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Infection with cowpox virus decreases female maturation rates in wild populations of woodland rodents

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Sublethal effects of parasitic infection, such as reductions in reproductive rate, can significantly affect host population dynamics. Here we show that in wild populations of both *Clethrionomys glareolus* (bank vole) and *Apodemus sylvaticus* (wood mouse), females infected with cowpox virus are likely to delay maturation and therefore reproduction – in most cases until the following breeding season. Some infected bank voles do mature in their year of birth but still take longer than uninfected females. Together with our previous demonstration that individuals infected with cowpox virus in the summer survive better than uninfected individuals, these results support the prediction that hosts that develop an acute infection may best optimise their fitness by decreasing current reproduction to maximise the probability of surviving infection. Moreover, as the proportion of individuals infected increases with density, the reduction in host fecundity may have significant consequences for host dynamics.

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There is now widespread recognition of the possible role of parasites in both host population dynamics (Tompkins et al. 2002) and life history evolution (Schmid-Hempel 2003). Theoretical studies have demonstrated that regulation of host populations is possible if parasitism influences demographic rates in a density dependent manner (Anderson and May 1978), and that sublethal (e.g. fecundity) effects can have greater regulatory effects than mortality (Dobson and Hudson 1992). Life history theory indicates that infection may change optimal patterns of resource allocation and result in individual hosts modifying their reproductive strategies to reduce the costs of infection (Agnew et al. 2000).

The evolutionarily optimum for hosts with acute (i.e. nonpersistent) infections may be to down-regulate current reproduction, increasing the chances of survival and future reproduction (Forbes 1993, Perrin et al. 1996). To date, no study has demonstrated such a pattern in wild populations or in a host–microparasite system. Microparasites are parasites that tend to reproduce within the host such as bacteria, viruses and protozoa. As one feature of many microparasitic infections is host recovery and development of life long immunity, a strategy that increases the chance of surviving the infection may well maximise reproductive success. Adaptive changes in reproductive effort appear particularly feasible in small rodents, since most exhibit phenotypic plasticity in reproductive traits (Krebs and Myers 1974). Recent work from our group has demonstrated that wild populations of rodents in Britain are infected with several endemic microparasites (Birtles et al. 2001, Noyes et al. 2002, Begon et al. 2003, Bown et al. 2003).

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Previous work has examined the effect of cowpox virus on survival of *Clethrionomys glareolus* Schreber (bank vole) and *Apodemus sylaticus* L. (wood mouse) in the field (Telfer et al. 2002). Cowpox virus causes endemic infections with no clinical symptoms and an infectious period of 4 weeks. The effect on survival varies seasonally: infected individuals survive better in summer but suffer higher mortality in winter. One possible explanation is that in summer, infected animals cease reproduction and consequently enjoy better survival than uninfected but reproductively active animals, whilst in winter, when very few individuals are reproductively active, animals that become infected have poorer survival when compared with uninfected individuals. Previously the only evidence of cowpox affecting reproduction came from laboratory studies (Feore et al. 1997).

Here we investigate the effect of cowpox virus infection on the rate of female maturation, which is known to have important consequences for rodent population dynamics (Agrell et al. 1992). As the impact on population dynamics will be greatest if prevalence is density-dependent, we also investigate how prevalence varies with current and past densities.

Methods

Bank voles and wood mice were trapped, monthly, within 1 ha plots at two mixed woodland sites in north-west England (Rake Hey: N53:20 W03:02 and Manor Wood: N53:19 W03:03). Rake Hey was trapped from April 1995 to December 1998 and Manor Wood was trapped from April 1995 to June 2002. At each site a 10×10 grid was marked out with 100 trap stations, permanently situated at 10 m intervals. Two Longworth traps (Penlon Ltd., Oxfordshire, U.K.) were placed at each trap station. Each site was trapped approximately monthly for a period of 2–3 days, with traps checked daily. All bedding material and obvious waste was removed from traps containing animals and they were cleaned with 70% ethanol prior to being reset. Traps were sterilised in an autoclave between trapping sessions. All animals captured were tagged using subcutaneous microchips. On first capture within a monthly trapping session sex, reproductive condition and weight were recorded and a 20–40 µl blood sample was taken from the tip of the tail. Females were defined as mature if they had, or had previously been recorded with, a perforate vagina. Sera were separated from blood samples and the presence of cowpox virus antibody determined by immunofluorescence assay (Bennett et al. 1997).

