J. Hyg., Camb. (1979), **83**, 521
Printed in Great Britain

Laboratory breeding of the European rabbit flea, *Spilopsyllus cuniculi* (Dale)*

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(*Received* 1 *February* 1979)

SUMMARY

A method is described for the laboratory breeding of the rabbit flea in which the immature stages are reared at constant temperature and humidity.

Eggs are obtained by confining fleas taken from a rabbit and her nest shortly after parturition with two of her nestlings in an incubator for 24 h. The eggs are transferred to an artificial diet medium on which the immature stages are reared. On average a female flea produces 50 eggs during the first six days post-partum. At 25 °C, 95% of eggs hatched at 79% RH and 98% at 84% RH. Most eggs hatched on the third day after laying and hatching was completed by the fourth day. Significantly more fleas of both sexes were obtained when larvae were reared at 25 °C on a medium containing powdered 41B rodent diet than on one containing terrier meal. Both diets also contained yeast and dried rabbit blood. There was no significant difference between the numbers of fleas obtained at 79% RH and 84% RH. Significantly more fleas were also obtained when larvae were reared at 27 °C, 84% RH, than at 25 °C. Female fleas emerged sooner than males at both 27 °C and 25 °C. Fleas from the laboratory culture were heavier than those from wild nests. Female fleas were heavier than male fleas in both cases.

INTRODUCTION

A steady supply of the rabbit flea, *Spilopsyllus cuniculi*, is needed at this laboratory for studies on its role as the major vector of myxomatosis in Britain. Fleas have been obtained mainly from wild rabbit nests collected throughout England and Wales and also from carcasses of wild rabbits placed in fleaproof bags immediately after being shot. Supplies from both sources tend to fluctuate as the first is limited to the breeding season of the rabbit and the second is affected by the marked seasonal variation in the numbers of fleas on rabbits (Mead-Briggs, Vaughan & Rennison, 1975). A laboratory culture reduces dependence upon these sources, providing a means of smoothing the fluctuations or of supplementing the supply when demand is heavy.

The usual method of culturing fleas is to confine adults with a host, provided with food and water, on or above a suitable rearing medium for the larval and pupal stages. This technique, with variations in details, has been used successfully

0022–1724/79/0012–1979 $01.00

for a number of species of mammal fleas (Leeson, 1932; Edney, 1945; Linduska & Cochran, 1946; Elbel, 1951, 1952; Burroughs, 1953; Hudson & Prince, 1958*a*, *b*; Krishnamurthy, 1966). This method requires modification for *S. cuniculi* as the reproduction of this flea is dependent upon the breeding of its host (Mead-Briggs & Rudge, 1960). Both sexes of flea are influenced by factors from the pre-partum female rabbit and the newborn young during their first few days of life (Mead-Briggs 1964*a*, Mead-Briggs & Vaughan 1969). Normally, ovarian maturation occurs only in fleas feeding on a pre-partum rabbit during the last 7–10 days of pregnancy or on newborn young during their first 7 days of life. Male fleas require probing contact with rabbits in the final stages of pregnancy or with newborn young before they can mate and successfully inseminate females; at parturition most fleas leave the female rabbit and enter the nest where they feed avidly upon the young, copulate, and the females lay eggs. It has been shown that the different stages in this sequence of events are influenced by changes in the concentrations of certain hormones in the bloodstream of the host and that an airborne factor from nestlings boosts copulation and impregnation of fleas (Rothschild & Ford, 1964, 1966, 1973; Rothschild, Ford & Hughes, 1970).

Sobey, Menzies & Conolly (1974) successfully cultured rabbit fleas by infesting pregnant rabbits and then allowing the larvae to develop in the maternal nest, i.e. by simulating the natural sequence of events as closely as possible. This method gave variable results in our hands, possibly because an animal house with a controlled environment was not available. Larvae were found in the nests, but the average yield of adults was low. It had been shown previously that rabbit flea larvae could be extracted from a nest and then reared successfully on an artificial medium comprising a diet of yeast, bran and dried rabbit blood in fine sand (A. R. Mead-Briggs, unpublished). In the method we have developed eggs are obtained and then transferred to an artificial medium on which the immature stages are reared.

