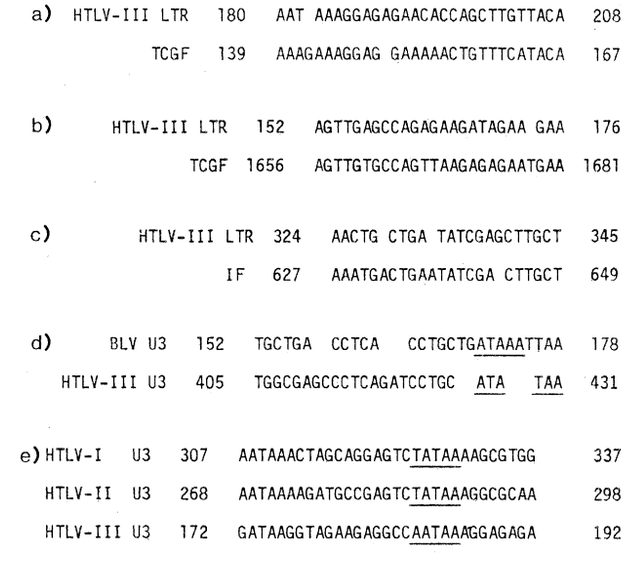

Fig. 4. (a) Sequence homology between the HTLV-III LTR and the TCGF gene. (b) Sequence homology between the HTLV-III LTR U3 region and the TCGF gene. (c) Sequence homology between the HTLV-III LTR U3 region and human γ-IF. (d) Sequence homology between HTLV-III U3 and BLV U3 regions. (e) Sequence homologies among the U3 regions of HTLV-I, HTLV-II, and HTLV-III. Gaps were introduced during complex analyses, to maximize the homologies (*35*).

immediately downstream from an enhancer-like sequence GTGGTTA.

Since the TCGF and γ-IF genes are expressed exclusively in T cells, and since the regions of homology are located downstream from their potential enhancer signals, the corresponding sequences in the HTLV-III LTR could play some role in host cell tropism or transcriptional regulation of this virus.

Some general features of the HTLV-III LTR are similar to those of HTLV-I, HTLV-II, and BLV. A 62 percent homology was found between HTLV-III U3 and BLV U3 sequences (*15*) that include the functional promoters of both viruses (Fig. 4d).

Homologies of 61 and 55 percent were also found between HTLV-III and HTLV-I and HTLV-II U3 sequences, respectively (*11, 12*). These sequences include the functional promoter signals of HTLV-I and HTLV-II and a promoter-like signal of HTLV-III U3 (AATAAA) (Fig. 4e) (position 527 in Fig. 2). One can speculate that this promoter-like sequence was actually utilized in an ancestral virus. If so, the R region of this virus would be extended and thus be more similar in length to those of HTLV-I, HTLV-II, and BLV. Furthermore, in this extended R region a stable loop structure would be positioned similarly to those shown for HTLV-I, HTLV-II, and BLV (*11, 12, 15*). These structural analogies suggest that HTLV-III has evolved from the same ancestor as other members of the HTLV/BLV family. The functional role of the transcriptional regulatory sequences in U3 as well as the involvement of the LTR in the cytopathic effect of HTLV-III remain to be demonstrated.

The recent finding of similarities between HTLV-III and visna virus in morphology, cytopathic potential, ability to infect brain tissue, and nucleotide sequence strongly suggests that these viruses are related (*7, 21*). It would be of interest to determine whether the LTR of visna is similar in structure to the LTR of HTLV-III.

