus. The experiments to test the compatibility of the blood of three of these hosts have yielded results to show that the fleas (X.cheopis and X.astia) can reproduce on all of them. But X.cheopis showed higher fecundity when fed on R.rattus and also a higher survival value. The laboratory mouse (M.musculus albino) may be said to have an equal host value, to that of R.rattus for X.cheopis. X.astia yielded no statistically significant results when tested between these hosts, but a general observation is that when fed on mouse the fecundity was very low. This differential fecundity may be the result of differences in the compatibility of blood of different hosts or to other reasons as morphological limitations or differential response due to differences in the "host-factor". The very fact that both the species did mature their ovaries when allowed to feed on the three hosts offered, is sufficient indication to show that the blood requirements are not too narrow. But blood-specificity should not be ignored altogether. Specific blood requirements of the flea may some-times evolve it into a state of hormonal dependency to the host. Rabbit flea S.uniculi is an example. Buxton (1948) made an attempt to show this in X.cheopis also, but the work carried out in the present attempt show no such relation. On the contrary, there may be seen a tendency to produce more eggs when fed on young mice (age between 3 weeks and 4 weeks). All these factors point out that specificity of blood requirement (if it exists)

of X.cheopis is not too narrow but this does not rule out the possibility of a specific blood requirements to reach the normal rate of fecundity. However, one fact is evident, that the presence of a host which is not the elected one of the flea species can considerably affect its reproductive potential which in turn may affect the magnitude of the population.

Other possibility which may reflect on the fecundity of the flea may be the morphological adaptation to particular type of host which may tend to restrict the blood intake from other hosts. There have been indications to show that the morphological adaptation may some-times speak of the habits of the flea. Rothschild (1914) showed that the species with the broadest and most heavily serrate mandibles (maxillary laciniae) are stationary parasites fixing themselves firmly in the skin of the host by means of the piercing organs. He showed the relation of the type of host (man) and the morphological modifications of the mandibles (maxillary laciniae) of P.irritans. Such morphological adaptations are of immense value for a flea species with regard to achieving its nutritional requirements and hence from an evolutionary point of view, forms an inseparable element of physiological adaptation. Comparative morphological studies carried out in the present work do not project any differences in the morphological make-up of the mouth-parts or any characteristic differences in the mechanism of feeding which may eventually influence the blood feed and thus be advantageous to the species as an adaptation. X.astia may be said to possess

comparatively bigger mouth-parts, a natural consequence of its bigger body size. This difference in size may not give any advantage to them over X.cheopis.

The third factor in the host-parasite relationship is the ethological relations of the parasite to the host. Olfactory conditioning of the flea, in this case, is of extreme importance. This may regulate the frequency of host visitations of the flea species under consideration. A flea species, accustomed to the smell of one type of host, when confronted with a new type will hesitate to feed readily on it unless driven out of sheer hunger. Experiments conducted, in the present work, indicate that frequency of feeding of X.cheopis will not only influence the daily egg production but the time taken for the maturation of ovaries as well. The same situation may develop when the flea is confronted with a host with which it is not familiar. Thus when offered a host to which the flea species is not attracted to the extent it does to its natural host, feeding frequency may be reduced thereby reflecting on their fecundity. Since the two species of *Xenopsylla* experimented did lay eggs when fed on the three rodents, it may be a good conjecture to suggest that the apparent difference in fecundity by the fleas may be mainly due to the differences in the degree of attraction to these different hosts. It may be also possible that the differences may be due to the fact that the nourishment obtained from the blood sample may be inadequate to meet the daily metabolic needs on one hand, and the egg production, on the other. This may reduce the average egg production of the

species. It may also be that the difference in fecundity may be an effect of the combined action of these two factors (degree of attraction and blood compatibility). It is also possible that given enough opportunities to the fleas to acquaint themselves with the new host, olfactory conditioning may modify these differences. Laarman (1955) showed that when a man-parasitizing *Anopheles maculipennis atroparvus*, was reared consistently on rabbits, there was an increased tendency to parasitize the latter host. However, a complete reversal of preference was not observed. Bacot (1914) also had realized the importance of the frequency of feeding and the amount of blood ingested in relation to the fecundity of X.cheopis. He found that X.cheopis required more than one feed a day on man to start laying eggs; but even then the number of eggs laid was so small that it was insufficient to maintain a natural population. From these results it is rather hard to judge the autochthonous host of X.cheopis and X.astia. As to X.cheopis, it is an accepted fact that R.rattus acts as its natural host. But this is judged mainly from their natural preferences. Here the point has been brought to light that mouse (M.musculus var. albino) also forms a suitable host for X.cheopis. Hirst (1927) aptly remarked that it is very difficult to judge the natural host of X.astia.

The different degrees of host-parasite relationships can have important bearings ecologically and epidemiologically. Audy (1958) suggests that the "parasite-pattern" could be used as an ecological label in many cases which may reveal the movements and activities of the hosts. He adds that the parasite-pattern also explains the disease pattern of a deme, as the disease pattern of a deme is a small part of its total parasite-pattern.

An example may be taken from the field survey of the present work to show how the parasite-pattern may reveal the movements of the hosts of these parasites in nature and how this information could be employed as an ecological label. Every animal in a community has a particular niche to which it is mostly confined and fleas are no exception to it. It has already been pointed out that X.cheopis prefers nests of house-rats while X.astia prefers the burrows of field rodents. Thus the parasite-pattern of house-rats and the house-shrew in Bombay show a predominance of X.cheopis while that of field varieties like the Bandicoot groups (B.bengalensis, B.indica, B.gigantia) showed predominance of X.astia. Though this is the general pattern of distribution, it is seen that a small proportion of X.astia occurs on the house-rats and shrew, while a certain proportion of X.cheopis occurs on the field rodents as well. The problems connected with the urbanization have forced the one-time field variety of rodents to seek shelter and food in human habitations. This necessity increased the possibility of mixing of the field and house-rats and thereby increasing the chances of accidental occurrences of flea ectoparasites on these hosts. When the percentage occurrence was calculated, it was found that only very small proportion of X.astia occurred on the hosts confined to the houses. Distribution of X.astia among R.rattus, Bandicoots and S.murinus was 6.6%, 86.98% and 6.5% respectively (Table X, Chapter II). But the proportion of X.cheopis on the field rodents was not that low. X.cheopis distribution among R.rattus, Bandicoots and S.murinus was found to be 31.1%, 23.54% and 45.2% respectively (Table X, Chapter II).

