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Kielder review

SUMMARY

INTRODUCTION

Almost ninety years ago, Charles Elton drew attention to the potential importance of parasites in the dynamics of natural populations (Elton 1924; Elton *et al.* 1931). Nonetheless, for many decades in the twentieth century, both this and the dynamics of the parasites themselves were Cinderella subjects in ecology, neglected in comparison with their sister interactions, predation and competition. The tide was turning, though, in the 1970s and early 80s, and may be said to have done so decisively with the publication of seminal papers by Roy Anderson and Robert May at that time (Anderson & May 1979; May & Anderson 1979). However, these papers and many that followed were theory-oriented, and field data with which to confront these theories were then - and remain still - in relatively short supply.

In Liverpool in the 1990s, separate lines of research were being pursued on host-parasite dynamics in laboratory populations of moths (Begon *et al.* 1996) and the distribution of a zoonotic pathogen, cowpox virus, in natural populations of rodents (Crouch *et al.* 1995). There were obvious attractions in moving from the laboratory to the field, in looking more deeply into the ecology of cowpox virus, and in re-visiting host-parasite dynamics in the favoured hosts of Charles Elton (himself a Liverpudlian). Work was initially on bank voles (*Myodes glareolus*)

and wood mice (*Apodemus sylvaticus*) in woodland habitats on the Wirral, near Liverpool, where both species exhibited clear annual (autumn) peaks in abundance, but no demonstrable multi-annual patterns and indeed only moderate variation in abundance from year to year. Then, from the early 2000s, the focus started to shift to populations of field voles (*Microtus agrestis*) in Kielder Forest on the England-Scotland border, living in grassland habitats and exhibiting multi-annual cycles in their abundance. These latter are the focus of this review. However, where related work on bank voles and wood mice sheds light on the Kielder field vole system, this too is described.

Studying hosts with cyclic dynamics offers two particular advantages in the context of host-parasite dynamics in natural populations. First, where the aim is to investigate the role of parasites in driving host dynamics, it is necessary to know what is 'signal' in those dynamics (and hence liable to explanation) and what is simply noise. In cyclic systems there is a clear dominance of signal over noise. Second, many key aspects of the dynamics of the parasites themselves, including those concerned with transmission and host condition, are dependent on host density. In order to have the statistical power to study these effects in a natural population, a system must provide observations across a whole spectrum of densities. Systems with cyclic dynamics are likely to do this and to do this in a predictable way that allows scientific investigation to be planned.

BACKGROUND TO THE SYSTEM

The Kielder Forest area had a long history of being affected by irruptions of field voles, sometimes of plague proportions (Elton *et al.* 1935; Elton 1942). Indeed,

some of the first steps in studying the population ecology of disease by researchers in Charles Elton's Bureau of Animal Populations were made on field voles in or near Kielder Forest (Chitty 1954, 1996). The move from Liverpool to work on field voles at Kielder was greatly facilitated by the initiation of a collaboration with Xavier Lambin's group at the University of Aberdeen. Prior to the collaboration, the group had been investigating the processes behind temporal and spatial dynamics of field voles (e.g. Mackinnon *et al.* 2001), including the role of predators in shaping spatial dynamics (**Sherratt, 2001**), and had been utilising both monitoring and field-experimental approaches (Graham & Lambin 2002; Ergon *et al.* 2004). In addition, a study of raptor dynamics has entailed monitoring field vole abundance since 1983 (**Petty 1992)** and thus the spatio-temporal dynamics of field voles in Kielder are exceptionally well characterised. Data reveal a pattern of multi-annual fluctuation with peak vole densities occurring at a 3-4 year interval, followed by steep population collapses usually taking place in summer during the vole breeding season, and followed by up to a year with little noticeable population growth. Within a single grass patch, maximum densities span 5 to 770 voles/ha (Burthe et al., 2006), but at a landscape scale the span is 50-220 voles/ha (Lambin et al., 1998, Lambin et al., 2000, Mackinnon et al., 2001). Critically, there is no evidence that voles ever go extinct at any spatial scale in the system. This has implications for the dynamics of species linked to voles. Neither predators nor pathogens are expected to experience extinction-recolonisation dynamics in the system.

The population cycles are generally asynchronous among populations across Kielder, although populations situated close together often fluctuate in a

synchronous manner (Lambin *et al.* 1998). Indeed, vole spatial dynamics in Kielder Forest were, at least for a time, spatially organised in travelling waves (Lambin *et al.* 1998). These spatial dynamics, in addition to their intrinsic interest, provide scope for substituting space for time in increasing the range of host densities that can be sampled over a given time interval.