BRUNO STARCICH
LEE RATNER
STEVEN F. JOSEPHS
TAKASHI OKAMOTO
ROBERT C. GALLO
FLOSSIE WONG-STAAL

Laboratory of Tumor Cell Biology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland 20205

References and Notes

1. R. C. Gallo *et al.*, *Science* **224**, 500 (1984); J. Schüpbach *et al.*, *ibid.*, p. 503.
2. M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *ibid.*, p. 497.
3. M. G. Sarngadharan, M. Popovic, L. Bruch, J. Schüpbach, R. C. Gallo, *ibid.*, p. 506.
4. M. S. Gottlieb *et al.*, *N. Engl. J. Med.* **305**, 1425 (1981).
5. L. Ratner *et al.*, *Nature (London)*, in press.
6. S. Oroszlan *et al.*, in *Human T-Cell Leukemia/Lymphoma Virus*, R. C. Gallo, M. Essex, L. Gross, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1984), p. 101.
7. M. A. Gonda *et al.*, *Science* **227**, 173 (1985).
8. B. H. Hahn *et al.*, *Nature (London)* **312**, 166 (1984).
9. G. M. Shaw *et al.*, *Science* **226**, 1165 (1984).
10. J. Lenz and W. A. Haseltine, *J. Virol.* **47**, 317 (1983); L. Des Groseillers, E. Rassart, P. Jolicoeur, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4203 (1983).
11. M. Seiki, S. Hattori, Y. Hirayama, M. Yoshida, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3618 (1983).
12. J. Sodroski *et al.*, *ibid.* **81**, 4617 (1984).
13. L. Ratner *et al.*, unpublished observations.
14. H. W. Temin, *Cell* **27**, 1 (1981).
15. N. Sagata, T. Yasunaga, Y. Owaga, J. Tsuzuku-Kawamure, Y. Ikawa, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4741 (1984).
16. H. Weiher, M. König, P. Gruss, *Science* **219**, 626 (1983).
17. T. I. Bonner, C. O'Connell, M. Cohen, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4709 (1982).
18. N. J. Holbrook, M. Lieber, G. R. Crabtree, *Nucl. Acids Res.* **12**, 5005 (1984).
19. T. Fujita, C. Takaoka, H. Matsui, T. Taniguchi, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7437 (1983).
20. P. W. Gray and D. V. Goeddel, *Nature (London)* **298**, 859 (1982).
21. G. M. Shaw *et al.*, *Science* **227**, 177 (1985).
22. A. M. Maxam and W. Gilbert, *Methods Enzymol.* **65**, 499 (1980).
23. A. J. Berk and P. A. Sharp, *Cell* **12**, 721 (1977).
24. E. P. Reddy *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2500 (1983).
25. R. Swanstrom, W. J. DeLorbe, J. M. Bishop, H. E. Varmus, *ibid.* **78**, 124 (1981).
26. D. E. Schwartz, R. Tizard, W. Gilbert, *Cell* **32**, 853 (1983).
27. R. Villemur, E. Rassart, L. Des Groseillers, P. Jolicoeur, *J. Virol.* **45**, 539 (1983).
28. T. M. Shinnick, R. A. Lerner, J. G. Sutcliffe, *Nature (London)* **293**, 543 (1981).
29. R. Dhar *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3937 (1980).
30. J. H. Elder and J. I. Mullins, *J. Virol.* **46**, 871 (1983).
31. A. H. Hampe, M. Gobet, J. Even, C. J. Sherr, F. Galibert, *ibid.* **45**, 466 (1983).
32. K. Shimotohno, S. Mizutani, H. M. Temin, *Nature (London)* **285**, 550 (1980).
33. N. Kennedy, G. Knedlitschek, B. Groner, N. E. Hynes, P. Herrlich, R. Michalides, A. J. J. van Ooyen, *ibid.* **295**, 622 (1982).
34. S. Devare, E. P. Reddy, J. D. Law, S. A. Aaronson, *J. Virol.* **42**, 1108 (1982).
35. C. L. Queen and L. J. Korn, *Methods Enzymol.* **65**, 595 (1980).
36. We thank G. Shaw, B. Hahn, S. K. Arya, and G. Chan in our laboratory for the HTLV-III clones, W. A. Haseltine for helpful discussions, J. Maizel for secondary structure analysis, and A. Mazzuca and L. Bruening for editorial assistance. B.S. is a fellow of the Italian Association for Cancer Research.

30 November 1984; accepted 31 December 1984

The Pollination of *Zygogynum* (Winteraceae) by a Moth, *Sabatinca* (Micropterigidae): An Ancient Association?