As has already been explained this type of parasite-pattern may be because the chances of the hosts confined to houses wandering into X.astia breeding grounds are less than those of the field varieties coming into contact with X.cheopis breeding areas. This strengthens the view that since houses to which R.rattus and S.murinus are confined afford enough shelter and food, seldom do they leave the houses or not at all. Whereas circumstances force the field rats to seek food and shelter in houses. Observations of Petrie and Todd (1923) in Egypt on the movements of R.rattus revealed that the movements of the recaptured animals over a period of three months were limited, consisting, for the most part, of oscillations between two or three adjacent houses. Audy (1958) explains that in Malaya, "the house rat R.r.diardi on the main land of Malaya is practically confined to houses, not poaching on the preserves of the field rat R.argentiventer so that the former will be infested by the Chigger A.indica and the plague flea X.cheopis while the latter will be infested by the scrub-typhus vector Chiggers T.deliensis and T.akamushi and will have no fleas". Thus the proper knowledge of the distribution pattern and ecological preferences of ectoparasites can yield very valuable information.

Rodent populations in a locality are not always stable. Food requirements may bring together different species of rodents occupying different ecological niches or it may cause mass migrations of rodents to new territories. Elton (1924) has shown that there are certain regular laws governing the fluctuations of many small mammals. Regular periodic fluctuations are controlled by two types of climatic pulsations: a short one with a period of 3 to 4 years and a longer one with a period of 10 to 12 years. Such movements and fluctuations in the rodent population are known to have serious

consequences in the epidemiology of plague. Hirst (1953) states how the mass migrations of R.norvegicus, in 1727 which crossed the volga river and spread west-ward over Russia (described by Barrett Hamilton and Hinton 1916) have caused the cessation of plague in Europe. Elton (1925) states "It is worth noticing that human plague outbreaks associated with wild rat plague in England occurred in 1906-7; 1909-11 and 1918. All these three periods are lemming maxima in Norway. It is therefore possible that mouse and wild rat maxima coincide in England and Norway".

Since the host population in a locality influences its flea population also, any such changes in a community will have corresponding effect on their ectoparasites. The habits of the synanthropic rodents, the degree of their association with man, their parasite populations and their state of immunity to different zoonotic conditions are all factors of utmost epidemiological importance. In Bombay, urbanization has caused an influx of field rats R.bengalensis into houses and nearby areas and now ^{they} show a tendency to replace house rats in many places. At Calcutta Seal (1961) has also recorded a similar change in the rat population. Deoras (1960) showed that R.rattus are at large immune to plague while R.bengalensis are highly susceptible. The fact that the vector X.cheopis has been found to attack bandicoots which are now moving in closer relation to man, is a situation pregnant with possibilities. The gradual replacement of the resistant house-rat with the susceptible Bandicoots should be looked up-on with suspicion. The influence of the type of blood ingested by the fleas on the

blocking phenomenon is a factor which needs further extensive study. Needless to repeat that block formation and eventual regurgitation^t are significant epidemiological factors. Thus whether the type of blood or the presence of immune bodies in the blood of the rat which is sucked up by the flea will have any retarding effect on the multiplication of the organisms in the gut of the flea is yet to be answered. For instance, Eyles (1952) who studied factors in the blood of vertebrate host influencing mosquito infection showed that "antibodies present in the infected blood may prevent development of some of the gametocytes potentially able to infect mosquitoes", and also discusses the importance of various blood components in promoting the growth of plasmodium gallinae cœum in mosquitoes. Taylor and Chitre (1923) found differences in the vector capacity of the fleas when two different hosts, guinea-pig and rats were used. Hirst (1926) pointed out that this difference is due to the differences in their susceptibility to plague. However, Goyle (1928) mentions that since the sex of the fleas left with sick rats in the experiments of Taylor and Chitre (1923) were not mentioned, the results cannot be taken into account. Kartman, Prince, Wan and Stark (1958) have stated that "By the use of artificial flea-feeding techniques (Kartman, 1954) we have found for instance, that certain strains of plague will grow normally and produce proventricular blockage in fleas when the bacteriae are suspended in rat blood, but will not multiply when the medium is rabbit blood". It needs further investigation before a proper conclusion^g may be arrived at.

The spread of plague by human agencies like ships and other means of transport was known as early as 1127. It is a fairly well-known fact that X.cheopis could be introduced into localities hitherto uninvaded by them. This was many a time followed by plague outbreaks as has been shown by Hirst(1927), Ali(1938) and others. Sharif (1948) showed that X.cheopis larvae could survive and metamorphose into adults even in the absence of dried blood in their food. This lend additional support to the prevalent belief that introduction of X.cheopis into new localities through human agencies is possible. Occurrence of X.cheopis in the interior of the U.S. (Ewing and Fox, 1938; Roudabush, 1939) shows the capacity of these fleas to adapt themselves to different environments. This also helps them in getting themselves established in new localities. Spread of a flea species may also be effected through the migration of the rats which are infested with these fleas. There exists no iota of doubt that X.cheopis had been and would still be introduced into new localities. This explains the present world-wide distribution enjoyed by X.cheopis. As has been evidently shown by researchers, X.cheopis has no too narrow specificities which may confine them to one biocenosis. Their ability to reproduce successfully on hosts other than their natural ones will certainly be a boon to their world-wide spreading. Though X.astia also did not show much of a difficulty in colonizing on hosts which are not their normal elected ones, (demonstrated by Hirst-1927 also) the narrow preferences of larval diet and climatic conditions may tend to restrict their distribution.