For each female recorded as perforate, age at maturity was estimated as the interval between the individual's estimated birth date and its first perforate capture. The latest possible birth dates were calculated by subtracting 14, 42 and 70 days from the date of first capture for individuals of juvenile, subadult and adult weight respectively (Telfer et al. 2002). This may result in underestimates of age at maturity. Due to the asymptotic nature of growth curves, individuals first caught at an 'adult' weight are most likely to have error associated with their estimated birth dates, with some individuals being older than the 10 weeks assumed. If this is a considerable problem in the data, individuals first caught as adults are expected to show higher variance in their estimated times to maturity and we tested for homogeneity of variances between individuals classified as juvenile, subadult and adult at first capture. The underestimation of age at maturity due to errors in assigning birth dates will tend to be counteracted by the fact that trapping is not continuous. Individuals may have reached maturity before their first recorded perforate capture, leading to overestimation of age at maturity. Hence, to minimise errors in the estimated age at maturity, females had to conform to strict rules before being included in the data set. We established the usual breeding season for each species from the estimated birth dates of individuals first caught as juveniles or subadults. For bank voles this was April-October (97%, $n = 163$): for wood mice May-November (91%, $n = 328$). Individuals with estimated birth dates from other months were excluded. The following were also excluded: individuals perforate on first capture, and individuals with a missing capture or recorded as pregnant prior to their first perforate record. These precautions will have reduced statistical power (sample size) but not introduced bias.

In both species the distribution of age at maturity was bimodal (Fig. 1). First, therefore, we investigated what factors influence the probability of delaying maturation to the second peak. This is predominantly a distinction between animals maturing in the calendar year of birth and the following year. However, as age at maturity is estimated in days, wood mice born late in a calendar year and maturing the following year appeared within the first peak, and one bank vole born early in the breeding season that bred late on in its year of birth appeared in the second. Consequently, based on the observed distributions, a threshold of 140 days at maturity was used in both species to distinguish individuals that delayed. As the threshold created a binary dependent variable, we used generalized linear modeling with a logit link and binomial errors.

Secondly, we investigated what factors influence the age at maturity of individuals in the first peak. With discrete trapping sessions, age at maturity is not a continuously distributed variable. We used an ordinal regression with 3 levels of the response variable: <2 months, 2–3 months, >3 months.

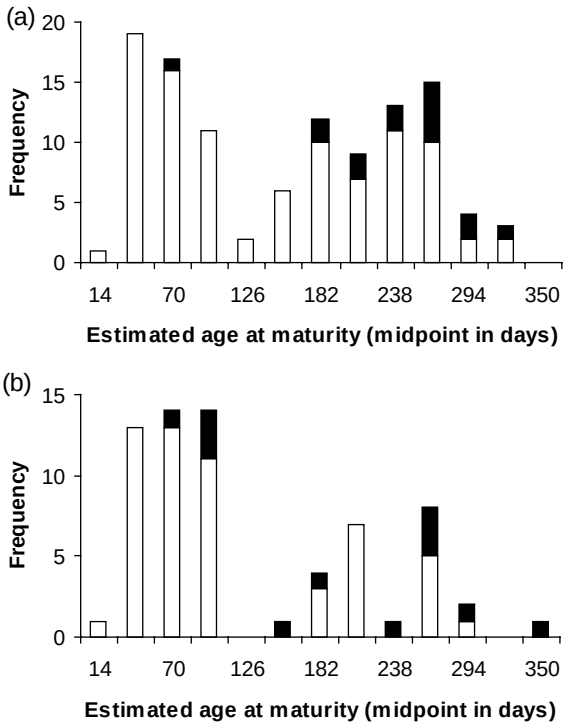

Fig. 1. Frequency distribution of the estimated age at maturity for (a) wood mice and (b) bank voles. Individuals that become infected with cowpox before maturing are indicated by black bars.