Abstract. *The primitive and vesselless angiosperm* Zygogynum *(Winteraceae), which is restricted to New Caledonia, is pollinated by a moth,* Sabatinca *(Micropterigidae). Fossil records of both the moth and the plant families extend to the Early Cretaceous. Adult* Sabatinca *have grinding mandibles and usually feed on the spores of ferns and on pollen. The insects use the flowers as mating sites and eat the pollen which is immersed in a dense pollenkitt. This mode of pollination in which flowers serve as mating and feeding stations with floral odors acting as cues may have been common in the early evolution of flowering plants.*

A complex flower-insect relation between two groups of archaic organisms in New Caledonia has been elucidated by field observations and chemical analysis of the flower odors. The life cycles of specific moths (*Sabatinca*) and trees (*Zygogynum*) are linked; aggregations of moths use the flowers as mating and feeding stations, and their activities result in pollination. This interaction is

particularly important since both genera belong to families with fossil records extending to the early Cretaceous (*1, 2*).

Micropterigid moths occur throughout the world with a concentration in the Southwest Pacific (*3*). In the fossil record the family is represented from Eocene Baltic amber and Early Cretaceous Lebanese amber (Aptian and Neocomian), the latter being the earliest known lepidopteran record. The fossil genus *Parasabatinca* from Lebanon is similar to the extant genus *Sabatinca* from New Zealand and New Caledonia, differing in only minor characteristics (*1*). The moths are small (5- to 15-mm wingspans) (Fig. 1) and have grinding mandibles, which they use to eat pollen and the spores of ferns. The larvae browse on liverwort thalli, angiosperm seedlings, or detritus (and possibly fungi) within soil and rotten logs.

Zygogynum consists of about seven species (*4*) all endemic to New Caledonia; it is one of ten genera of vesselless angiosperms. Winteraceae, like the Micropterigidae, are thought to represent a relictual assemblage with a fossil record from the Aptian-Albian of Israel (late Early Cretaceous). This is the earliest known record of an extant family of magnoliod angiosperms (*2*).

Zygogynum species are small evergreen trees (~4 to 8 m tall) that occur throughout the moist-tropical forest of New Caledonia (*4*). We studied populations of *Z. baillonii* v. Tieghem in the Dzumac mountain range (800 to 950 m) and *Z. bicolor* v. Tieghem on Plateau du Dogny (850 to 1000 m). Both species are allopatric and differ in their flowering periods. *Zygogynum baillonii* flowers from April through December peaking in November and *Z. bicolor* from August to January peaking in the last 2 months of the season. Although both species produce abundant flower buds, the number of open flowers displayed per tree per population daily, throughout the flowering season, is extremely low (Table 1). The functional floral life-span of the protogynous, nectarless flowers is 2 days with an individual plant producing all female- or all male-phase flowers on a given day; these adaptations enforce outbreeding.

The female-phase flowers (those in the first day) emit a strong fragrance; the yellow-red flowers of *Z. baillonii* produce a "burnt orange" fragrance whereas the red-white flowers of *Z. bicolor* smell "musty." They bear receptive stigmas, but their anthers remain indehiscent. No edible reward is available to the insects during this floral phase. The flowers are fragrant throughout the day but the floral odor ceases when the petals close in the evening (*5*). The odor recommences during the male phase (beginning the second day) after the second expansion of the petals at about 7 a.m. This is always accompanied by the spreading and elongation of the stamens which extrude pollen through terminal slits (*4*). The pollen hangs in strands, often gradually accumulating at the base of the petals. At the end of the day the petals and stamens usually abscise leaving the central gynoecium of fused carpels with their crusty black or brown stigmatic crests. These floral movements probably operate by water pressure (*6*).

Table 1. Flower production and insect activity in a population of 16 trees of *Zygogynum baillonii* on Mount Dzumac in 1983. Two other populations on Mount Dzumac and one on Montagne des Sources displayed a similar flowering pattern and insect activity. Populations of *Z. bicolor* also conform to this pattern.