Thus when a flea species is introduced into a new locality, it may or may not come across a biocenosis akin to its original one. It is natural that when the conditions and the hosts prevalent are not very suitable, the success with which the flea gets established in that new locality will certainly depend upon its ability to adapt to the new environment and to the new type of host. When they do so, one may find certain biological differences (from their original strain) in this group of fleas established in the new locality. Even in the same locality, if one species of flea colonizes on different hosts inhabiting different ecological niches, there again certain biological differences may be expected. This phenomenon may be designated as "biological race formation". Thorpe (1930) has defined a biological race as follows. "A biological race may be said to exist where the individuals of a species can be divided into groups usually isolated to some extent by food preferences, occurring in the same locality and showing definite differences in biology, but with corresponding structural differences, either few and inconstant or completely absent". This biological differentiation may be associated with geographical isolation but with little or nothing in the way of structural or pigmentary differences. Thorpe (1930) continues to suggest that there are three ways of explaining regional differences in the behaviour of a parasitic species.

(1) As the expression of characteristic of germinally fixed biological races.

(2) As a direct effect of general environmental differences.

(3) As a direct effect of biological differences either germinal or environmental between the races of the host species in the two areas. Biological races defined by Thorpe (1930) have now been unmasked as geographical races or ecophenotypes or sibling species or host-races. Here the term "host-race" may be used. Thus the accidental transfers of fleas or their introduction into new localities may bring different groups of fleas of the same species in closer association with hosts occupying different ecological niches and may form "host-races" which may or may not exhibit morphological and physiological differences.

Roubaud (1920) explains the relation of biological differentiation among anopheles and absence of malaria from North Western Europe despite the presence of this malarial vector. The development of zoophilic race among these mosquitoes gave man a certain degree of protection from the bites of these mosquitoes. Such biological differences may or may not be correlated with apparent morphologic differences. Human louse-Pediculus- and itch mites Sarcoptes scabiei have also been known to form such biological races. Some researchers have gone to the extent of recording over fifteen subspecies of S. scabiei, but now they are all known to be only different host-races of the same species. Rozeboom (1952) has shown how closely related species and subspecies of Anopheles exhibit variations in their activities and how it could affect the problems of disease transmission and control. Andrewartha and Birch (1960) point out the existence of four races of the Argasid tick *Ornithodoros moubata* differentially adapted to their special habitats in East Africa. These changes are observed in their host relations also. Form A feeds on man, Form B on domestic fowls, Form C on Wart hogs and Form D on man and domestic fowls.

Though no specific work has been so far done in this direction which may yield data leading to such indication of such a race formation among fleas, certain very suggestive observations^{which have} hitherto been produced may be cited here. Hopkins and Miriam Rothschild (1953) state that "there appear to be two biological strains of X.cheopis which are indistinguishable morphologically. In the Sudan, upto Nile Valley as far as the approximate line of the Victoria Nile in Uganda and the adjacent parts of Kenya and the Belgian Congo, and probably in Ethiopia, the colony of the Niger and that part of North Africa which lies within the Palaearctic Region, cheopis is found abundantly on field-rats as well as on house rats. This form, which may be called the Nilotic strain, has obviously spread Southwards, mainly up the Nile valley, by natural means; it is of importance to note that R.rattus is still absent from much of the Southern portion of the range of this strain, while R.norvegicus is absent from the whole southern portion. The other strain has been carried about the world so freely by the agency of ship-rats, it is difficult to decide what part of its territory it occupied without the aid of man. It seems probable that this part of its range does not include more than the Oriental region and the form may be conveniently called the Oriental strain. Throughout the territory it occupies, the Oriental strain is associated with the domestic R.rattus and R.norvegicus, and it may be doubted if it occurs normally and permanently on any other host in striking contrast to the Nilotic strain".

D.montanus while proving, in the experience of Wheeler and Douglas (1941) an even more efficient vector than X.cheopis, figured last among the transmitters given in Burroughs' list (1947). Burroughs (1947) discussing this problem was inclined to believe that the discrepancy between his results and those obtained by Wheeler and Douglas (1941) was due to biological differences between the strains used in the two experiments. There was no doubt that the fleas used by Wheeler and Douglas (1941) came from a locality different from that where Burroughs (1947) obtained his material. The contention of Burroughs was supported by the observations of Holdenried (1952) who found that three of his D.montanus strains, obtained from different plague-foci as well as a fourth strain from an apparently plague-free region, yielded vectors at a rate of 4% and 5% respectively.

"A question of great interest and possible importance is whether the vector capacities of different strains of the same flea species may be at variance" (Pollitzer 1954). Pollitzer continues that "considering the limited scope of these observations, one must agree with Holdenried that the evidence concerning the existence of racial differences in the vector capacity of fleas belonging to the same species, although suggestive, is not fully convincing".

Haas (1964) studying the comparative suitability of the four murine rodents R.norvegicus, R.rattus (white bellied), R.exulans and Mus musculus, to rat flea X.cheopis and

X.vexabilis found that these two species showed low fertility when fed on M.musculus. In the present work it was noticed that fecundity was high for X.cheopis on R.rattus and M.musculus (var.albino). Though Haas (1964) has not given the data to support his conclusions nor did he mention what technique he used in evaluating the fertility rate among the fleas, it may be presumed that the differences found in the present work from that of his may be due mainly to biological differences in the group of fleas used.

The ability of these fleas to breed and colonize on different varieties of hosts makes these possibilities of differential race formation still more strong. This type of strain differentiation is important not only from the evolutionary as well as from epidemiological point of view.