The field voles have 'fast' life histories typical of microtine rodents, with high fecundity (average litter size of five), a low age at maturation for some seasonal cohorts (28 days old for spring and early summer born females), and iterated reproduction at typically 21 day intervals by members of overlapping cohorts during a breeding season that, for the most part, coincides with the plant growing season. However, for individuals born in the second half of breeding season, reproduction is typically delayed until the next year. There is thus a strong seasonality to reproduction and the production of cohorts of susceptible individuals. The spacing system of juveniles and subadults, but also of females breeding in the year of birth, is characterised by non-defended home ranges. Dispersal is primarily by subadults. Thus there is also a distinct seasonal pattern to the spatial range and the number of individuals with who individuals make potentially infectious contacts.

Kielder Forest is intensely managed for timber production. Hence, grassland areas are restricted to roads and unplanted river margins, and restock sites where dense grass cover establishes 2-3 years after rotational timber harvesting and persists for 10-12 years after planting. Restock sites are typically 5-25 ha in size and are embedded in a matrix of dense spruce plantation with no grass

cover. The field vole is a grass-eating species that therefore relies more than bank voles on well-vegetated areas. The bank vole, common shrew (*Sorex araneus*) and least shrew (*Sorex minutus*) share many arthropod disease vectors and some pathogens with field voles and also mostly occupy grassy areas, whereas wood mice use both the forested landscape matrix and the semi isolated grassland patches.

THE DATA SETS

Many of the Kielder studies used all or part of two data sets, and so the nature and derivation of these are described first in outline. The one used most often was a longitudinal, time series data set. Field voles were trapped in four similar-sized clearcuts, in two areas of the forest approximately 12 km apart, between May 2001 and March 2007. Two sites in the Kielder catchment, Kielder Site (KCS) and Plashett's Jetty (PLJ) are situated 4 km apart. Two further sites in the Redesdale catchment, Black Blake Hope (BHP) and Rob's Wood (ROB) are 3.5 km apart. Thus, these four populations are far enough apart, with sufficient forest between them, to be considered as effectively independent replicates. Populations were trapped in 'primary' sessions every 28 days from March to November, and every 56 days from November to March. Each site had a permanent 0.3 ha live-trapping grid consisting of 100 Ugglan Special Mousetraps (Grahnab, Marieholm, Sweden), in optimal habitat dominated by *Deschampsia caespitosa, Agrostis tenuis,* and *Juncus effuses* grasses. Traps were set at 5 m intervals and baited with wheat and carrots. Traps were pre-baited with a slice of carrot and a few grams of oats 3 days before each trapping session, set at approximately 18:00 on the first day and checked five times ('secondary

126 sessions') at roughly 12 h intervals starting and ending at dawn and dusk,
127 respectively. Mass, sex, body condition and reproductive status (assigned
128 according to the external appearance of reproductive organs) were recorded at
129 the time of first capture in each primary session. Individual animals were
130 identified using subcutaneous microchip transponders (AVID plc, East Sussex,
131 UK) injected into the skin at the back of the neck. Total population size was
132 estimated by capture-recapture methods assuming a closed population from
133 data within a primary session.
134
135 A 20–30 μL blood sample was taken from the tail tip of each individual each
136 primary session, again usually in the first secondary session in which it as
137 caught. These provided the material for pathogen diagnoses and haematological
138 measurements (see below). The presence and (in some cases) the number and
139 identity of ectoparasites were also noted. In addition, the presence of external
140 skin lesions characteristic of late-stage tuberculosis (caused by the bacterium
141 *Mycobacterium* microti) (Cavanagh *et al.* 2002; Burthe, Bennett, *et al.* 2008) was
142 noted in the field. Hence, in those cases where individuals were recaptured in
143 one or more primary session, the time course of infections in individuals and
144 transitions in individual status could be monitored, as well as the profiles of
145 whole populations being followed over time..
146
147 The second, cross-sectional data set was derived from traps set bi-annually in
148 March (spring) and September (autumn) in 27 grass-dominated clearcut sites
149 (5–12 ha) within the three adjacent catchments of Kielder Forest. There were 12
150 sites located in the Kielder catchment, 10 within the Kershope catchment and 5

sites within the Redesdale catchment. The minimum and maximum inter-site distances were 0.4 km and 36.9 km respectively. Within each clearcut, small mammals were sampled using the small quadrant design (Myllymaki *et al.* 1971): a 15 m by 15 m trapping square was established in good quality field vole habitat and three Ugglan traps were set at each corner. Other procedures were as described for the longitudinal study. This cross-sectional approach carries with it the disadvantages of being intrinsically correlational, but sets against this the advantages of the additional data that can be obtained from individuals when they are sacrificed (e.g. larger blood specimen volume, organ samples, confirmed reproductive status, etc.).