Date (1983)	Trees in flower	Flowers	Moths	Beetles
28 Oct.	2	3	1	1
9 Nov.	3	5	50	1
14 Nov.	2	2	30	0
16 Nov.	2	2	21	0
17 Nov.	3	3	29	0
19 Nov.	1	3	31	0
29 Nov.	2	2	5	0
30 Nov.	2	2	0	0
8 Dec.	4	4	0	1
28 Dec.	1	1	1	0
4 Jan.	2	2	0	5

The anthers produce such a copious pollenkitt that the pollen tetrads (30 to 40 μm in diameter) are virtually embedded in an oily matrix which stains positively for lipids with Auramine O and Scarlet R. The lipid coat serves as a food for the moths and encourages successful attachment of the tetrads to the insect's body. During the process of feeding, the mixture of pollenkitt and tetrads adheres to the moth; the coat dries to an elastic, white-colored substance.

The male and female moths, an undescribed species of *Sabatinca*, congregate on opening buds in numbers of up to 30 or more (Table 1). The insects rapidly crawl over the flower, twigs, and leaves in the general area of the flower. At times all the moths are stationary. Eventually, an individual begins to crawl rapidly in counterclockwise looping circles, usually on a leaf. Other individuals then begin to crawl in similar patterns until most of the individuals are moving about rapidly. After a few such loops the moths mate. The moths also crawl inside and on the outer surface of the flower and it is not unusual to find 7 to 10 moths in either a male- or female-phase flower; the mating behavior occurs on both male and female flowers of *Z. baillonii* and *Z. bicolor*. The moths are strong fliers for their size and, upon the slightest disturbance, cease their activities. In many instances, they leave in a tight-flying group and may well visit flowers as a swarm.

The moths eat the pollen-matrix of male-phase flowers. Their wings, legs, and lower abdomen become coated with oily tetrads. Even when all the extruded pollen has been consumed, the moths continue to visit each stamen, removing the few stray tetrads. The moths were never observed feeding on the stigmatic crests. When the moths finally leave a male-phase flower, it has been denuded of pollen. If the moths visit female-phase flowers next, they will crawl over the stigmatic crests searching for pollen or mates, and thus pollinate the flowers (*5*). Coleoptera have been collected in flowers of both *Zygogynum* species, but their occurrence is most infrequent and they

Fig. 1. Male phase flower of *Zygogynum baillonii* with *Sabatinca* sp. removing pollen from the stamens; arrow indicates a stigma (×~1.2).

cannot be considered primary pollen vectors (Table 1). Fruit production is hard to determine quantitatively, since maturation takes over a year while the successive stages are hard to discern; however, it appears safe to claim that it is low. The percentage of flowers setting fruit is estimated to be 2 or 3.

Opening floral buds produce a strong fragrance which apparently serves as the primary attractant. At this early stage of anthesis the colorful petals are not visible yet 5 to 10 moths may gather on these buds. The female-phase flowers are devoid of the edible reward (pollen matrix). In a sense, the attraction of the moths to such flowers amounts to "pollination-by-deceit," as reported in some *Acacia* species in Australia (*7*). The floral odor of *Zygogynum* must act as an assembling scent that attracts both male and female moths, both of which assemble on the open flowers. The unusually low number of flowers presented on the trees at any one time enhances this process by concentrating a majority of the moths in the area on relatively few flowers and encourages trapline foraging.

The same species of *Sabatinca* has been recorded from flowers of both *Z. baillonii* and *Z. bicolor*. The chemical composition of the floral odors of the species has been determined (*8*) and is presented in Table 2.

Micropterigid moths are also attracted to the pink-yellow flowers of *Exospermum stipitatum* (Baill.) v. Tiegh. ex Pilger (orange-banana fragrance) (*9*), the sole species of another genus of Winteraceae, also endemic to New Caledonia, and closely related to *Zygogynum*. Moths, however, cannot gain entry to the female-phase flowers of *Exospermum* because the inner petals do not open but instead form a chamber around the gynoecium. Beetles enter the floral chamber by way of the overlapping petal seams and pollinate these flowers. The chemical compositions of the floral odors of *E. stipitatum* and *Z. baillonii* and *Z. bicolor* are quite similar considering the number of compounds shared and the classes of chemicals (Table 2). All species attract beetles and moths, but the functioning of their flowers determines the mode of pollination.

