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FRENKELIA PARASITES IN A SMALL MAMMAL COMMUNITY. DYNAMICS OF INFECTION AND EFFECT ON THE HOST

FICHET-CALVET E.*****, KIA E.B.******, GIRAUDOUX P.***, QUÉRÉ J.P.*, DELATTRE P.* & ASHFORD R.W.**

Summary:

A community of small mammals, *Clethrionomys glareolus*, *Arvicola terrestris*, *Microtus arvalis*, *M. agrestis*, *M. subterraneus*, *Apodemus* spp. and *Sorex* spp., was studied as hosts of *Frenkelia glareoli* and *F. microti* in Franche-Comté (France). They were monitored in spring, summer and autumn on an area of about 1,350 ha comprising open field, hedgerow network and forest. Among 1,714 small mammals examined between July 1992 and October 1993, 47 % (178/376) of *C. glareolus*, 9.9 % (14/139) of *A. terrestris* and 1.3 % (4/311) of *Apodemus* spp. were infected by *F. glareoli*. The prevalence of infection with *F. microti* was 9.2 % (66/716) in *M. arvalis* and 8.2 % (6/73) in *M. agrestis*. *M. subterraneus* and *Sorex* spp. were not infected. The maintenance of each parasite in a rural landscape is assured both by a forest and a grassland host. Multiple logistic regression showed that prevalence was highly age-dependent, with an apparent seasonal pattern. Prevalence varied between 30 % in summer and 60 % in early spring for *F. glareoli* in *C. glareolus* and between 3 % in autumn to 30 % in early spring for *F. microti* in *M. arvalis*. The year, habitat, host sex, relative density had no impact on prevalence. In *M. arvalis* only, sexually active voles were preferentially uninfected, indicating a possible impact of this parasitism on fertility.

KEY WORDS : voles, population dynamics, *Frenkelia* spp., Coccidia, prevalence, age effect, agroecosystem, mid-mountain.

MOTS CLÉS : campagnol, dynamique de population, *Frenkelia* spp., coccidie, prévalence, effet de l'âge, agroécosystème, moyenne montagne.

Résumé :

DES *FRENKELIA* CHEZ UN PEUPEMENT DE PETITS MAMMIFÈRES : DYNAMIQUE DE L'INFESTATION ET IMPACT SUR L'HÔTE
L'infestation par *Frenkelia glareoli* et *F. microti* a été étudiée au niveau d'un peuplement de petits mammifères composé de *Clethrionomys glareolus*, *Arvicola terrestris*, *Microtus arvalis*, *M. agrestis*, *M. subterraneus*, *Apodemus* spp. et *Sorex* spp. Les populations ont été suivies au printemps, en été et en automne dans un agroécosystème comprenant des champs ouverts, du bocage et de la forêt. Parmi les 1714 petits mammifères examinés entre juillet 1992 et octobre 1993, 47 % (178/376) des *C. glareolus*, 9,9 % (14/139) des *A. terrestris* et 1,3 % (4/311) des *Apodemus* spp. étaient infestés par *F. glareoli*. La prévalence de *F. microti* était de 9,2 % (66/716) chez *M. arvalis* et 8,2 % (6/73) chez *M. agrestis*. Aucune infestation n'a été observée chez *M. subterraneus* et *Sorex* spp. Dans un tel paysage rural, la maintenance de chaque parasite est assurée par deux hôtes, l'un fréquentant les habitats prairiaux, l'autre les habitats forestiers. Une analyse par régression logistique multiple a montré que les prévalences sont étroitement liées à l'âge de l'hôte alors que les fluctuations saisonnières (30-60 % pour *F. glareoli* chez *C. clethrionomys*; 3-30 % pour *F. microti* chez *M. arvalis*) de la prévalence ne sont qu'apparentes et ne dépendent que de la structure en âge de la population hôte. L'année, l'habitat, le sexe de l'hôte et sa densité relative n'ont pas d'influence sur les prévalences. Chez *M. arvalis*, les individus sexuellement actifs sont préférentiellement ceux qui sont indemnes de *F. microti*, suggérant ainsi un possible impact de ce parasitisme sur la fertilité de ces rongeurs in natura.

INTRODUCTION

Parasites have long been considered as possible factors in the regulation of rodent populations (Elton *et al.*, 1935). Around 1980, hypothetical

models were produced, considering host-parasite systems as a special case of predator-prey interaction (Anderson & May, 1978; Holmes, 1982). Paradoxically, very few studies in nature have tested these models. This was true long ago (Wiger, 1977), and there have been few relevant studies since that time. Ecological studies on the effects of parasites on host populations are particularly appropriate in the applied study of the management of irruptive rodent species (Jäkel *et al.*, 1999). We have carried out a long-term study of agricultural pest rodents in mid-mountain zones in France (Delattre *et al.*, 1992, 1996, 1999), and have previously linked a study of the cestode parasites of these rodents (Giraudoux, 1991; Le Pesteur *et al.*, 1992). Additional results are presented here, on the protozoan parasites *Frenkelia* spp., of the brains of these rodents. *Frenkelia microti* was first discovered (As "M organism", thought to be closely related to *Toxoplasma*) in Wales,

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by Findlay & Middleton (1934), in *Microtus agrestis* at the time of a population crash.