MATERIALS AND METHODS

Eggs are obtained by confining fleas taken from a domestic rabbit and her nest shortly after parturition with two of her nestlings in a small box for 24 h. Mating takes place in the presence of the nestlings and the fertile eggs are laid in batches adhering to paper lining the box. The eggs are then transferred to the larval medium which is kept at a controlled temperature and humidity until adults emerge. Rabbit nestlings can be removed from the nest for 24 h without risk, provided they are kept warm in an incubator, as they are fed by the mother only once every 24 h and receive their first feed shortly after birth. No deaths occurred during the experiments nor were any nestlings rejected by the mother after being returned to the nest.

Egg-laying

New Zealand White rabbits are mated each week to provide litters and matured fleas. The rabbits are infested with 50–75 fleas of each sex at least 10 days pre-partum, and provided with a metal nestbox containing sawdust and hay 2–3 days

before the expected date of parturition. As soon as possible after parturition any fleas remaining on the female are combed off, added to those recovered from the nest and nestlings and the combined group sexed and counted. A glass-topped museum box $15 \times 10 \times 5$ cm with a folded paper towel placed in the bottom to absorb urine is lined with green blotting paper; coloured paper is used as the pearly white eggs are more easily seen against a tinted background. Two nestlings are selected from the litter, placed in the box and 30–50 fleas of each sex released onto them. The lid is quickly replaced, sealed with cloth tape to prevent the fleas escaping and the box placed in an incubator at 25 °C for 24 h in the dark.

After 24 h the fleas are aspirated into a $7{\cdot}5 \times 2{\cdot}5$ cm tube and kept in case further batches of eggs are required. The nestlings are marked so that they are not used again and returned to the nest; if further egg-batches are required the fleas are set up again in another box with a fresh pair of nestlings. In experiments made to investigate daily egg production a second box containing one nestling and spare matured fleas is set up each day if sufficient nestlings are available. Any losses of fleas from the experimental box are replaced each day from these spares. If there are insufficient nestlings to provide a fresh pair for the experimental box each day a pair is used for a second 24 h period only after an interval of at least two days in the nest.

Diet medium

The egg-batches are cut from the lining on slips of paper, grouped into lots containing 50 eggs and placed on the larval rearing medium, which is made up of 2 g larval diet and 2 g heat-sterilized sawdust thoroughly mixed, in a 100 ml beaker. Two larval diets have been tested, the first made up of finely ground dog biscuit, dried de-bittered brewer's yeast and powdered dried rabbit blood in the ratio of 5:1:1 parts by weight. In the second diet the dog biscuit is replaced by an equal weight of powdered 41B rodent meal. The dog biscuit, rodent meal and yeast are obtained from commercial sources. The blood is prepared by drying a thin film taken from a rabbit immediately after death at 40 °C for 3–6 h and then grinding the film finely in a pestle and mortar. Blood dried at a higher temperature forms a hard film which is difficult to grind and was associated, in early experiments, with reduced yields of fleas, suggesting that the particles were either too large or too hard for the larvae to ingest. The adverse effects arising from the use of baked blood have been reported previously (Sharif, 1937; Edney, 1947). The diets and sawdust are kept over saturated salt solutions for 24–48 h to equilibrate with the humidities used for rearing the immature stages.

Rearing conditions

Rearing is carried out at 25 °C or 27 °C and at 79 % or 84 % RH, humidity being controlled by saturated solutions of KBr or KCl respectively. The beakers containing the larval rearing medium stand in a plastic food storage box on a perforated sheet of Perspex which is supported above two dishes each containing 60 ml of saturated salt solution. Humidity is measured with cobalt thiocyanate paper, compared against permanent colour standards (Solomon, 1957). The boxes are

Table 1. *The mean number of eggs/flea/day laid by groups of rabbit fleas during the first six days after parturition of the host*

	Age of nestlings (days)					
	1	2	3	4	5	6
Mean no. of eggs/flea	18·3	14·1	8·5	4·7	3·4	1·0
S.E. ±	1·51	1·16	1·32	1·06	—	—
Range	11·2–29·1	5·9–22·0	2·4–17·4	0·6–11·5	—	—
n	15	15	15	14	2	2

Table 2. *The hatching success and % of hatch/day of rabbit flea eggs at two humidities at* 25 °C

	Humidity	
	79% RH	84% RH
	(10 batches of 10 eggs at each humidity)	
Total % of eggs hatched ...	95	98
Days after laying	% of hatch/day	% of hatch/day
2	8·4	1·1
3	89·4	93·8
4	2·1	5·1

sealed inside polythene bags as the lids are not airtight, and are held in incubators at the required temperature.