A surprising number of magnolioid flowers exhibit pollination in which the floral odor attracts large numbers of insects to the flowers where they remain for substantial periods of time, using the flowers as sites for mating, feeding, and subsequent pollination (*10*). Indeed, this mode of pollination may have been common in the early evolution of the angiosperms (*11*).

Table 2. Volatile components of *Zygogynum bicolor*, *Z. baillonii*, and *Exosperum stipitatum* collected from flowers in different phases. Abbreviations: T, terpenoids; F, short chain fatty acid derivatives; B, benzoid compounds; and Tr, trace (from selective ion monitoring).

Compound	Retention time (minutes)	Compound class	Percentage in		
			Z. bicolor	*Z. baillonii*	*E. stipitatum*
Ethyl acetate	3.5	F	81	31	80
Propyl acetate	5.4	F	0.4	0.2	1.8
2-Methylpropyl-acetate	6.4	F	0.5	27	8.1
α-Pinene	6.5	T	3.0	<0.1	Tr
Camphene	8.1	T	<0.1	Tr	
Butyl acetate	8.3	F	<0.1	Tr	Tr
2-Methyl-1-propanol	8.4	F		0.2	Tr
β-Pinene	9.3	T	0.9	Tr	
2-Methylbutyl acetate	9.6	F	0.6	4.0	3.8
3-Carene	10.5	T	0.6	<0.1	
Myrcene	11.1	T	0.5	<0.1	Tr
α-Phellandrene	11.2	T	0.6		
2-Methyl-1-butanol	12.2	F		<0.1	Tr
Limonene	12.3	T	3.0	<0.1	<0.1
β-Phellandrene	12.4	T	2.9		
cis-β-Ocimene	13.3	T	0.2	0.1	
trans-β-Ocimene	13.6	T	0.5	<0.1	
Hexyl acetate	14.3	F	<0.1	<0.1	<0.1
Dimethylbenzene isomer	14.4	B	0.7	Tr	
Terpinolene	15.0	T	0.4	Tr	
6-Methyl-5-hepten-2-one	16.3	T		0.2	
Unidentified (related to 6-methyl-5-hepten-2-ol)	17.5	T		0.2	
6-Methyl-5-hepten-2-yl acetate	18.5	T		34	
Acetic acid	19.2	F	0.2	<0.1	<0.1
6-Methyl-5-hepten-2-ol	19.5	T		0.7	
Linalool	22.0	T	0.2	<0.1	
Caryophyllene	24.0	T	0.2	<0.1	
Humulene	25.5	T	<0.1	<0.1	
Unidentified sesquiterpene	26.5	T	<0.1	<0.1	0.2
α-Farnesene	27.3	T			0.3
(*E*)-6,10-Dimethyl-5,9-undecadien-2-one (Geranylacetone)	29.5	T		<0.1	<0.1
(*Z*)-6,10-Dimethyl-5,9-undecadien-2-yl acetate	30.0 30.4	T		<0.1	<0.1
(*E*)-6,10-Dimethyl-5,9-undecadien-2-yl acetate	31.2	T		<0.7	<4.1
(*Z*)-6,10-Dimethyl-5,9-undecadien-2-ol	31.2	T		<0.1	
(*E*)-6,10-Dimethyl-5,9-undecadien-2-ol	31.5	T			<0.1

LEONARD B. THIEN
Department of Biology, Tulane University, New Orleans, Louisiana 70118
PETER BERNHARDT
Plant Cell Biology Research Center, University of Melbourne, Parkville, Australia 3052
GEORGE W. GIBBS
Zoology Department, Victoria University of Wellington, Wellington, New Zealand
OLLE PELLMYR
Department of Entomology, Uppsala University, Uppsala, Sweden S-75122
GUNNAR BERGSTRÖM
INGA GROTH
Department of Ecological Chemistry, Göteborg University, Göteborg, Sweden S-40033
GORDON MCPHERSON
Missouri Botanical Garden, St. Louis, Missouri 63166