Frenkelia spp are heteroxenous coccidia with sexual reproduction in the intestines of birds of prey, especially the Buzzard *Buteo buteo*, and asexual multiplication in the brains of rodents. The full life history was first described, for *F. glareoli* in the bank vole, *Clethrionomys glareolus*, by Rommel & Krampitz (1975). Resistant sporocysts are excreted in the faeces of Buzzards from 7-9 days following infection, for a period of 7-57 days. Sporozoites emerge from the sporocysts when these are ingested by a rodent, and migrate to the brain, where they produce cysts that are visible 17 to 18 days following infection (Geisel *et al.*, 1978; Laarman *et al.*, 1979). Over a period of weeks, the cysts grow to 350 μm in diameter, and contain many thousand bradyzoites, which are infective to Buzzards when the rodent host is eaten. Heavily infected rodents contain numerous cysts, which occupy a considerable proportion of all parts of the brain, and infection lasts for the life of the host (Tadros & Laarman, 1976; Laarman *et al.*, 1979). The earliest age at which the rodents can be infected is unknown.

Two species of *Frenkelia* are known to occur in Europe, *F. glareoli*, mainly in *C. glareolus*; and *F. microti*, mainly in *Microtus* spp. (Tadros & Laarman, 1982).

Vorisek *et al.* (1998) have shown evidence that infected rodents are more likely to be predated than uninfected individuals, as happens in certain other host-parasite combinations (review by Combes, 1995). Transmission of the parasite is thereby facilitated, and the longevity of infected rodents is reduced. Reduced longevity of some individuals does not necessarily have any regulatory effect on populations. In order to assess any regulatory effect of the parasite on intermediate host populations, information is first required on the distribution of the parasite in the host community at a local scale.

The aim of this study is to test for any effect of extrinsic (year, season, habitat) and intrinsic (host age, sex and relative density) factors on the infection rates in each species of the rodent community. Then, the possibility of an effect of the parasites on the hosts was investigated by comparing the body weight and sexual activity of infected and uninfected individuals.

MATERIALS AND METHODS

STUDY SITE

The study area occupies about 1,350 ha, in Franche-Comté, 10 km north-west of Pontarlier (47.10° N, 6.24° E, 850 m above sea level) with mean annual rainfall of 1,500 mm. The landscape is

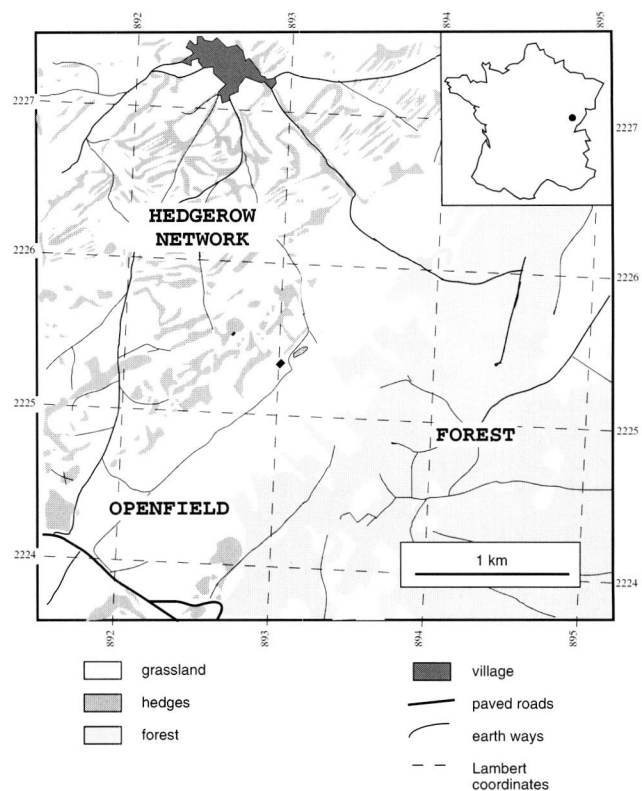


Fig. 1. – Landscape composition of the study site located in the Jura plateau.

composed of forest and agricultural land. The forest is mostly semi-natural, composed of mixed beech *Fagus sylvatica*, oak *Quercus robur*, and fir *Abies alba*, and there are some spruce *Picea abies* plantations. The agricultural land is either improved grassland or permanent pasture (Delattre *et al.*, 1988; Giraudoux *et al.*, 1997), and is either, open over wide areas (open field), or enclosed by hedgerows in plots of ca 1 ha (Fig. 1).

TRAPPING AND SAMPLING

Trapping was carried out in forest (deciduous, mixed, coniferous), hedgerow network (hedge, hedge edge, enclosures) and open field (permanent grassland) habitats. Because this study is part of a rodent survey for outbreak management, small mammals were sampled during the reproduction period: in July and October 1992, and April, July and October 1993. INRA (French Agronomic Research Institute) trap lines were used (Spitz *et al.*, 1974). Thirty-four traps were placed at 3 m intervals in each line of about 100 m. The numbers of lines set on each occasion, and the distribution by habitat of the 214 trap lines (21,828 trap nights) are shown in Table I. Traps were left in place for three consecutive nights, and were visited twice daily. Animals were killed by cervical dislocation according to Mills *et al.* (1995).