In both analyses we included individual and population level covariates, examining the effects of site, year of birth, cohort, population density and cowpox infection prior to maturity. 'Cohort' was based on the estimated birth date and on bimonthly cohorts assigned a priori (starting with January-February). Three (Jolly–Seber) estimates of population density were investigated: density in the month of birth, density in the month after birth, and density two months after birth. As errors associated with estimating the time to maturity may differ depending on the mass and subsequent age classification at first capture (above), we repeated the analyses using only individuals first caught as juveniles or subadults.

As voles born in the same month in the same site shared the same population level covariates, analyses should have included the interaction between site and month of birth as a random effect. Random effects were successfully predicted in the analysis of reproductive delay in wood mice, but the bank vole data proved too sparse and only fixed effects were examined. Nor was it possible to predict random effects in the ordinal regressions. For analyses without random effects, model selection was based on Akaike information criteria (AIC), with an AIC difference of 2 being considered significant (Burnham and Anderson 1992). In the absence of model selection criteria such as AIC for generalized linear mixed models (GLMM), we conducted a backward stepwise selection procedure for analyses with random effects, retaining only terms significant according to t-tests for the parameter coefficients. Results should be treated with some caution where it was not possible to predict random effects.

For each species we investigated whether the proportion of infected individuals was related to site, current population density, density 3 months previously and/or density 6 months previously, using Jolly–Seber estimates of population size. To reduce the problem of non-independence in successive samples, analysis was limited to August trapping sessions (when cowpox prevalence tends to peak). We conducted logistic regressions on the proportion of infected individuals (Telfer et al. 2002) with model selection based on AICs.

Results

Overall, 36% of bank voles (n = 66) and 55% of wood mice (n = 112) delayed breeding and did not mature until greater than 140 days old. The distributions of age at maturity in non-delay individuals were similar in both species (Fig. 1). There was no evidence of increased variance in the estimated time to maturity for individuals first caught at an adult weight (Bartletts test for homogeneity of variances: bank voles $\chi^2_2 = 0.358$, $p = 0.836$; wood mice $\chi^2_2 = 4.53$, $p = 0.10$).

Probability of delaying maturation

For wood mice, due to problems estimating coefficients, observations were omitted for individuals born in May or June (none delayed breeding; n = 11) and for 1998 (only one individual conformed to the rules for inclusion) leaving 100 individuals, of which 15 became infected before maturing. The best model (Table 1) had no effect of cohort (with May-June omitted), but females were significantly less likely to delay breeding in 1995 than in other years; the probability of delay increased significantly with the population density at age 1 month (odds ratio 1.05; 95% C.I. 1.01–1.09); and also increased significantly in females that became infected with cowpox virus (odds ratio 122.29; 95% C.I. 2.86–5231.78). When only individuals first caught at a juvenile or subadult weight were included, the best model remained the same (data not shown).

For bank voles 66 animals were included, of which 12 became infected before maturation. In the best model (Table 2), the probability of delay increased significantly for cohorts born later in the season (Table 1), and was again associated with both high population densities at age 1 month and cowpox virus infection (odds ratios:

Table 1. Parameter estimates (logit scale) and standard errors (s.e.) for the best models of (a) delayed breeding in female wood mice (b) delayed breeding in bank voles and (c) age at maturity in bank voles that did not delay. For (a) model selection was based on a backward stepwise procedure, retaining only terms significant according to t-tests for the parameter coefficients. For (b) and (c) random effects could not be estimated (text) and model selection was based on the AIC (see Table 2). 0 = reference group for categorical variables. '.' = no significant effect. NA = no data for that category in the analysis. The variance component (σ_u) reflects differences in the intercepts of the different site*month groupings.