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S Y N O P S I S

Some studies on rat-fleas and their bearing on host specificity

Fleas exhibit a wide range of host specificity. Holland (1964) states in this connection "Some species or groups of fleas are rigidly associated with a particular taxonomic group of hosts. Some species of fleas will breed on only a single species or genus of animal; others may live on more-or-less related groups of hosts; others again apparently breed indiscriminately on hosts sharing a common habitat. Distribution of fleas is governed, in part, by factors other than the hosts".

Though the type of host present in a locality may influence the breeding potentialities of the flea ectoparasites, fleas may survive for considerable time on blood of host other than their normal ones, even if they fail to reproduce. These promiscuous feeding habits exhibited by many groups of fleas is of great advantage to them, in the sense that this helps to reduce the period of starvation while searching for a proper host. But this habit also makes them efficient vectors of flea-borne diseases and the host specificity probably plays a key role in the spread of a disease. To cite an example, in places where sylvatic plague is prevalent, the flea transfer between wild rodents and domestic ones is said to play an important role in the spread of this disease.

Apart from this flea transfer between hosts, the difference in the vector capacity of fleas also makes it important to know the factors which control the prevalence and abundance of a flea species in a locality, as this has also been shown to influence the trend of epizootic and/or epidemic.

Among other factors as climatic conditions, type of larval food etc. hosts, their habits and habitats also deserve mention as this can undoubtedly influence the flea population in a locality. Sharif (1948) explained the differential distribution of fleas on rats and their prevalence as an effect of the difference in the larval food requirements. Stark and Miles (1962) showed the effect of the host prevalence on the occurrence of the different species of fleas.

Field studies at Bombay and at other places in India have revealed the distinct association of X. cheopis (Rothe) with Rattus rattus the house rat and X. astia (Rothe) with Bandicota bengalensis. Bombay has been witnessing a dramatic change in the rat population, an effect brought about by the urbanization of the place. B. bengalensis originally a field rodent is now found near human habitations and could be trapped from houses. It has been well established by the previous workers that as a result of this mixing up of rat population, the flea distribution on them also have been showing changes. This mixing up of the rat populations may bring about changes in the food and associated factors of the flea parasites as they depend on the host's blood for

their normal metabolism and reproduction.

The present work is an attempt to understand this delicate host-parasite relationship of fleas on Bombay rats.

The work carried out is divided into three parts.

First part deals with the collection of fleas by trapping rats using wonder-traps and single door traps. This is to get a good picture of the relative distribution of the two species of Xenopsylla found in Bombay. This also gives a fairly good account of their natural host preference in a place where the rats mingle. As has been explained before, the predominance of X.cheopis on Rattus rattus and X.asiaticus on B.bengalensis has been noticed, a fact which cannot be overlooked inspite of the contagious distribution of the fleas on rats.

Second part has been devoted for the relative reproductive potentials of these fleas on different rats. Reproduction of the fleas, oocyte development and yolk deposition also have been considered. Influence of the age of the host on the breeding capacity of the flea also have been experimented using laboratory mouse as the host.

In explaining the differences in the reproductive potentials, it is essential to study any difference in the feeding mechanism or the morphology of the mouth parts which

can influence the blood intake of the fleas. This has been worked out to show that the feeding mechanism is fundamentally similar in both the species of fleas. The mouth parts do not differ morphologically and functionally in the two species of fleas, but with some differences in size between the two species and the two sexes.

In conclusion it may be stated that the specific association shown by the fleas to their natural hosts, reflects well on their breeding potentials also, whereby more larval production per female may be observed when given an opportunity to feed on the natural host of the flea species concerned. This consequently will have an effect on the spread of the diseases caused by these Pulicine insects.

Statement I

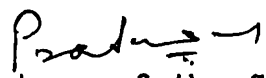
Studies on the host parasite relationship worked out in the present work is of great significance from the epidemiological point of view. The fact that B.bengalensis, which is highly susceptible to plague carried X.cheopis, an efficient vector of plague, adds colour to this epidemiological significance. Host specificity has a double role to play in the spread of a disease. Firstly through the state of immunity of the different hosts to the disease and secondly through the influence it has on the breeding of the parasites and thereby controlling the prevalence and abundance of the vector. These findings would throw light on the intricate host-parasite relationship of rats and fleas.

Statement II

The sources from which the information is derived to complete this work have been the publications of the previous workers in this field of study and allied subjects.

Statement III

None of the parts of this work has so far been published.


Signature of the Candidate

Forwarded through the Research Guide

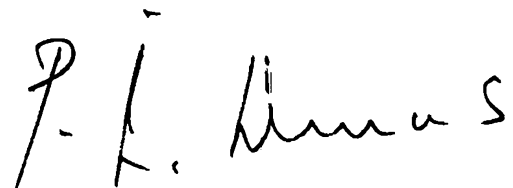

Signature of the Guide

PLATE I
MOUTH-PARTS OF X.CHEOPIS
AND X.ASTIA

Fig. 1 to 6 - X.cheopis Male

Fig. 7 to 12- X.cheopis Female

Fig. 1 - Labrum

Fig. 2 - Epipharynx; (A) Post pharyngeal pump; (B) Pharyngeal pump
(C) Cibarial loop; (D) Epipharyngeal stylet;
(E) Nodules; (F) Sensory bristles at the tip.

Fig.3- Maxilla; (A) Palp; (B) Lobe; (C) Extensor muscles of
the tip.

Fig.4 - Lacinia; (A) Lever arm; (B) Blade; (C) Midrib;
(D) Denticles.

Fig.5 - Hypopharynx; (A) Salivary pump; (B) Dilators of the pump;
(C) Hypopharyngeal arms; (D) Drawn out tip
of the hypopharynx.

Fig.6- Labium; (A) Postmentum; (B) Prementum; (C) Median
muscles of the prementum; (D) Palps.