Species	Habitat	Jul 92	Oct 92	Apr 93	Jul 93	Oct 93	
	Forest	deciduous mixed coniferous	0	22 $\begin{cases} 5 + 1^* \\ 3 \\ 13 \end{cases}$	21 $\begin{cases} 1 \\ 6 \\ 14 \end{cases}$	0	20 $\begin{cases} 4 + 1^* \\ 4 \\ 11 \end{cases}$
	Hedgerow network	hedge hedge edge grassland	5 $\begin{cases} 4 \\ 1 \\ 13 \end{cases}$	8 $\begin{cases} 2 \\ 6 \\ 12 \end{cases}$	18 $\begin{cases} 1 \\ 17 \\ 21 \end{cases}$	5 $\begin{cases} 4 \\ 1 \\ 13 \end{cases}$	7 $\begin{cases} 2 \\ 5 \\ 12 \end{cases}$
	Open field	fence edge grassland	7 $\begin{cases} 2 \\ 5 \end{cases}$	7 $\begin{cases} 2 \\ 5 \end{cases}$	8 $\begin{cases} 3 \\ 5 \end{cases}$	8 $\begin{cases} 2 \\ 6 \end{cases}$	7 $\begin{cases} 3 \\ 4 \end{cases}$

Table I. – Distribution of the 214 trap lines by habitat and by season. Arrows design the habitats in which the rodent relative abundances are calculated. * corresponds to a forest clearing.

HOST POPULATION PARAMETERS

Relative abundance was estimated for each species as the number caught per trap line. For *C. glareolus*, captures in forest and in hedgerow network were analysed separately. For *Microtus arvalis*, captures in hedgerow network and in open field were analysed separately. For *Microtus agrestis*, which was less abundant, captures in forest and in hedgerow network were combined. *Arvicola terrestris* numbers were not estimated as only juveniles were sampled, adults being too big to enter the traps.

The weight of the desiccated eye lens (ELW) gives the best indication of age for small mammals (Lord, 1959; Martinet, 1966, rev. in Morris, 1971). Eyes were removed and preserved for a minimum of two weeks in 10 % formalin, then the lenses were extracted, dried for two hours at 100°C, and weighed to a precision of 0.1 mg. Females were classified as sexually active if they were pregnant or lactating, as were males with seminal vesicles over 40 mm² (length × breadth). Litter size was estimated by the number of embryos.

PARASITES

Carcasses were preserved in 10 % formalin before examination. The brain was removed by dissection of the skull, and stained for at least 24 h in undiluted Semichon's acetic carmine. They were then washed in distilled water, transferred to 1 % HCl in 70 % ethanol to differentiate, until the brain material was very pale pink in colour (usually a few hours), and placed in glycerine to clear. The stained, cleared brains were then sliced with a scalpel and the slices were examined with a dissecting microscope (× 100) to detect any parasites. Lobulated cysts were identified as *F. microti*, and large round cysts as *F. glareoli* (Tadros *et al.*, 1972; Tadros & Laarman, 1978). A few very small round cysts were regarded as unidentifiable except in juvenile *A. terrestris* in which 2/102 were *Toxoplasma gondii*, and 3/102 were *Frenkelia glareoli* (Kia *et al.*, in press).

STATISTICAL ANALYSIS

Year, season, habitat, host sex, age and relative density effects on prevalence were analysed with a multiple logistic regression using a binary factor (infected = 1, non infected = 0) as the dependent variable and year (two levels: 1992, 1993), season (three levels: spring, summer and autumn), habitat (three levels: open field, hedgerow network and forest), host sex (two levels), host age (continuous ELW) and host relative density (continuous abundance index) as independent variables. The strategy of data treatment was first to enter all the variables in a global model, and to perform a forward stepwise regression to select the non redundant variables. The second stage was to enter these selected variables with their interactions in a restricted model as recommended by Kleinbaum & Klein (2002). This analysis was performed with Systat 9, SAS Institute Inc. (1999).

The effect of infection on weight was analysed using ANCOVA with weight as the dependent variable and the host sex and infection (two levels: infected, uninfected) as the independent variables. Host age (continuous ELW) was entered as the covariate in the model. The effect of infection on sexual activity was analysed using multiple logistic regression including sexual activity (active = 1, inactive = 0) as the dependent variable and the infection (two levels: infected, uninfected), year (two levels), season (three levels), habitat (three levels), host sex, host age (continuous ELW) and host relative density (continuous abundance index) as the independent variables. As the goal of this analysis was to obtain a single estimate of the *Frenkelia* infection, adjusted for year, season, habitat, host sex, host age, and host relative density, the interactions were not included in the model (Kleinbaum & Klein, 2002). The effect of infection on fertility in each sex was analysed using ANCOVA with seminal vesicle size or litter size as the dependent variable and infection (two levels: infected, uninfected), season (three levels) and host age (continuous ELW) as independent variables (Legendre & Legendre, 1998; Sokal & Rohlf, 1998).

RESULTS

HOST RANGE

Of 2,848 animals collected, 1,714 were examined for *Frenkelia* infection (Table II). *F. glareoli* was mainly found in *C. glareolus*, secondarily in *Arvicola terrestris*, and rarely in *M. arvalis*, *M. agrestis* and *Apodemus* spp. *F. microti* was most frequent in *M. arvalis* and *M. agrestis* and was also found rarely in *C. glareolus*. *Microtus subterraneus* and *Sorex* spp were never found infected. Further analysis is restricted to the infection in the four

most important hosts, *C. glareolus*, *A. terrestris*, *M. arvalis* and *M. agrestis*.