	(a) Delayed breeding in wood mice			(b) Delayed breeding in bank voles	(c) Age at maturity in bank voles that did not delay
Effect	coefficient (se)	t-value	prob > t	coefficient (se)	coefficient (se)
fixed					
Intercept 1	−6.34 (2.26)	−2.80	0.005	−0.94 (1.97)	−2.78 (1.19)
Intercept 2	–	–	–	–	0.10 (1.02)
Cohort: April	.	.	.	−5.99 (1.68)	1.96 (1.35)
May/June	.	.	.	−3.98 (1.96)	0.36 (0.77)
July/Aug	.	.	.	−1.03 (1.46)	0
Sept	.	.	.	0	NA
year: 1995	0	–	–	.	0.35 (0.71)
1996	2.05 (1.20)	1.71	0.087	.	0.70 (0.80)
1997	5.74 (1.92)	2.99	0.003	.	−2.16 (0.85)
1998	NA	–	–	.	1.48 (1.08)
1999	3.19 (1.81)	1.76	0.078	.	−2.40 (0.97)
2000	4.61 (2.03)	2.27	0.023	.	NA
2001	5.75 (2.06)	2.79	0.005	.	0
density at 1 month	0.05 (0.02)	2.58	0.010	0.05 (0.03)	.
cowpox	4.81 (1.92)	2.51	0.012	3.02 (1.56)	1.79 (0.74)
random					
σ_u	1.27 (0.60)			.	.

density 1.05; 95% C.I. 0.99–1.12; cowpox 20.45; 95% C.I. 0.96–437.31). Unfortunately, when only individuals first caught at a juvenile or subadult weight were included it was not possible to estimate the effects of cohort due to lack of data. However, with cohort removed, both density at age 1 month and cowpox virus infection remained significant (data not shown).

Age at maturity in non-delay individuals

To enable estimation of cohort and year effects, the single bank voles from the September/October cohort and from 2000 that did not delay were excluded. Hence, 40 bank voles were included, of which 4 became infected before they matured. Only one wood mouse that became infected did not delay, so wood mice were not analysed further. Among bank voles (Table 1, 2), individuals born early in the season took significantly longer to mature; females matured significantly faster in 1997 and 1999 than in other years; and individuals infected with cowpox virus took significantly longer to mature (odds ratio 5.99; 95% C.I. 1.40–25.54).

Density dependence in prevalence

In both species, the proportion of individuals infected in August increased with current density (Table 2; parameter estimates on logit scale (SE): wood mouse intercept −4.97 (0.67); current density 0.023 (0.007); bank vole intercept −4.48 (0.76); current density 0.025 (0.010)). Also, the proportion of bank voles infected was lower at Manor Wood (−0.98 (0.20)).

Table 2. Model selection for analyses without random effects. For each analysis the best model is shown in bold and all models with fewer parameters and an AIC within 3 of the best model are shown. np = number of parameters. Parameter estimates for the best models for analyses (a) and (b) are shown in Table 1 and for analyses (c) and (d) are given in the text.

Analysis	Model	Deviance	np	AIC
(a) delayed breeding in bank voles	**cohort + density(1month) + cowpox**	**24.80**	**6**	**36.80**
	cohort + cowpox	29.24	5	39.24
	cohort + density(1month)	29.46	5	39.47
(b) age at maturity in bank voles that did not delay	**cohort + year + cowpox**	**58.69**	**10**	**78.69**
	year + cowpox	29.46	8	81.07
(c) effect of density and site on cowpox prevalence in wood mice	**current density**	**214.79**	**2**	**218.79**
(d) effect of density and site on cowpox prevalence in bank voles	**site + current density**	**286.92**	**3**	**292.92**

Discussion

In wild populations of both bank voles and wood mice, we found that cowpox virus infection was strongly associated with an increased age at maturity in females. In principle, individuals in poor condition could have been more prone to both infection and delayed maturation, but two observations point strongly to infection causing the delay. First, a key determinant of condition is likely to have been density (resource shortage), and this is accounted for in the analyses quite separately from infection. Second, the results support entirely our previous laboratory experiments, where infection increased the time to first litter by 20–30 days in both species (Feore et al. 1997). Indeed, it appears that in the natural, seasonal environment the effects are more dramatic. In the laboratory, infected bank voles and wood mice produced litters at mean times of 61 and 75 days respectively, whilst in the wild many infected females of both species matured in the following breeding season, taking more than 140 days. For bank voles that did not delay for more than 140 days, infection was associated with increases similar to those in the laboratory. Thus, although in 2 of the 3 analyses it was not possible to predict random effects, the consistency of the results provides compelling evidence of an effect of cowpox virus infection in both species.