Adult collection

In experiments on adult production, the beakers are covered with squares of nylon organdie secured with rubber bands when pupation starts 8–10 days after egg-lay. They are then checked daily from the 15th day after egg-lay to record the date of first emergence. Newly emerged adults are removed from the beakers, sexed, counted and stored at 4 °C, 95% RH until required.

RESULTS

Egg production and hatching

Table 1 gives the results of experiments investigating the daily rate of flea egg production during the first six days after parturition of the host. As it was not practicable to obtain eggs from individual fleas the number of eggs laid during each 24 h by several replicate groups of 30–68 female fleas was used to calculate the daily means and SEs. The results show that during this period each flea produces on average 50 eggs of which 64·8% are laid during the first 48 h. The rate of egg production is highest on day 1 and declines steadily until day 6 by which time egg-lay has nearly ceased.

Hatching success and the proportion of the hatch occurring on each day after

https://doi.org/10.1017/S0022172400026371 Published online by Cambridge University Press

Table 3. *The mean numbers of ♂ and ♀ fleas obtained at 25 °C on two larval diets and at two humidities from batches of 50 eggs*

	Diet							
	Terrier meal				41B rodent meal			
% Relative humidity	79		84		79		84	
Sex	♂	♀	♂	♀	♂	♀	♂	♀
Mean no. of adults	11·9	8·9	11·7	10·2	17·0	11·1	14·5	13·1
S.E. ±	1·39	1·24	1·26	1·60	0·97	0·92	1·01	1·37
n	13		13		13		13	

Analysis of variance of √ transformed numbers of ♂ and ♀ fleas obtained from each batch of 50 eggs

Source of variance	df	M.S.	F	
Between diets	1	7·3140	13·6269	$P < 0·001$
Between sexes	1	5·3645	9·9947	$P < 0·01$
Between humidities	1	0·0527	0·0981	ns
Sexes × humidities	1	0·9386	1·7487	ns
Humidities × diets	1	0·1191	0·2220	ns
Sexes × diets	1	0·1219	0·2270	ns
Error	97	0·5367		

laying were investigated using 10 batches of 10 eggs at both 79% and 84% RH at 25 °C. The results, given in Table 2, show that at both humidities few eggs failed to hatch and that hatching began on the second day after laying, most frequently occurred on the third day and was complete after four days. It was found, using the Mann–Whitney U-test, that the daily percentage hatch did not differ significantly between humidities ($P > 0·05$). Subsequent checks on mass rearing experiments involving several hundred eggs showed an average hatch of 95%.

Comparisons of rearing conditions

Table 3 shows the average number of ♂ and ♀ fleas obtained when larvae from batches of 50 eggs were reared at either 79% or 84% RH on the alternative diets described above. The numbers of ♂ and ♀ fleas obtained under each set of conditions were compared by analysis of variance after transformation to square roots. The appropriate transformation was selected by the method of Jeffers (1960). The analysis showed that there was a highly significant difference between diets ($P < 0·001$) and a significant difference between sexes ($P < 0·01$) but no significant difference between humidities. None of the interactions was significantly large, showing that the difference between diets was the same for both sexes and at both humidities and similarly that the difference between sexes was the same on both diets and at both humidities. Examination of the means in Table 3 shows then that significantly more fleas were obtained on the larval medium containing 41B rodent

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Table 4. *The mean numbers of ♂ and ♀ fleas obtained from batches of 400 eggs reared at 84 % RH and 25 or 27 °C on diet containing 41B rodent meal*

	Temperature			
	25 °C		27 °C	
Sex	♂	♀	♂	♀
$\bar{x}$	145·0	143·8	163·1	154·3
S.E. ±	5·37	5·94	6·93	7·06
n	10		10	

Analysis of variance of √ transformed numbers of ♂ and ♀ fleas obtained from each batch of 400 eggs.