PREVALENCE

• *Frenkelia glareoli* in *Clethrionomys glareolus*

The influence of year, season, habitat, host sex, age and relative density on prevalence was analysed in a global model by forward stepwise regression. The main effect on prevalence was due to host age ($\chi^2 = 19.204$, $p < 0.0001$), whereas the other factors were not significant. Host age is highly significant with an odds ratio of 1.043 ($p < 0.0001$), indicating an increase of prevalence with

Host species	No. collected	No. examined	<i>F. glareoli</i> (%)	<i>F. microti</i> (%)
<i>Clethrionomys glareolus</i>	537	376	178 (47)	3 (1)
<i>Arvicola terrestris</i>	210	139	14 (9.9)	0
<i>Microtus arvalis</i>	981	716	1 (2)	66 (9.2)
<i>Microtus agrestis</i>	136	73	1 (1.4)	6 (8.2)
<i>Microtus subterraneus</i>	20	12	0	0
<i>Apodemus</i> spp. ¹	761	311	4 (1.3)	0
<i>Sorex</i> spp. ²	203	87	0	0

¹ *Apodemus flavicollis* predominated, but *A. sylvaticus* also occurred; no attempt was made to distinguish juvenile specimens, so both species are grouped together. ² *Sorex coronatus* and *S. araneus* were not distinguished for the present study.

Table II. – Prevalences and host range in a small mammal community infected by *Frenkelia glareoli* and *F. microti*.

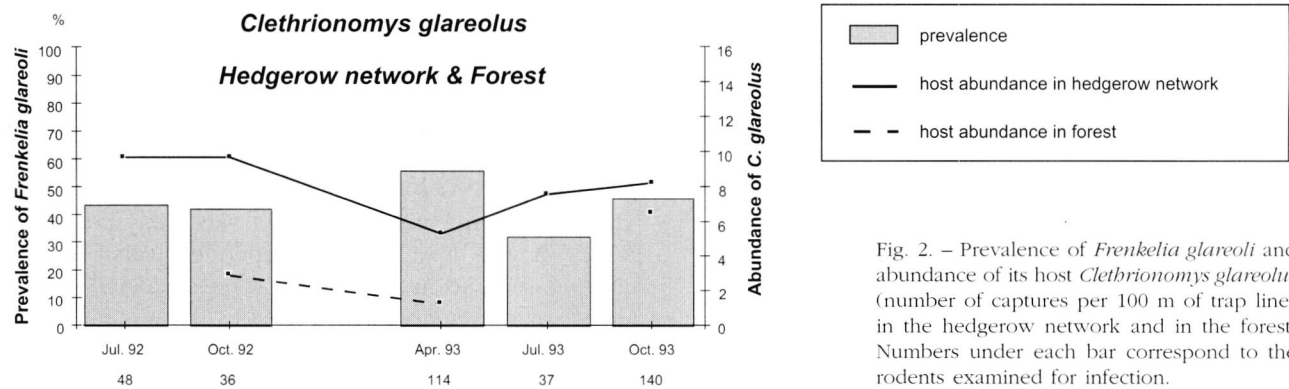


Fig. 2. – Prevalence of *Frenkelia glareoli* and abundance of its host *Clethrionomys glareolus* (number of captures per 100 m of trap line) in the hedgerow network and in the forest. Numbers under each bar correspond to the rodents examined for infection.

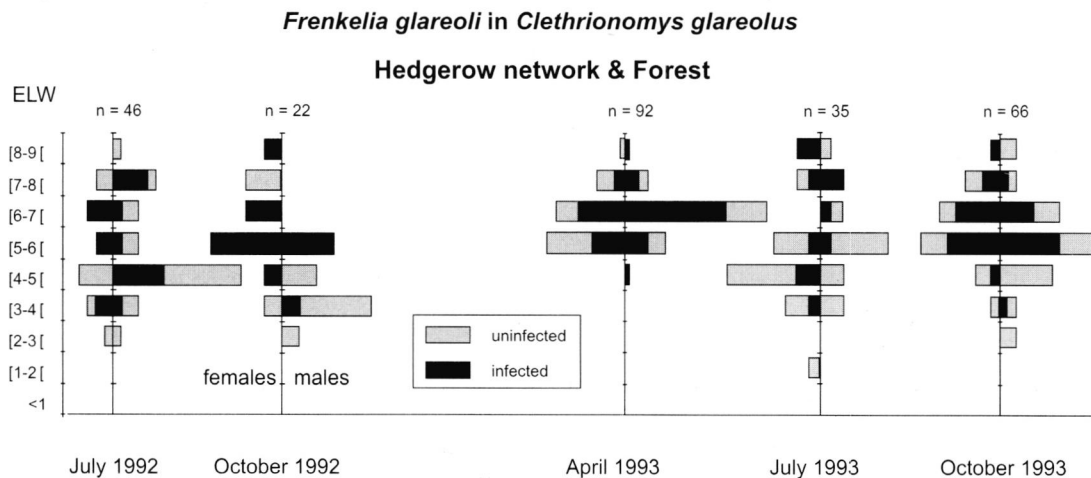


Fig. 3. – Distribution of *Frenkelia glareoli* in *Clethrionomys glareolus* by eye lens weight (ELW in mg) and sex of host.

age. The host age effect is illustrated in Figure 3 where the age structure is presented for each session. Infected individuals were present at each session and in each ELW class over 3 mg. Prevalence remained high throughout the year (Fig. 2) with the lowest prevalence in July 1993 (32 %) and the highest in April 1993 (62 %). These seasonal variations were not significant when host age was taken into account. The model explains only 4 % (250.475 – 240.577/250.475) of the total variation, suggesting that many other factors than host age explain 96 % of the variation of prevalence of *F. glareoli* in *C. glareolus*.