Maturation rates in bank voles and wood mice also showed patterns in relation to other variables in line with previous studies: early cohorts were less likely to delay breeding (Green 1979, Bujalska 1983); and high densities decreased maturation rates (Krebs and Myers 1974, Agrell et al. 1992, Lambin 1994). There was evidence of variation between years, probably attributable to factors not considered in the present study such as food availability (Flowerdew 1973).

In several rodent species, the vagina is sealed during anoestrus and as trapping was conducted monthly we may have missed the first perforate capture of some individuals. We tried to minimise this by excluding animals recorded as pregnant prior to their first perforate record. However, clearly individuals that switch back to being imperforate but do not successfully breed may be wrongly assigned as non-mature animals. In the context of this study, however, the important point is whether infection could influence this probability of mis-assignment. Vaginal opening is related to receptivity to mate and can be influenced by the social environment of an animal (e.g. Féron and Gheusi 2003). Thus, even if infection is influencing the probability of being recorded as imperforate following maturation, rather than the probability of maturing, infection is still having a significant effect on reproductive status.

A reduction in fecundity in parasitised hosts has been observed in several studies of endemic macroparasites in wild populations (Tompkins and Begon 1999). Studies showing this effect with endemic microparasites are much less common (but see Schall 1983), and it appears never to have been observed previously for an acute infection. After their first litter, bank voles and wood mice produce litters at regular intervals of 20–30 days during the summer (Clarke 1985). Consequently, the probability of delaying breeding until the following year and the time to maturation are critical demographic parameters, and infection with cowpox virus will have an important effect on individual fitness.

Reductions in the fecundity of infected hosts are usually interpreted simply as a cost inflicted by the parasite. However, such reductions are also consistent with manipulation by the parasite or with hosts modifying their reproductive strategy to optimise fitness. Models predict that hosts infected with parasites where impact increases with time should preferentially allocate resources to reproduction (Gandon et al. 2002); but hosts with parasites that cause acute infections from which the host may recover, like cowpox virus, should allocate resources to maximising survival, possibly through reduced reproduction (Perrin et al. 1996). In our system, it seems particularly plausible that hosts have evolved to optimise fitness following infection, since we have demonstrated that time to maturation and survival both increase in infected hosts. In the only previous study reporting both a reduction in fecundity and an increase in survival, the relative roles of host response and parasite manipulation are uncertain (Hurd 1998), because the tapeworm parasite may increase its own transmission by increasing host (beetle) survival (Hurd et al. 2001). However, an increase in cowpox virus transmission due to a reduction in maturation rate seems unlikely.

Thus, female bank voles and wood mice infected with cowpox mature at a slower rate than uninfected females. Relative to an infected individual that attempts to reproduce and must suffer both the costs of mounting an immune response and reproduction, an infected individual that delays breeding may optimise its fitness. However, due to the high mortality rates of small mammals and the frequency with which they can produce litters, an individual that delays reproduction following infection is still predicted to have lower fitness than an individual that remains uninfected and matures rapidly. Unfortunately, as it is difficult to assign juveniles to parents from field data, it is not possible to measure lifetime reproductive success. Importantly, as cowpox prevalence increased with density in both species and populations with a high prevalence of cowpox are predicted to have reduced fecundity, cowpox virus infection has the potential to significantly influence population dynamics. Additional work with other pathogens is required to determine whether our finding is a common response by small mammals

to acute infections, and whether this contrasts with the response to chronic infections, as predicted by theory.

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