References and Notes

1. P. Whalley, *Ann. Transvaal Mus.* **31**, 71 (1978).
2. J. W. Walker, G. J. Brenner, A. G. Walker, *Science* **220**, 1273 (1983).
3. G. W. Gibbs, *GeoJournal* **7** (No. 6), 505 (1983).
4. W. Vink, *Blumea* **23**, 219 (1977).
5. L. B. Thien, *Biotropica* **12**, 1 (1980).
6. S. Carlquist, *Bull. Mus. Nat. Hist. Nat.* **3**, 18 (1981); S. Carlquist, *Aliso* **10** (No. 2). 277 (1982).
7. P. Bernhardt, J. Kenrick, R. B. Knox, *Ann. Mo. Bot. Gard.*, in press; P. Bernhardt, in *Pollination 82*, E. G. Williams, R. B. Knox, J. H. Gilbert, P. Bernhardt, Eds. (Melbourne Univ. Press, Melbourne, 1982), p. 84.
8. Flowers were put in contact with Porapak Q, 50 to 80 mesh in an enclosure for 6 hours ("enfleurage"). Then the polymers were separated from the flowers and stored in a glass vial. Desorption was done by extracting the Porapak portions with 2 ml of redistilled diethyl ether each. Gas chromatography, with FID/NPD (flame ion detection nitrogen phosphorus detection) 1:1, and gas chromatography–mass spectometry were performed with OV-351 and Superox FA fused Silica capillary columns. Temperature was programmed from 50°C (isothermal for 4 minutes)

to 210°C, at 5° per minute. Compounds were identified by comparisons of retention times (in some cases co-injection) and mass spectra with those of reference compounds in our position. In a few cases reference compounds were synthesized (by J. Bergström, Göteborg University). Percentages were calculated from total ion chromatograms and trace amounts were detected by selective ion monitoring. For further description of techniques for the isolation and enrichment of plant volatiles, see G. Bergstrom *et al.* [*Chem. Scripta* **16**, 173 (1980)].

9. L. B. Thien and G. McPherson, in preparation.
10. G. Gottsberger, *Osterr. Bot. Z.* **118**, 237 (1970); L. B. Thien, *Am. J. Bot.* **61**, 1037 (1974).
11. O. Pellmyr and L. B. Thien, in preparation.
12. Plant voucher specimens (collected by G.M.) are deposited in the herbarium at the Missouri Botanical Garden as follows: *Z. baillonii*, 5833; *Z. bicolor*, 5873; *Z. pomiferum* subsp. *balansae*, 5767; *Z. mackeei*, 5933; and *Exospermum stipitatum*, 5929. Moth specimens, which are not numbered, are stored at the Department of Zoology, University of Wellington, Wellington, New Zealand. L.B.T. thanks J. P. Cherrier, the head of Forests and of Ecology in New Caledonia for permission to work on the island. P. Morat and J. Gutierrez provided research facilities via ORSTOM. P. Raven inspired the research. The following individuals critically read the manuscript: B. Sampson, V. Grant, P. Raven, S. Carlquist, D. Davis, and B. J. D. Meeuse. The National Geographic Society provided a grant making the research possible.

13 July 1984; accepted 24 September 1984

Transovarial Transmission of Murine Typhus Rickettsiae in *Xenopsylla cheopis* Fleas

Abstract. *It has been generally accepted that infected fleas do not pass on* Rickettsia mooseri, *or indeed any other known pathogen, to their progeny. It is reported here that such transovarial transmission does occur in laboratory-infected* Xenopsylla cheopis *fleas. By means of the direct fluorescent antibody test,* Rickettsia mooseri *was observed in cells of the hemolymph of infected fleas. As many as 11 percent of the adults and 2.9 percent of the larvae of the generation reared therefrom, had demonstrable rickettsiae. Moreover, batches of the F_1 fleas were capable of transmitting the infection to more than 18 percent of the rats they infested. The data support the contention that* Xenopsylla cheopis *fleas play an important role in the maintenance of murine typhus in rats in nature.*

In the classical studies on murine typhus that incriminated fleas, particularly *Xenopsylla cheopis*, as vectors, it was reported that infected fleas do not transmit the etiological agent, *Rickettsia typhi* (referred to here as *R. mooseri*), to their progeny (*1*). This statement has remained unchallenged for more than 50 years (*2, 3*).