• *Frenkelia glareoli* in *Arvicola terrestris*

Because *A. terrestris* were captured almost exclusively in enclosed grassland, the variable “habitat” was excluded from this analysis. In addition, relative density of this species was not evaluated since only juvenile specimens were caught. Among the four remaining variables, year, season, host sex and host age (1.6 ≤ ELW ≤ 11.1), the multiple logistic regression shows that only the two last had an effect on prevalence (Chi² = 3.983, p = 0.046 and Chi² = 21.200, p < 0.0001 respectively). Infection was twice as common in females (8/61) than in males (4/58).

• *Frenkelia microti* in *Microtus arvalis*

First, the forward stepwise regression showed host age, relative density, and year to be significant variables having an effect on the *Frenkelia* infection. The other variables, season, host sex and habitat, were not correlated with prevalence. Host age is highly significant with an odds ratio of 1.069 (p < 0.0001), indicating increasing infection with age. This effect is illustrated in Figure 5 where the age structure is presented for each session. Infected individuals were present at each session, with very young ones in summer with ELW between 2 and 3 mg. In autumn, the youngest infected vole had ELW over 3.5 mg. Host relative density is significant with an odds ratio of 0.807 (p < 0.001) indicating that the prevalence of *F. microti* is negatively correlated with the abundance of its host. The year effect is described by an OR of 0.635 indicating a lower prevalence in 1993 than 1992. Figure 4 shows prevalence to be highest in spring (29 % in April 1993), when the vole population was at its lowest; prevalence declined in the breeding season (17 % in July 1992; 3 % in July 1993), reaching its lowest in autumn (7 % in October 1992; 3 % in October 1993), when the host population was at its greatest. These seasonal variations were not significant when host age was taken into account. In the restricted model containing the main factors, host age, host relative density and year, and their interactions, the 2-way interactions, i.e., “year × host relative density” and the 3-way interaction “year × host age × host density” were significant, whereas the main factor turned to non-significant (Table III). This means

Variables	Chi ²	Residual deviance	p
Null		208.882	
Host age (1.2 ≤ elw ≤ 7.5)	60.084	181.277	< 0.0001
Host relative density (2.8 ≤ ai ≤ 14.4)	20.610	169.946	< 0.0001
Host age × host relative density	8.083	165.854	0.004
Year × host relative density	4.462	163.815	0.035
Year × host age × host relative density	3.969	161.922	0.046
Year × host age	0.093	161.922	0.761
Year	0.028	161.922	0.868

Table III. – Logistic regression results for *Frenkelia microti* infection in *Microtus arvalis* in a hedgerow network (HNW) and open field model. Elw = eye lens weight in mg, ai = abundance index in number of voles trapped per 100 m. n = 670.

that these variables were not additive, and also that the combined effect of host age and host density on prevalence has to be considered year by year. This restricted model explains 22 % (208.882-161.922/208.882) of the total variation, suggesting that other factors are involved in *Frenkelia* infection in *M. arvalis*.

• *Frenkelia microti* in *Microtus agrestis*

As the *M. agrestis* sample was not large enough to segregate captures between hedgerow network and forest, the data were pooled, and habitat was excluded from the model. Here, the main effect on infection is due to host age only (Chi² = 7.851, p = 0.005) whereas year, season, host sex and relative density are not significant.

EFFECT OF PARASITES ON WEIGHT AND SEXUAL ACTIVITY OF THE HOST

To assess the possible impact of parasitism on weight and sexual activity in rodents, infection in the two numerous and well sampled hosts, *C. glareolus* and *M. arvalis* was analysed.

• *Frenkelia glareoli* in *C. glareolus*

Table IV shows the effect of *F. glareoli* infection on the weight of *C. glareolus* with season, host sex and

Source of variation	<i>C. glareolus</i> model		<i>M. arvalis</i> model	
	F	p	F	p
Host infection	0.019	0.889	0.583	0.445
Host sex	9.857	0.002	13.991	0.0002
Season	36.474	< 0.0001	131.277	< 0.0001
Infection × sex	0.882	0.348	4.749	0.297
Infection × season	4.649	0.010	1.421	0.242
Sex × season	26.024	< 0.0001	4.672	0.009
Infection × sex × season	0.668	0.513	0.856	0.425
Host age (elw)	184.219	< 0.0001	546.805	< 0.0001

Table IV. – Intrinsic and extrinsic sources of variation in the body weight in *Clethrionomys glareolus* infected with *Frenkelia glareoli* and in *Microtus arvalis* infected with *Frenkelia microti* through ANCOVA. Host age, estimated by the eye lens weight (elw), is entered as a covariate in each model.

