TECHNIQUES FOR REARING AND HANDLING
BODY LICE, ORIENTAL RAT FLEAS, AND CAT FLEAS

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SYNOPSIS

The authors describe techniques for handling and rearing large numbers of body lice (Pediculus humanus humanus L.), oriental rat fleas (Xenopsylla cheopis (Rothschild)), and cat fleas (Ctenocephalides felis felis (Bouché)).

Body lice may be fed on man or on domestic rabbits. In the latter case, the lice are kept on woollen patches in glass dishes at 30°C and 60% relative humidity. The patches are placed on the clipped belly of a rabbit once a day and the lice allowed to feed. Eggs are deposited on the patches, and the adult lice are transferred to new patches every two or three days.

The adults of oriental rat fleas are fed on white rats, and the larvae are reared in a medium of dry sand and powdered beef-blood. Cocoons are placed in emergence funnels from which pure cultures drop into collecting jars. Cat fleas are raised in a very similar manner, but the adults are fed on dogs.

The body louse (Pediculus humanus humanus L.), the oriental rat flea (Xenopsylla cheopis (Rothschild)), and the cat flea (Ctenocephalides felis felis (Bouché)) have been reared in large numbers at the Orlando, Florida, laboratory of the Bureau of Entomology and Plant Quarantine for use in studies of insecticides and insect repellents. Certain aspects of the methods employed have been in general use by entomologists for some time, but all the methods have been modified at Orlando to produce large numbers of insects of uniform age and vitality with a minimum of handling. The most successful procedures are presented here in the hope that their publication may save time and effort for other workers seeking to establish and maintain colonies of these species.

The Body Louse

Several techniques have been described for rearing the body louse on either a small or a large scale. It is not a difficult insect to rear. A good
colony can usually be produced if the lice are allowed to feed on man once or twice daily and if reasonable care is given to rearing conditions. Although man is the only natural host of the body louse, it has been reared successfully on domestic rabbits. The rearing technique is usually determined by the particular needs of the individual or laboratory. Some of the more important or commonly used procedures are discussed in the following pages.

Rearing on man

Much of the biology of the body louse has been worked out by rearing lice in pill boxes fastened to the arms or legs of human subjects. The lice feed through a fine-mesh cloth covering a hole in the bottom of the pill box. The boxes are usually worn during the day and removed at night. Metal or paper boxes may be used, but metal boxes require a ventilator, protected with gauze, on the side away from the skin. Several methods of this type are discussed by Buxton.1

The pill-box methods are not considered practical for rearing large numbers of lice, but they are useful for conducting various biological studies and also for producing sufficient lice for small-scale tests of insecticides.

Moore & Hirschfelder 6 were among the first to demonstrate a laboratory method of rearing body lice in fairly large numbers. They kept the lice on cloth in glass containers at approximately 86°F (30°C). The lice were fed twice daily on the arms of volunteers, and up to 4,000 lice were fed on a single individual.

The methods used by Moore & Hirschfelder were much improved by Culpepper.2 3 The lice were fed twice daily and held in an incubator at 30°-32°C and at a relative humidity of approximately 60%. By this method hundreds of thousands of eggs were produced for experimental purposes from May 1942 to October 1943, thus supplying average daily requirements of over 1,000 lice and eggs during this period. The technique as described by Culpepper 2 is as follows:

"The lice are kept on loosely woven patches of woolen suiting cut approximately 1½ inches square with pinking shears. The preferred color is dark blue or black, since both lice and eggs are more readily visible on dark than on pastel shades. In addition to furnishing favorable footing, loosely woven patches adhere to one another better than smoothly woven or had-finished materials, which is an aid to the technician in the feeding process.

Each patch of cloth supports approximately 100 adults. Obviously the smaller the lice, the more can be maintained on a cloth patch. The patches with the lice are kept in crystallizing dishes 8 to 10 inches in diameter... Forty to 60 patches containing a total of 4,000 to 10,000 lice, depending on the size of the lice, are carefully placed so they will cover a circular piece of half-inch-mesh hardware cloth cut to fit the bottom of the dish. The wire meshes hold the patches off the bottom of the dish so that the parasites
have freedom of movement on both sides of the cloth. Crystallizing dishes are used for
the reason that lice cannot crawl up the sides of glassware.

"The colony is separated into lots according to age by removing the adult lice from
the egg patches every 48 hours to assure a continuous supply of eggs for rearing the
colony. The separated egg patches become a new egg lot, and are placed in a beaker of
appropriate size, on which the date is labeled so that the time of hatching may be
anticipated.