Fig. 13 to 18. X.astia Male

Fig. 19 to 24. X.astia Female

PLATE I

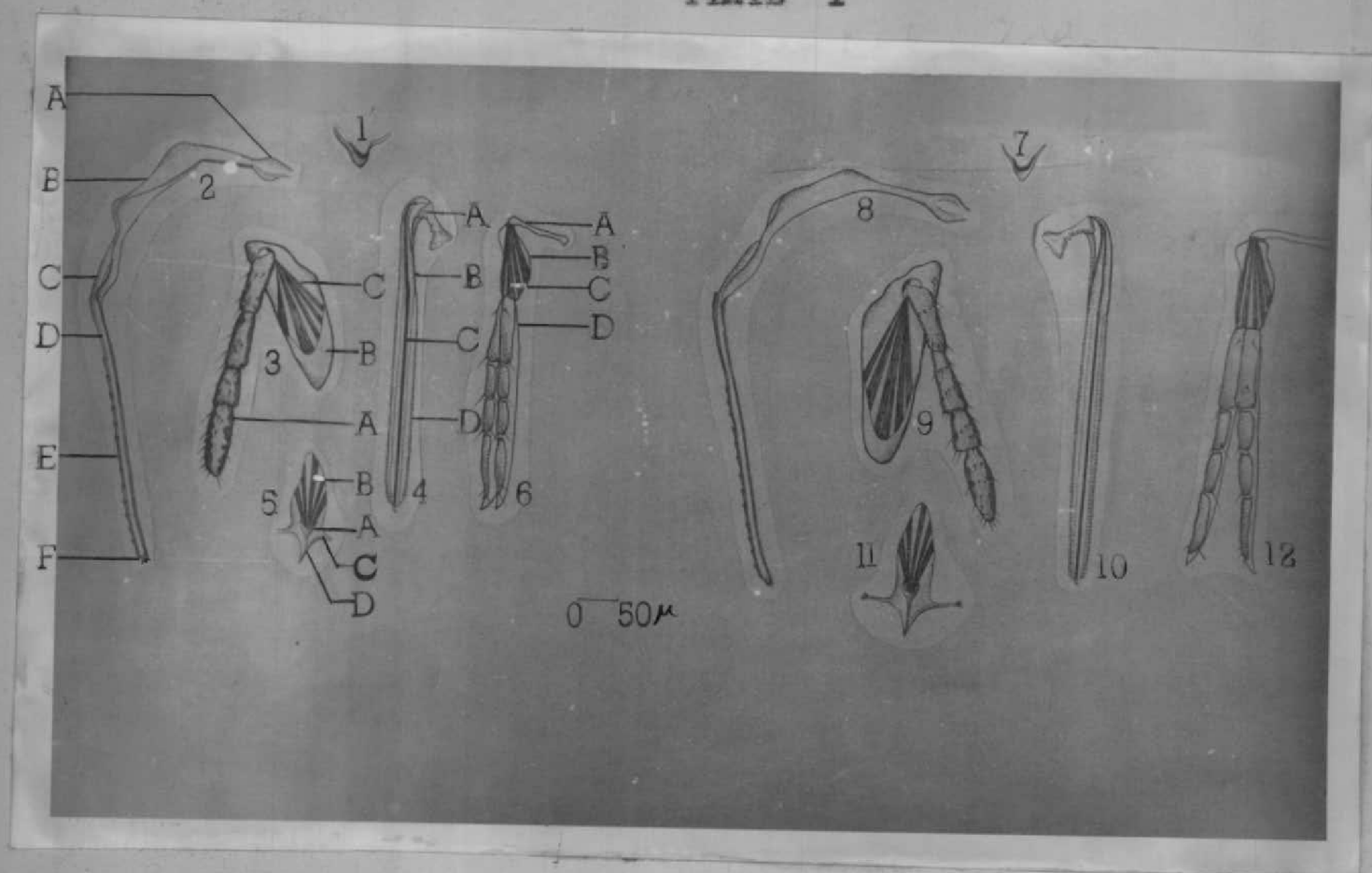


Fig. 1 to 12

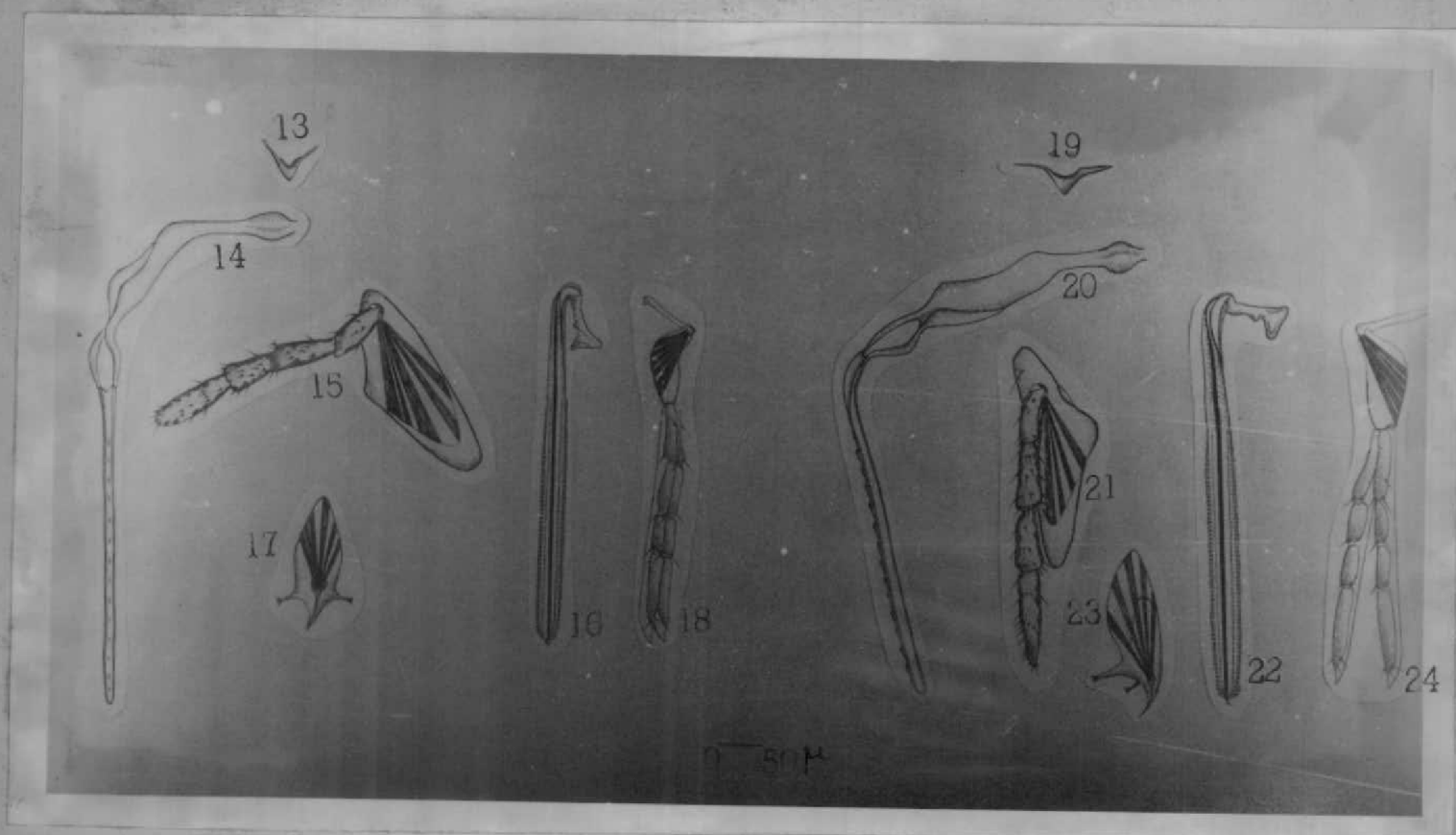


Fig. 13 to 24.

PLATE II

Fig. 25 & 26. Musculature of the Mouth-parts of the flea

- (A, B and C) Cranial flexors (Lacinial retractors)
- (D) Lacinial protractor
- (E) Muscles of the palp
- (F) Muscle running between the head and the base of the lacinial lever arm.
- (G) Lacinial blade
- (H) Lacinial lever arm
- (I) Maxillary lobe

Fig. 27. Musculature of the Mouth-parts of the flea

- (A) Epipharynx; (B) Cibarium; (C) Pharynx;
- (D) Muscles of the hypopharyngeal arm
- (E) Hypopharyngeal arm; (F) Dilator muscles of the salivary pump; (G) Drawn out tip of the hypopharynx; (H) Stitophore.