It has become generally accepted that all the other microbial pathogens of humans that are transmitted by fleas also remain wholly restricted to the confines of the alimentary canal while resident in the flea, thereby excluding the possibility of transovarial transmission (TOT) (*4, 5*). This so-called gut barrier has even been proposed as the reason why fleas do not serve as true vectors of viruses (*4*), which, like rickettsiae, are intracellular parasites. Since TOT occurs in at least 7 of the 15 arthropod-borne rickettsial diseases of man or domestic animals, it is somewhat surprising that over so many decades no questions have been raised about the purportedly invincible nature of the barrier posed to human pathogens by the gut of fleas. In this report we present evidence that not only may transovarial transmission of *R. mooseri* to the next generation take place, but that the F_1 fleas are capable of transmitting the infection to rats.

Murine typhus is believed to be contracted by contact with the crushed bodies or feces of infected fleas and not by the bite of these insects. In earlier papers (*6, 7*), in which we presented details on methodology, we confirmed that when *X. cheopis* fleas are infected with *R. mooseri*, the rickettsiae penetrate and develop in the cells of the midgut epithelium, the only part of the intestine that lacks a cuticular lining. It seemed likely that any escape of rickettsiae from the gut would have occurred when the infection had become so massive that the lumen of the midgut and hindgut were packed with the organisms. We could even observe rickettsiae in the proventriculus and foregut about 15 days after the fleas had fed on infected rats or later (*7*).

In the present study, 17 percent (10 of 60) of the smears of the hemolymph of fleas examined 19 to 22 days after they had fed on infected rats were positive for intracellular rickettsiae by the direct fluorescent antibody test (DFA), whereas no rickettsiae were observed in 50 fleas examined earlier. Further, as shown in Table 1, not only do some of the progeny of infected fleas harbor *R. mooseri*, but TOT occurred in significantly greater numbers of offspring derived from eggs deposited late in the course of the infection of the parents than from those laid shortly after the infectious meal; for example, 7.5 to 11.3 percent for days 26 and 31, in contrast to 2.9 percent for days 5 to 11. The corresponding data for the minimum number of infected F_1 fleas in the pools tested are 1 in 125 or 132 compared to <1 in 180 (*8, 9*).

Further analysis of the data for the groups 17 to 31 days after infective feeding showed that there were no major differences between males and females or between fed and unfed fleas, even though the fed fleas had an additional 4 to 6 days for the growth of any transovarially transmitted rickettsiae (Table 2) (*10*).

As shown in Table 3, the F_1 generation of fleas was capable of infecting rats after being allowed to infest and feed upon them during a period of 24 hours. The highest rates of transmission (15.5 to 18.4 percent) occurred in fleas that had fed (on uninfected hosts) on one or two previous occasions and were 7 to 18 days of age, suggesting that the more time available for growth of rickettsiae within TOT-infected fleas, the greater the chance for transmission of rickettsiae by those fleas (*11*).

Five of the seven known rickettsial infections in which TOT occurs are transmitted by ticks and two by mites (*12, 13*). This phenomenon has not been demonstrated in the two that are louse-borne. In scrub typhus, the trombiculid vector also serves as the reservoir of the infection because of TOT in "naturally

Table 1. *Rickettsia mooseri* infection rates among F_1 progeny of *X. cheopis* fleas reared from eggs collected at various dates after infection of the parents (*8*).

Days after infective feeding of parental fleas	Direct fluorescent antibody test			Mouse antibody test			
	Number of fleas		Percentage of fleas infected	Number of fleas examined	Number of		Minimum infection rate
	Examined	Positive			Pools tested	Positive pools	
5 to 11*	305	9	2.9	180	15	0	<1 to 180*
17	493	19	3.8	568	29	4	1 to 142
26	252	19	7.5	250	10	2	1 to 125
31	398	45	11.3	395	12	3	1 to 132

*The apparent discrepancy probably reflects the small number of infected fleas present.