"After the eggs hatch, the nymphs are allowed to remain on the same cloth patches
until they become adults, when they are transferred to clean patches. When the nymphs
become crowded as they increase in size, additional patches are added to the lot. If the
lice are maintained on the same cloth during nymphal life, it becomes soiled with molted
skins and excreta, which necessitates cleaning. Cleaning is accomplished by first spreading
the cloth patches containing the lice in a shallow pan and drying them by use of an
ordinary electric lamp. The dry patches are then rubbed lightly together with the hands,
and the debris is shaken out while the lice hold to the cloth. In addition to the patches,
all glass containers are cleaned at regular intervals.

* The lice are fed on research subjects, who are used also for other assignments
connected with the projects. As nearly as practicable, the colony is fed at 12-hour
intervals, once in the early morning and once in the late afternoon. The feeding process
consists of placing the cloth patches containing the lice systematically across the back
of the subject, leaving a space approximately 1 inch wide between the different lots. After
the cloth patches are placed on the subject, most of the adult lice immediately go under
the cloth and begin feeding... the entire operation, which includes placing and removing
the lice and dressing the subject, is finished within a period of 30 to 45 minutes. As
many as 30,000 to 40,000 lice may be fed ... at the same time on the back of one subject.
As many as 100,000 lice and an equal or greater number of eggs have been carried in
the colony at one time. With few exceptions, the research subjects have not been required
to feed the colony more often than once per week. In four instances, however, research
subjects voluntarily fed approximately 40,000 lice each on two successive days without
any apparent ill effect. Two subjects have been observed sleeping soundly during the
process of feeding the lice.

"There is a wide difference in individual reaction to louse feeding. Some subjects
display no visible reaction and experience only a slight, transient sensation of itching,
while others develop from slight to conspicuous dermatitis, which persists for two or
three days. Relatively few subjects... who have been employed to feed the lice reacted
so severely that they could not serve again in this capacity, but all apparently were normal
in 7 to 10 days."

Rearing on domestic rabbits

Attempts to rear body lice on animals other than man have usually
met with little success. Davis & Ilansens 3 succeeded in rearing two
generations of lice on rabbits, but failed to produce a thriving colony.
Culpepper 3, 4 reared body lice on rabbits with very good success.
The body-louse colonies at Orlando are now reared exclusively on domestic
rabbits. Human subjects are used only in starting new colonies from lice
collected in nature or for special studies. Although the size of the main
colony varies from time to time, depending on the demand, at present it
consists of several hundred thousand nymphs, adults, and eggs. This
number is maintained by feeding the lice only once each day on only eight
rabbits. The method of rearing given below is otherwise essentially the same as that described by Culpepper.4

The lice are kept on patches of loosely woven woollen suiting approximately 4-cm square. Blue or black cloth is used, since both lice and eggs are more visible on dark than on light backgrounds. The patches are kept in beakers or in petri dishes and held in cabinets at a constant temperature of about 30°C and at a relative humidity of 60% or less.

**FIG. 1. FEEDING BODY LICE ON CLIPPED BELLY OF RABBIT**

The cloth patches have been removed from the rabbit's left side to reveal the attached lice. After feeding, or when disturbed by rubbing with the patches, the lice cling to the cloth and may be easily picked up with the patches.

Lice are fed by placing the patches on the ventral side of the rabbits immobilized on their backs in specially designed stanchions (fig. 1). The entire ventral area is closely clipped with fine electric clippers before the patches are placed on the rabbit.

The ventral area of the average rabbit will accommodate 16 to 22 patches, each bearing 100 to 200 lice. Several such lots can be fed in succession on the same rabbit. One rabbit is capable of supporting a colony of this size for several weeks without apparent ill-effects, but it is advisable to rest each rabbit for several days after each feeding. At the Orlando laboratory rabbits are used once every 4 days.
Tests should be run with a number of rabbits to determine which are suitable louse hosts. The favourable and unfavourable hosts usually can be determined after a single feeding. As a rule, on favourable hosts the lice attach and feed to repletion in 12 to 20 minutes, whereas on the unfavourable ones they attach more slowly and require 15 to 30 minutes for repletion. The mortality of lice feeding on favourable rabbits is negligible but may be high on unfavourable ones. A stock of favourable rabbits may be ensured by mating and rearing the offspring of favourable males and females.

Starting new colonies of body lice

Body lice collected in nature vary considerably in their ability to adapt themselves to a new environment. Some new colonies have been established with very little effort, whereas others have been lost completely without any apparent reason. Newly collected lice are usually rather "wild" and reluctant to feed, and those that do feed require much more time for engorgement than do lice from an established colony. The lice should be fed at least twice daily until the colony is well established; then one or two feedings may be given each day, depending on the size of the colony needed and other requirements. In establishing a new colony it is important to make sure that the lice are given sufficient time to engorge fully. Partial blood-meals do not produce thriving colonies. If rabbits are to be used or are desired as hosts, all new colonies should be established first on men and then transferred to rabbits.