Fig. 28. Musculature of the Mouth-parts of the flea

- (A) Cibarial dilator muscles; (B) Epipharynx;
- (C) Pharyngeal dilator muscles; (D) Frontal ganglion
- (E) Lateral dilators of pharynx; (F) Pharynx.
- (G) Post pharyngeal pump; (H) Dilators of the post-pharyngeal pump; (I) Tentorial bridge.

Fig. 29. Section through the head of the flea showing the C.S. of epipharynx passing through the base of the stylet.

- (A) Dorsal piece
- (B) Ventral piece

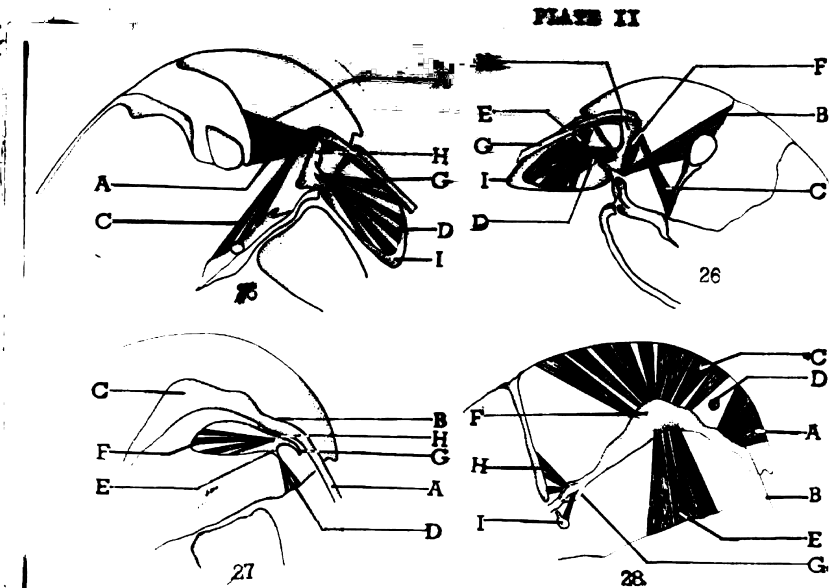


Fig. 25 to 28



Fig. 29

PLATE III

Fig. 30 and 31. C.S. of the various components of
the mouth - parts of flea.

Fig. 30 - C.S. at the level of prementum.

- (A) Prementum
- (B) Lacinia
- (C) Epipharynx
- (D) Maxillary lobe
- (E) Maxillary palp.