Source of variance	df	M.S.	F	
Between temperatures	1	3·2833	4·966	$P < 0·05$
Between sexes	1	0·4162	< 1	ns
Temperatures × sexes	1	0·2220	< 1	ns
Error	36	0·6612		

meal. The mean productivity, measured as the percentage of eggs completing development to adults, was 55·7 % on the 41B rodent diet compared with 42·6 % on the terrier meal diet. Significantly more males than females were obtained with an average ratio over both diets and both humidities of 1:0·78.

Table 4 gives the results of a set of experiments comparing the numbers of fleas of each sex obtained when the rearing conditions were 84 % RH and 25 or 27 °C. The numbers of fleas, from four batches of 100 eggs in each replicate, were again compared by analysis of variance after transformation to square roots. The mean productivity was 72·5 % at 25 °C and 79·25 % at 27 °C. The analysis showed that the difference between numbers of fleas produced at the two temperatures was just significant ($P < 0·05$) and that the temperature × sexes interaction was not significant. Therefore, significantly more males and more females were obtained when rearing was done at 27 °C than at 25 °C. In contrast to the results of Table 3, the difference between numbers of males and females was not significant; the non-significant interaction showed that this applied at both temperatures. The cumulative flea counts obtained during these experiments were used to calculate the results given in Table 5. This Table gives the number of days elapsed at 25 °C and 27 °C from egg-lay to first emergence of adults and to emergence of 50 % and 90 % of the total number obtained of each sex. As the fleas were collected at 48 h intervals the results are only correct to ± 1 day. The results show that at both temperatures 50 % and 90 % emergence occurred first for females. The results of Table 5 also indicate that the development of both sexes proceeded more quickly at the higher temperature. The larval rearing medium containing 41B rodent meal has been adopted as standard for the laboratory culture, at 84 % RH and 25 or 27 °C. The average productivity under these conditions during a period of 20 months was 65·7 %, with a ♂:♀ ratio of 1:0·95.

The weights of fleas reared in the laboratory culture were compared with those

https://doi.org/10.1017/S0022172400026371 Published online by Cambridge University Press

Table 5. *The time taken from egg-lay to first emergence, 50 % and 90 % emergence of adults when reared at 25 or 27 °C, 84 % RH on diet containing 41B rodent meal*

	Temperature			
	25 °C		27 °C	
Sex	♂	♀	♂	♀
Mean no. of days to 1st emergence	17		16	
50 % emergence	24	20	22	19
90 % emergence	30	28	28	26

obtained from wild nests by taking the mean weights of 5 batches of each sex from each source. Each batch contained 14–50 fleas which had emerged overnight; the total numbers weighed were 148 ♂ and 168 ♀ from the laboratory culture and 191 ♂ and 228 ♀ from wild nests. The mean weight of males from the culture was 0·29 mg and of females 0·38 mg, compared with 0·27 mg for wild males and 0·31 mg for wild females.

DISCUSSION

Experience with the technique described has shown it to be a successful laboratory method for producing a regular supply of fleas using limited facilities. A standard diet has been developed which gives a consistent yield of fleas reared at constant temperature and humidity. Taking average weight as the criterion, the fleas from the culture are at least comparable to those obtained from wild rabbit nests brought into the laboratory and kept in polythene bags until adults emerge; female fleas are heavier than males from both sources. The finding, obtained from Table 1, that the majority of eggs are laid during the first two days post-partum indicates that little is gained by setting up fleas with older nestlings. The average of 32·4 eggs per flea during days 1 and 2, the average development to adults of 65·7 % and the mean number of successful litters per month can be used in conjunction with the average loss of fleas pre-partum to estimate the input in terms of numbers of fleas placed on mated does required for a given monthly output.