Collecting and shipping body lice

The manner in which lice are collected and shipped has an important bearing on the success of colonization. When lice are desired by a certain group or laboratory, the sender sometimes ships an entire suit of clothing containing only a few lice and eggs, and frequently the nymphs and adult lice are dead upon receipt. Moreover, newly hatched nymphs are very difficult to recover from whole garments.

It is therefore best to collect adults, if they are available in sufficient numbers, and to confine them on a minimum amount of clean, dark cloth so as to obtain eggs. The lice should be offered a blood meal immediately after collection, placed on patches, and held in an incubator at 32°-34°C and at a relative humidity of not more than 75%. This temperature is higher than normal, but will serve to increase egg production. Feeding the lice two or three times a day is essential to maximum oviposition. Every two days the patches containing the eggs should be removed and shipped, and the lice placed on new patches.

Where plenty of adults are available, adults and eggs can be shipped every two days, but it is advisable to give the adults a good blood-meal just
before shipping. If this is done, the adults will continue to lay for 24-48 hours before dying of starvation.

The Oriental Rat Flea

Adult oriental rat fleas are fed on white rats, and the larval rearing medium is a mixture of sand and powdered beef-blood. Because the gravid female fleas leave the host to oviposit and return for additional blood-meals, the infested rat is confined in a screen cage, which is kept in a pan containing the larval medium.

FIG. 2. PANS FOR REARING ORIENTAL RAT FLEAS

The pan shown on the left contains the caudal rat ready to be infested and enclosed with the cloth cover in the left foreground.

The rearing pan (fig. 2) is made of heavy galvanized iron, 35 cm in diameter and 20-cm deep. About 100 cm³ of powdered beef-blood are mixed thoroughly with 1.5 litres of dry sand that has been passed through a 16-mesh screen, and the mixture is spread evenly over the floor of the pan to form a layer 3-6 mm in depth.

The cage is 18.5-cm long, 8.25-cm high, and 8.25-cm wide, and is made of 4- or 2-mesh galvanized-iron screen. One end is hinged at the bottom to form a door, which is held closed by wire hooks.
The cage containing the rat is placed in a tray on the sand in the centre of the pan. The tray is made of lightweight galvanized iron, and is 21-cm long, 9.5-cm wide, and 1.25-cm deep. The floor of the tray is covered with blotting-paper, to absorb the urine and to allow it to evaporate without wetting the sand in the pan. The blotting-paper is changed twice a week.

About 500 newly emerged adult fleas are released on the rat, and the pan is covered with bleached muslin held in place with an elastic band. The female fleas leave the rat to oviposit, and lay most of their eggs in the sand. A few are laid on the blotting-paper, but the number lost when the paper is changed is negligible.

FIG. 3. UNIT FOR EMERGENCE OF ORIENTAL RAT FLEAS

The unit is shown assembled on the right.

The pan is kept in an air-conditioned room at a temperature of 27°C and at a relative humidity of 80%. The rat is fed daily a piece of apple and a few pieces of dry commercial dog-food. After 21 days the rat is removed from the pan, disinfested, and placed in a large cage for a resting period of several weeks before being reinfested.

By this time, the female fleas have deposited about 90% of their eggs, and adults of the second generation are almost ready to emerge. The sand containing eggs, larvae, and pupae is also removed from the pan and sifted through 8- and 16-mesh screens. The coarser screen removes any large debris, and the finer removes the cocoons.
The remaining sand, containing eggs and larvae, is placed in a pupation pan. This is an enamelled or porcelain pan, 28-cm in diameter and 10-cm deep, covered with bleached muslin. Once a week the sand is sifted through a 16-mesh screen to remove the cocoons. When no more cocoons are found, usually after three or four weeks, the sand is discarded.

The cocoons are then kept in emergence units (fig. 3). Each unit consists of a funnel, a basket, wire hooks, a collar, a cover, and a collecting jar. The funnel is an inverted glass pickle-jar from which the bottom has been cut. The jar should be at least 16.5 cm in diameter and 23-cm deep, and the mouth should be designed for closure with a screw-cap 7 cm in diameter (the size of a fruit jar). The cocoons are placed in the basket, which is 12 cm in diameter and 4-cm deep and is made of 16-mesh screen wire reinforced by a ring of 4-mesh hardware cloth. The basket is suspended from the rim of the funnel by three wire hooks. The collar is made of two of the ring portions of fruit-jar caps, soldered together. The funnel is screwed into one ring and the collecting jar into the other. The collecting jar is a fruit jar with a disk of paper towelling in the bottom, and the cover is of bleached muslin held in place with an elastic band.