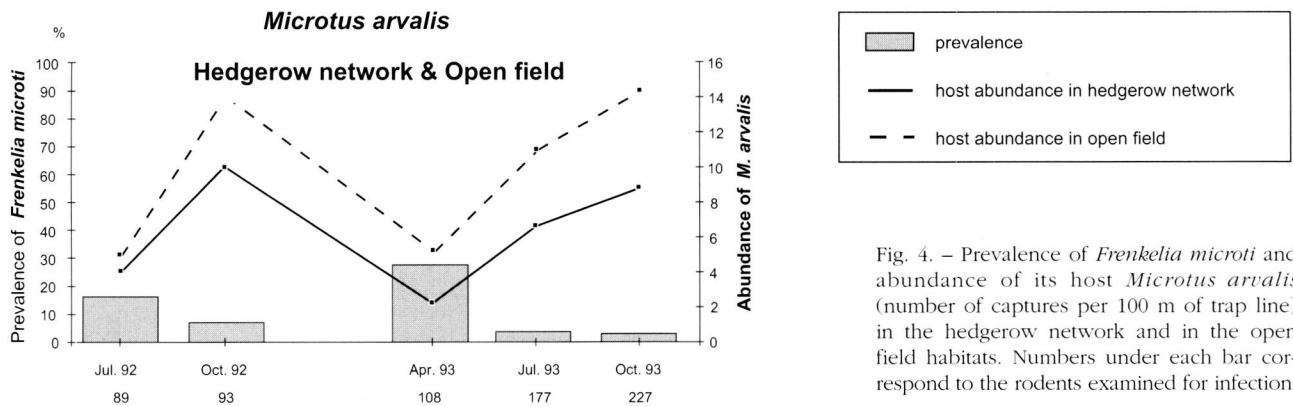


Fig. 4. – Prevalence of *Frenkelia microti* and abundance of its host *Microtus arvalis* (number of captures per 100 m of trap line) in the hedgerow network and in the open field habitats. Numbers under each bar correspond to the rodents examined for infection.

***Frenkelia microti* in *Microtus arvalis*
Hedgerow network & Open field**

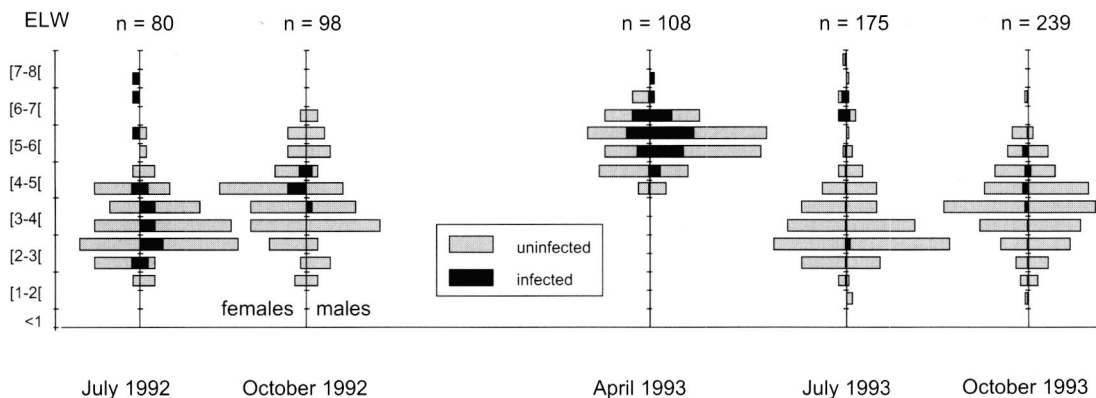


Fig. 5. – Distribution of *Frenkelia microti* in *Microtus arvalis* by eye lens weight (ELW in mg) and sex of host.

age taken into account through ANCOVA. Weight is significantly affected by age, sex and season but not by *Frenkelia* infection. The two way interaction, infection × season, is significant. This is illustrated in Figure 6

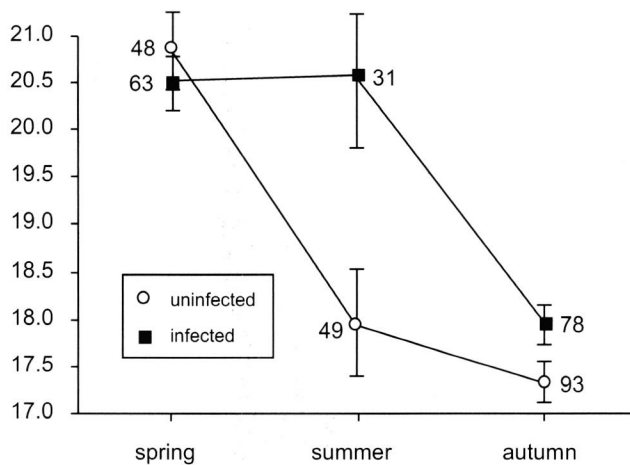


Fig. 6. – Mean body weight (in g with standard error bars) in infected and uninfected *Clethrionomys glareolus*, by season. Number close to each symbol indicates the sample size.

showing that, in summer, the infected voles are heavier than the uninfected ones.