The times for the duration of development at 27 °C, 80 % RH, given in Table 5, are similar to those obtained for three species of *Xenopsylla* by Sharif (1949). The time of 50 % emergence of *S. cuniculi* reared in batches was, for males, 22 days with a range of developmental duration of 16–28 days and for females 19 days, range 16–26 days. By comparison Sharif obtained mean development times of 22·7 days, range 21–26 for male *X. cheopsis* and 18 days, range 15–20 for females; for *X. brasiliensis* the times were 25·7 days, range 24–27 for males, and 20·2 days, range 18–22 for females, and for *X. astia* 24·9 days, range 23–27 for males and 19·4 days range 16–22 for females. *S. cuniculi* resembles the three *Xenopsylla* spp. in that females have a shorter mean development time than males, but differs in that the emergence periods of the two sexes overlap whereas in all three *Xenopsylla* spp. emergence of females is complete before males begin to emerge.

The laboratory breeding method of Sobey *et al.* (1974) was developed for the mass production of fleas for release on wild rabbits in Australia, whereas our method is designed to produce a comparatively small number of fleas reared under constant conditions. Both methods still require that fleas have access to pregnant does and subsequently to newborn nestlings for eggs to be produced. Sobey *et al.* used the multiplication rate, expressed as the number of fleas obtained divided by the number seeded onto does, as a measure of the output of their culture. They achieved an average multiplication rate of 3·6 over 4 years with the monthly averages varying between 0·2 and 10·5 compared with an average rate of 2·4 over 2 years with a monthly range of 0·6 to 5·0 for our culture in which the highest weekly rate achieved was 9·3. The lower average rate in our culture arose partly because for much of the period the number of eggs set up each week was limited to 800 although more eggs than this were laid instead of the current maximum of 1600, so restricting the output artificially.

The relatively low multiplication rate achieved in both culture methods is in marked contrast to results obtained from laboratory cultures of other flea species. Hudson & Prince (1958*a*) obtained an average yield of 194 pupae $♀^{-1}$ from their colony of *Ctenocephalides f. felis* during the first 22 days after emergence of the parental adults. The mortality of parental adults during this period was high. only 24% of females being recovered after 22 days. The average egg production was estimated at 28 eggs $♀^{-1}$ day^{-1} compared with our result for *S. cuniculi* of 50 eggs $♀^{-1}$ during the whole of an egg-laying period. The same authors obtained yields of 6·4–10·3 pupae $♀^{-1}$ wk^{-1} for a human strain of *Pulex irritans* and 11·0 pupae $♀^{-1}$ wk^{-1} for *P. simulans* (Hudson & Prince, 1958*b*). Our results show that the low multiplication rate of *S. cuniculi* in culture arises mainly from the comparatively small number of eggs laid rather than from heavy mortality during development. In addition to the contrast in multiplication rate *C. f. felis*, *P. irritans* and *P. simulans* differ markedly in life-style from *S. cuniculi*. The first three species are able to make use of a range of hosts and are all nest fleas, the adults spending relatively little time on the host. The mortality rate of these species in culture indicates that few reproduce more than once. *S. cuniculi* on the other hand is a monoxenous semi-sedentary flea having *Oryctolagus cuniculus* for its host although it may occur accidentally on hares, cats and foxes. Its reproduction is linked to that of one sex of its host and it is possible that *S. cuniculi* reproduces more than once, as the parental generation fleas return to the female rabbit from 7 days post-partum onwards.

The restriction imposed on *S. cuniculi* by the dependence of its reproduction upon that of its host is offset by the advantage that its eggs will be laid in the maternal nest of the rabbit (the only nest made by the species) in a favourable environment for larval and pupal development. Newly emerged adults show marked host-finding behaviour and a high proportion are likely to reach a host (Mead-Briggs, 1964*b*; Vaughan & Mead-Briggs, 1970). Host finding is necessary as rabbits less than four months old, which are those likely to be still using the nest burrow, appear to be unattractive to fleas and carry low numbers only (Rothschild & Ford, 1966). Hence an individual egg of *S. cuniculi* may have a

greater probability of giving rise to a reproductive adult by comparison with the other three species. Consequently the species can be maintained with a smaller number of eggs per female and a lower multiplication rate.

We wish to thank Dr A. R. Mead-Briggs for his encouragement and advice throughout this work and also Miss L. Terry, Miss P. Housden, Miss S. Boyce, Mr R. Fitzjohn, Mr C. Bain and Mr B. Crook for technical assistance. We are indebted to Mr B. D. Rennison for statistical advice and computation.

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