Collecting jars can be changed as often as necessary to obtain fleas of a desired age. If a fresh jar is placed under a unit at the peak of emergence and the cocoons are gently breathed on through the cloth cover to stimulate emergence, several hundred fleas will be obtained in a few hours.

Each rearing pan will produce from 1,000 to 5,000 fleas. Production sometimes declines during the winter months, even though the temperature and humidity are kept constant.

The Cat Flea

The method of rearing cat fleas is very simple. Dogs are confined during the day in a small yard infested with fleas. From 5 p.m. to 8 a.m. the flea-infested dogs are confined in cages about 1.2 metres square. The floor of each cage is of 2-mesh hardware cloth, with about one-third covered by a board on which the dog sleeps. About 2.5 cm below the floor is a sliding, removable, plywood panel, the full width of the cage. Flea eggs fall through the mesh floor on to the panel. Every two days, the panel is removed, and the eggs and debris are brushed off into a dish and sifted through 16-mesh screen. The eggs and debris that pass this screen are placed in an enamelled or porcelain pan, 28 cm in diameter and 10-cm deep, containing about 1.5 litres of dry sand mixed with 100 cm³ of powdered beef-blood. These rearing pans are kept at a temperature of about 27°C and at a relative humidity of about 80%. Under these conditions pupation begins after 10-13 days and the emergence of adults after 13-15 days. After the tenth day the sand in the dishes is sifted through 16-mesh screen twice a week to remove the cocoons. These cocoons are placed in emergence units, and the
The elements are, from left to right: jar, threaded collar, metal ring with attached rubber gasket, glass funnel, and plastic tubing and clamps.
newly emerged cat fleas are collected in the manner described for the rat fleas.

Handling Adult Fleas for Testing

Fleas can be removed from the collecting jar in any desired number by fitting the jar with a glass funnel opening into a short piece of transparent, flexible, plastic tubing, as illustrated in fig. 4 and 5. The glass funnel should have a maximum outside diameter of 65 mm. It should be seated on a metal ring, with attached rubber gasket, made by cutting the centre from the disk portion of a standard metal two-piece fruit-jar lid. The ring and funnel make a flea-proof closure when fastened to the jar with the threaded metal collar, which is the collar portion of a standard glass two-piece fruit-jar lid. The threaded collar of a metal two-piece lid is too short to grip the threads of the jar securely when the funnel is in place.

FIG. 6. FLEAS ISOLATED IN TRANSPARENT TUBING BETWEEN TWO CLAMPS FOR COUNTING

Fleas are shaken through the funnel into the plastic tubing and isolated between two clamps for counting, as shown in fig. 6. When the desired number have been isolated, the distal clamp is removed and the fleas are released into the test chamber.
RÉSUMÉ

L'auteur décrit les techniques employées avec succès pour l'élevage en grand nombre du pou de vêtement (*Pediculus humanus humanus* L.), de la puce indienne du rat (*Xenopsylla cheopis* (Roths.),) et de la puce du chien (*Ctenocephalides felis Bouché*).

Bien que l’homme soit le seul hôte naturel de *P. h. humanus*, l'élevage sur le lapin a donné des résultats satisfaisants. Les poux sont placés dans des boîtes de verre, sur des morceaux de tissu de laine, à 30° C et 60 % d’humidité relative. Une fois par jour, les morceaux d’étoffe sont placés sur le ventre rasé d’un lapin, pendant 20-30 minutes, afin que les insectes puissent se nourrir. Les œufs sont déposés par les femelles sur ces morceaux de tissu ; ces derniers sont nettoyés tous les 2-3 jours après que l'on en a retiré les poux.

Les poux indiens du rat, adultes, sont nourris sur des rats blancs ; les larves sont élevées sur un milieu composé de sable sec et de sang de bœuf. Les rats infestés sont maintenus dans des cages de treillis métallique, posées sur des plateaux de métal au-dessus du « milieu de culture » des larves, de sorte que les femelles gravides peuvent déposer leurs œufs sur le sable et retourner sur les rats. Les pupes sont aspirées à la pipette et placées dans des entonnoirs d’où les puces adultes, en « cultures pures », tombent dans les flacons, où elles seront prélevées pour les tests. On procède de façon analogue pour les puces du chat, si ce n'est que les cages contenant l'animal-hôte (le chien) ne se trouvent pas directement au-dessus des sables. Les œufs sont aspirés à la pipette et placés dans un milieu convenable. Pour faciliter le dénombrement et le maniement des puces adultes, on peut adopter aux flacons où elles sont recueillies lors de l'éclosion, un entonnoir de verre, un tube de plastique transparent et flexible, et deux pinces.

Références

5. Davis, W. A. & Hausch, F. J. (1945) *Amer. J. Hyg.* 41, 1