Using multiple logistic regression, sexual activity in both sexes is significantly correlated with year, season, host sex and age, but not with habitat, relative density or *Frenkelia* infection (Table V). For males, the length × breadth of the seminal vesicles is highly correlated with host age ($F_{1,200} = 58.980, p < 0.0001$) and

Variables	<i>C. glareolus</i> model		<i>M. arvalis</i> model	
	Chi ²	p	Chi ²	p
Infection	0.200	0.655	6.205	0.013
Year	3.980	0.046	32.940	< 0.0001
Season	18.867	< 0.0001	97.888	< 0.0001
Habitat	0.151	0.697	17.105	< 0.0001
Host sex	16.044	< 0.0001	0.470	0.493
Host age	16.578	< 0.0001	42.791	< 0.0001
Host relative density	0.145	0.703	11.802	0.0006

Table V. – Logistic regression results for sexual activity in *Clethrionomys glareolus* infected with *Frenkelia glareoli* and in *Microtus arvalis* infected with *Frenkelia microti*.

season ($F_{2,200} = 53.222$, $p < 0.0001$) but not with *Frenkelia* infection ($F_{1,200} = 2.506$, $p = 0.115$). There were insufficient pregnant females in the sample to test for differences in litter size.

• *Frenkelia microti* in *M. arvalis*

The same analysis as above shows that the variation in weight of *M. arvalis* was mainly due to host age and sex, and to season, but not to *Frenkelia* infection. The significant two way interaction, season \times host sex, is due to the increased weight of females in autumn (Table IV).

The sexual activity in *M. arvalis* also showed a multifactorial dependency pattern, significantly correlated with year, season, host age, relative density, habitat and infection (Table V). The most interesting correlations are those concerning habitat and *Frenkelia* infection. Their partial coefficients show that open field and infection are negatively correlated with sexual activity ($r = -0.131$, $p < 0.0001$ and $r = -0.069$, $p = 0.014$ respectively). This suggests a lower sexual activity in open field than in hedgerow network, and in infected voles than in uninfected ones. Seminal vesicle size was not influenced by *Frenkelia* infection ($F_{1,359} = 0.949$, $p = 0.330$) but only by age ($F_{1,359} = 102.341$, $p < 0.0001$) and season ($F_{2,359} = 22.567$, $p < 0.0001$). Litter size was related to the season only ($F_{2,91} = 10.478$, $p < 0.0001$) but not to *Frenkelia* infection ($F_{1,91} = 0.022$, $p = 0.871$).

DISCUSSION

OCCURRENCE OF *FRENKELIA* SPP. IN INTERMEDIATE HOSTS

The main host for *F. glareoli* is clearly *C. glareolus*, with almost 50 % prevalence overall. The other important host for this species is *A. terrestris*, which appears to be a new host record. Bearing in mind the fact that only juvenile animals of this species were sampled, the

10 % prevalence is probably an underestimate of the real prevalence.

Apodemus spp, *Microtus arvalis* and *M. agrestis* are incidental hosts, with low prevalence of infection. A similar result was found in the Czech Republic by Vorisek *et al.* (1998). It is not clear whether these low prevalences are due to innate resistance in most individuals, lower exposure to infection (unlikely for *Microtus* spp, as these are infected with *F. microti* which has the same transmission mechanism), or high mortality of infected animals. The main hosts of *F. microti* are confirmed to be *M. arvalis* (9 % prevalence) and *M. agrestis* (8 % prevalence). *C. glareolus* is clearly an incidental host for this parasite.

This study shows that each of the *Frenkelia* species is maintained by two main intermediate hosts, which inhabit wooded habitats such as forest or hedges in hedgerow network, and grassland such as open field or fields in hedgerow network.

VARIATIONS IN PREVALENCE

Host age is the main factor influencing the prevalence of *Frenkelia* in voles (Table VI). This positive relation has been pointed out in rodents infected with many parasites such as cestodes (Behnke *et al.*, 1993, 1999), trematodes (Duplantier & Sène, 2000), protozoa (Turner, 1986), bacteria (Godeluck *et al.*, 1994; Fichet-Calvet *et al.*, 2000) and viruses (Mills *et al.*, 1992). These results suggest that as the rodents age, the probability of infection increases. In *M. arvalis*, the infection can occur very early in its life, around 20-30 days in summer. This age was extrapolated from the ELW measures of captive-bred animals (Martinet, 1966). In *C. glareolus*, host age is the only factor correlated with the prevalence of *F. glareoli* whereas year and host relative density also showed a distinct influence on the prevalence of *F. microti* in *M. arvalis*. *F. microti* was more prevalent in 1992 than 1993 and during this time, the density of *M. arvalis* was stable. As the buzzard population declined in 1993 (pers. obs.), it is suggested

Source of variation	Prevalence				Sexual activity	
	<i>Fg</i> in <i>Cg</i>	<i>Fg</i> in <i>At</i>	<i>Fm</i> in <i>Ma</i>	<i>Fm</i> in <i>Mg</i>	<i>Cg</i>	<i>Ma</i>
Year	0	0	– (1993)	0	– (1993)	– (1993)
Season	0	0	0	0	+ (spring & summer)	+ (spring & summer)
Habitat	0	NI	0	NI	0	– (Open Field)
Host sex	0	+ (female)	0	0	+ (male)	0
Host age	+	+	+	+	+	+
Host relative density	0	NI	–	0	0	+
Infection					0	– (infected)

Table VI. – Summarized effects variables on the prevalence of *Frenkelia* infection and on sexual activity. 0 = not significant, + positive, – negative. Information between brackets indicates which level is source of variation for nominal factors. *Fg* = *Frenkelia glareoli*, *Fm* = *Frenkelia microti*, *Cg* = *Clethrionomys glareolus*, *At* = *Arvicola terrestris*, *Ma* = *Microtus arvalis*, *Mg* = *Microtus agrestis*. NI = non included.

that the reduction in prevalence may be related to the decrease in density of buzzards. The negative correlation between prevalence and host relative density indicates that when the voles are most numerous, the infection rate is lowest. The buzzard is a common predator of the two voles, but the density effect is not discernible in *C. glareolus*, probably because of the relative stability of their population.