Fig. 31 - C.S. at the level of labial palp

- (A) Labial palp
- (B) Lacinia and epipharynx
- (C) Maxillary lobe

PLATE III

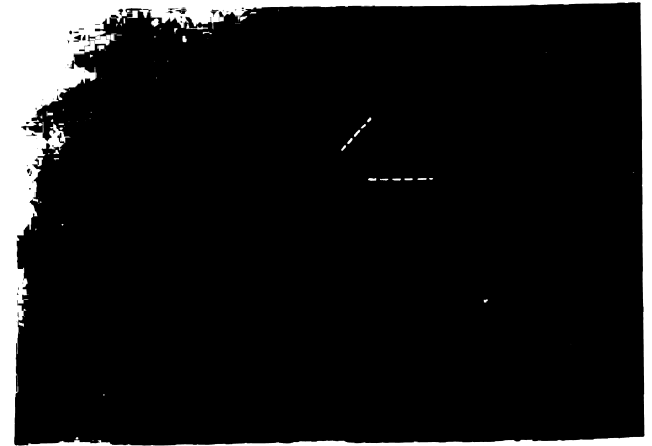


Fig. 30

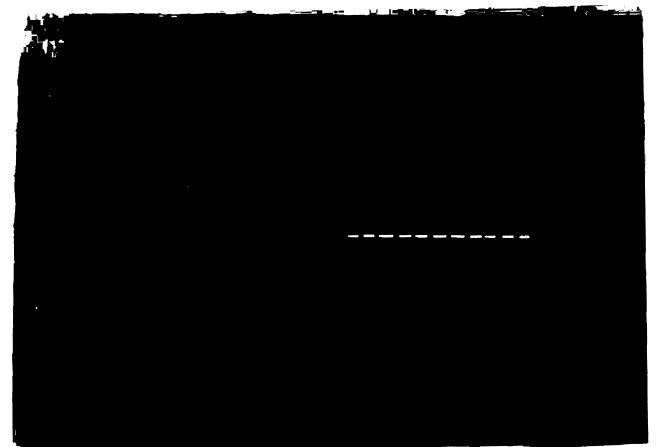


Fig. 31

PLATE IV

Fig. 32. Diagram of C.S. of proboscis.

- (A) Epipharynx
- (B) Lacinia
- (C) Food channel
- (D) Salivary channel

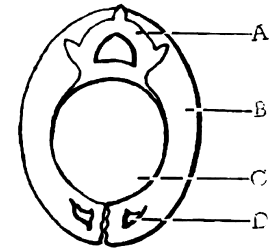


Fig. 32

Fig. 33. Diagram of the apparatus designed for the observation of the feeding mechanism of fleas.

- (A) Feeding chamber
- (B) Adhesive tape
- (C) Microslide - B
- (D) Microslide - A

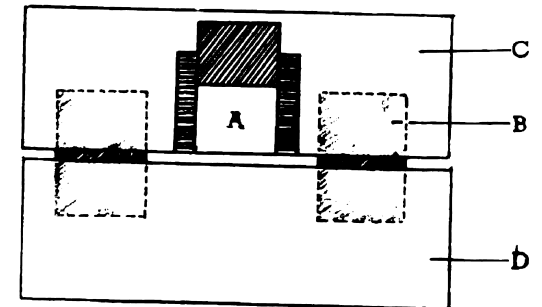


Fig. 33

PLATE V

Fig. 34 - Apparatus with mouse ear in the feeding chamber.

Fig. 35- Apparatus set on microscope ready for observation

PLATE V



Fig. 34

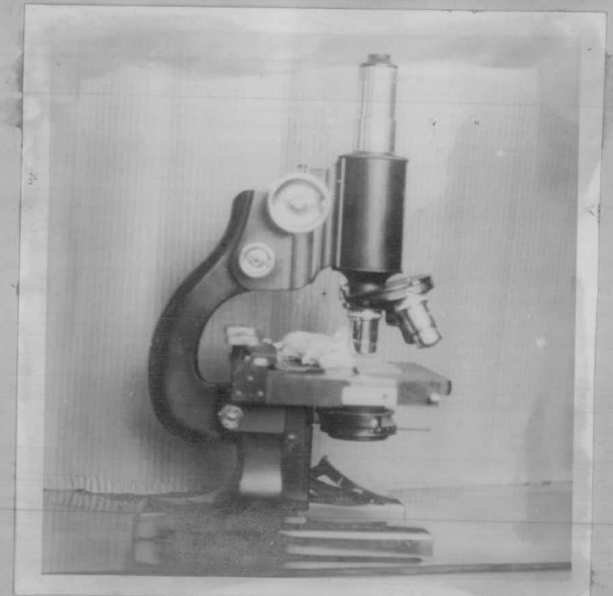


Fig. 35

PLATE VI

Fig. 36 - Feeding Posture of the flea - I

Fig. 36 to 39 represent in a sequence, microphotographs (taken with the help of the new apparatus) of the different stages in the act of piercing the skin of the host by the flea.

Fig. 37 - Feeding Posture of the flea - II

PLATE VI



Fig. 36

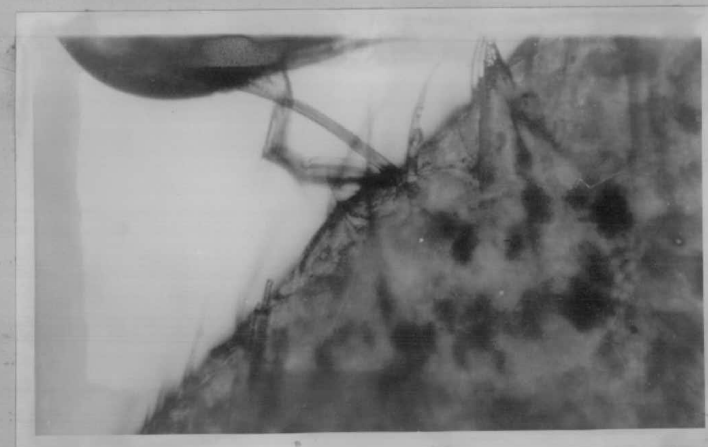


Fig. 37

PLATE VII

Fig. 38 - Feeding Posture of the flea - III

PLATE VII



Fig. 38

Fig. 39 - Feeding Posture of the flea - IV



Fig. 39

PLATE VIII

Fig. 40 - Section of the skin showing the tip of the
proboscis in the capillary

(A) Tip of proboscis

(B) Capillary

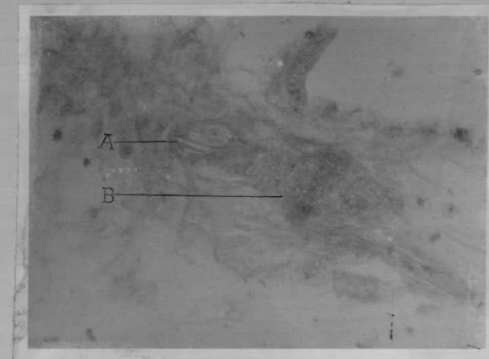


Fig. 40

Fig. 41 - Proboscis in the tissue showing the flexibility
bend in the form of S

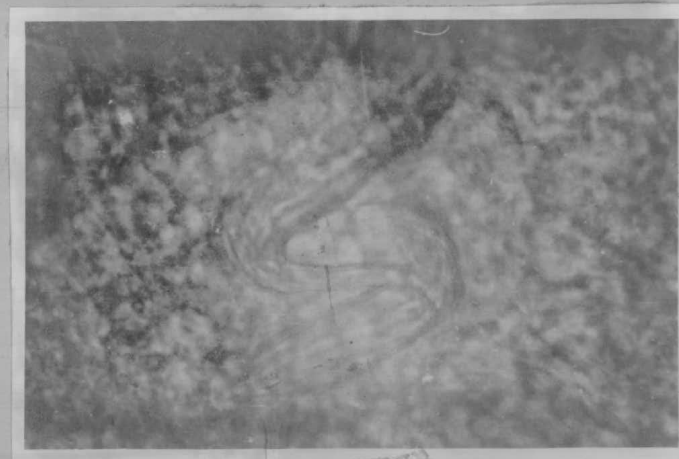


Fig. 41

PLATE IX

Fig. 42 - Salivary glands.

- (A) Lobes
- (B) Salivary duct
- (C) Common salivary duct
- (D) Hypopharynx
- (E) Epipharynx

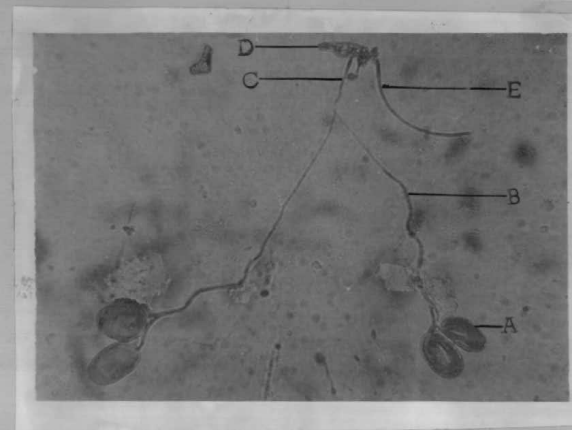


Fig. 42

Fig. 43 - L.S. of salivary gland

- (A) Salivary gland



Fig. 43

PLATE X

Fig. 44 - Bursa copulatrix of X.cheopix

- (A) Spermathecal duct
- (B) Ductus obturatus
- (C) Bursal chamber
- (D) Chitinous plate bordering the bursal opening

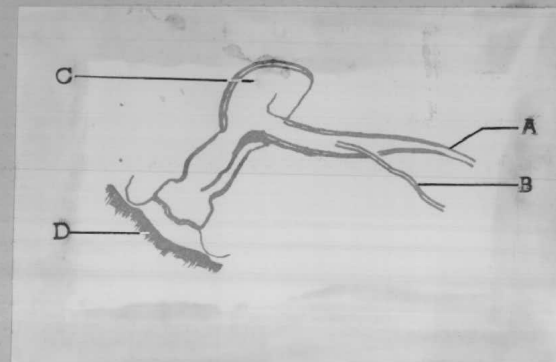


Fig. 44

Fig. 45 - Bursa copulatrix of X.astia

- (A) Spermathecal duct
- (B) Ductus obturatus
- (C) Bursal chamber
- (D) Chitinous plate bordering the bursal opening

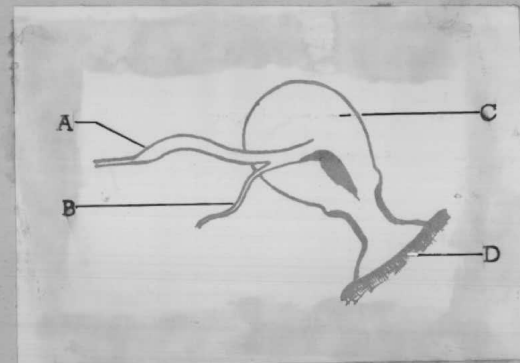
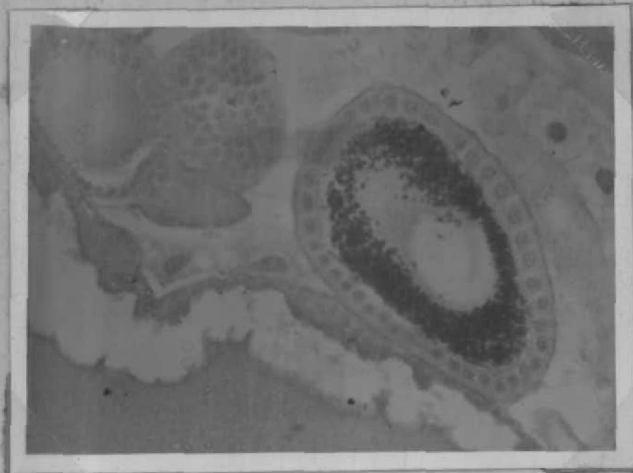


Fig. 45



Section through one egg chamber
showing yolk deposition. Yolk
granules stained with the Triple
stain for Polysaccharides
(Reddish granules); Protein (yellow)
and D.N.A. (violet)