Even though prevalence fluctuates seasonally, season has no impact on prevalence when host age is taken into account. In spring, when the population of *C. glareolus* and *M. arvalis* was at its minimum, consisting only of old adults that had survived the winter, prevalence of both *Frenkelia* spp. was maximal (*F. glareoli*: 62 %, *F. microti*: 29 %). Infected animals were then diluted by newly born individuals between spring and autumn and, as the older individuals died off, overall prevalence declined (*F. glareoli*: 32 %, *F. microti*: 3 %). These findings agree with those of Laarman *et al.* (1979) in the Netherlands where, in winter and early spring, most of bank voles were infected whereas only 25-30 % were infected in summer. In the Czech Republic, Vorisek *et al.* (1998) found a mean prevalence of 16 % in spring which is similar to that observed here in the *C. glareolus* living in forest. The declining prevalence of *F. microti* between July and October is explicable partly by the extension of the breeding season into the autumn, and continuing dilution with young individuals.

The data suggest that *F. microti* and *F. glareoli* are equally transmitted all through the year. The high prevalence of *F. glareoli* in young of both *C. glareolus* in July and *A. terrestris* in April, indicating a high rate of transmission in spring and early summer, supports this hypothesis.

In *M. agrestis*, the prevalence of *F. microti* in April 1993 (22 %) was greater than that observed in Finland (6 %) in the same season (Soveri *et al.*, 2000). In the winter of 1992-1993, buzzards were unusually abundant on the Jura plateau, which may explain this difference.

Habitat had no impact on the prevalence of *Frenkelia* spp. The bank voles were equally infected in hedgerow network as in the forest. The irregularity of the forest boundaries and clearings make the forest a mosaic where the permeability of the parasite is equal to that in the hedgerow network. A comparative study in a landscape with larger areas of unbroken forest would be necessary to show any impact of habitat on prevalence. Infection in *Microtus* spp. is equally prevalent in enclosed and open grassland and, here again, a larger open field would be necessary to show any impact of the habitat in relation to the behaviour of the buzzard, which spends more time on open than closed habitats.

EFFECTS OF PARASITES ON HOST WEIGHT AND SEXUAL ACTIVITY

In the overall samples, when animals of all ages were represented, and before correcting for age, infected voles of both species were systematically heavier than uninfected individuals (*C. glareolus*: 19.3 ± 3.1 g, $n = 172$ vs 18.3 ± 3.5 g, $n = 190$; *M. arvalis*: 21.6 ± 6.3 g, $n = 63$ vs 18.2 ± 6.2 g, $n = 607$). For *C. glareoli* infected by *F. glareoli*, this trend was particularly true in summer when voles were reproducing. Hoogenboom & Dijkstra (1987) found a similar effect in another heteroxenous coccidian in the muscles of *M. arvalis*, *Sarcocystis cernae*, in which infection was associated with increased weight, but this study was not fully adjusted for age, and older, heavier animals are more likely to be infected. When the sample is restricted to similar season and age cohort, as for *Psammomys obesus* infected with *Bartonella* spp. or *Babesia* spp. in Tunisia, the weight is equal in, infected and uninfected animals (Fichet-Calvet *et al.*, 2000). In our study, infection with *Frenkelia* spp. had no impact on body weight when season, sex and age were taken into account. These last three factors are normally the main determinants of body weight. More interesting are the results concerning sexual activity, which also depends on season and age (Table VI). Sexual activity was also dependent on year, with a higher probability of inactivity in 1993. This lack of sexual activity could explain why the *M. arvalis* population crashed in 1994 following a period of high density lasting three years (unpublished data). *M. arvalis* was less sexually active in open field than in hedgerow network, indicating that the crash began in open field before continuing in hedgerow network. Sexual activity was positively related with density, reflecting continuation of reproduction into the autumn and an accumulation of several cohorts born in the previous spring and summer when *M. arvalis* was abundant. In males, the infection was not correlated with the size of the seminal vesicles. In pregnant females, the infection did not affect the litter size. However, infected individuals of *M. arvalis* were less sexually active than uninfected ones. This suggests that *F. microti* may delay female sexual maturity. Mechanisms such as a delay in the first pregnancy or an increasing time between litters have been shown in *C. glareolus* infected with cowpox virus in UK (Feore *et al.*, 1997). Our result is consistent with a possible regulation of host population by parasitism. An additional regulatory effect on the intermediate host populations could operate through an increased risk of predation leading to reduced longevity and a reduced number of litters produced by predated individuals. Against this, there is no evidence of reduced prevalence in the oldest animals, indicating that longevity is not reduced in infected individuals.

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