The data set used for the immunological work and that provided data for genetic studies (see below) was separate from these. It came from repeated trapping at two spatially separate sites from February 2008 to March 2009 and a further two from April 2009 to March 2010 and had longitudinal and cross-sectional components. Each site contained a live-trapping grid (~ 0.375 ha) of 150 (10 × 15) regularly spaced traps (3-5m intervals) placed in optimal habitat for the longitudinal study. There were also satellite transects on each (with traps spaced at ≥5 m intervals) from which ten animals per month per site were sampled destructively (to allow a wider range of immunological measurements) for the cross-sectional component of the study. At each site, there were monthly trapping sessions from February/April to November, during which capture-recapture and destructive samples were taken. Then, in November and again in the following March, larger numbers of animals were destructively sampled both

from the transects and from the grid habitats. Other procedures were as described previously.

EPIDEMIOLOGY ON INDIVIDUAL PATHOGENS

The Kielder field voles are subject to infection by a number of endemic micro- and macroparasite species, of which the microparasite community has been the most extensively studied (Table 1). Many of these species are zoonotic or related to pathogens of medical or veterinary importance and include directly transmitted and vector-borne pathogens, and the ectoparasitic vectors themselves. A brief summary of the pathogen species most closely studied in Kielder follows.

Cowpox virus

Cowpox is an orthopoxvirus endemic throughout European and western Asian rodent populations (Baxby & Bennett 1999). Despite its name, cowpox virus rarely infects cattle and is actually most often diagnosed in domestic cats (Cavanagh *et al.* 2004). The virus is also a zoonosis, although human cases are rare (Baxby *et al.* 1994). Field voles, bank voles (*Myodes glareolus*) and wood mice (*Apodemus sylvaticus*) appear to be the primary reservoir hosts in the UK (Chantrey *et al.* 1999) with Kielder field voles exhibiting a prevalence of 28-100%, peaking in mid- to late summer (Burthe *et al.* 2006). A summer peak was also observed in the Liverpool populations of bank voles and wood mice, though at lower prevalences (Hazel *et al.* 2000). Animals infected with the virus develop an antibody response after around two weeks and remain infected for approximately four weeks (Chantrey *et al.* 1999).

Further analysis showed that both the number infected with cowpox virus (I) and the prevalence of infection (I/N) increased with total population size (N). (Begon *et al.*, 2009a) However, whereas previous work in bank voles had suggested a threshold abundance, below which infection was not found (Begon *et al.* 2003), evidence for such a threshold in field voles was at best equivocal, in spite of the wide range of abundances sampled.

Abundance in the Kielder field voles, N, was most strongly correlated with contemporary values of I and I/N, but in the case of the numbers of susceptible hosts (S), the strongest correlations were with values 1 to 2 months preceding the values of I and I/N. Thus, in transfer function analyses, as epidemiological theory would predict, values of S (which 'drive' new infections) were much more effective than those of N (of which S is only a component) in predicting future numbers infected (I). Nonetheless, while monitoring the number of susceptible individuals has most to offer, the results suggest that monitoring overall abundance, which is much commoner and more easily achieved, may nonetheless provide valuable insights into the dynamics of infection (Begon, Telfer, Burthe, *et al.* 2009).

The seasonality of cowpox virus dynamics was examined further by Begon *et al*. (Begon, Telfer, Smith, *et al.* 2009). The timing of seasonal peaks within the year was related to the multi-annual patterns of abundance displayed by the voles, which in turn was associated with both the number and the rate of recruitment of susceptible hosts. A plentiful and sustained supply of susceptible hosts

throughout the summer (March – September), such as occurs in the increase phase in the abundance cycle, gave rise to a steady rise in infected hosts and a peak late in the year – often October or November. However, a meagre supply of susceptible hosts more limited in time, such as occurs in a crash year, was often insufficient to sustain an increase in infected hosts, leading to an early peak, around June, followed by a decline. This was in contrast to more predictable seasonal peaks seen in some human infections (Stone *et al.* 2007), the lesson being that to understand seasonal disease dynamics in wildlife populations, the dynamics of the hosts themselves must be fully taken into account.

Vole tuberculosis

Vole tuberculosis (TB) is a chronic, endemic infection of field voles caused by *Mycobacterium microti,* a member of the *M. tuberculosis* complex (van Soolingen et al. 1998). TB is a zoonotic infection, having been infrequently recorded in both immunocompromised and immunosuppressed humans (van Soolingen *et al.* 1998; Niemann *et al.* 2000; Xavier Emmanuel *et al.* 2007). In voles, TB causes severe clinical pathology in the later stages of the disease, characterised by externally visible cutaneous lesions (Burthe *et al.*, 2008a; Cavanagh *et al.*, 2002; Cavanagh *et al.*, 2004). The definitive transmission route is unknown. However, wounding has been suggested due to the common occurrence of cutaneous lesions (Burthe, Bennett, *et al.* 2008) .

Prevalence of vole TB also showed evidence of delayed density dependence (Cavanagh *et al.* 2004). However, this approach underestimated TB prevalence (Wells 1946; Cavanagh *et al.* 2004; Burthe, Bennett, *et al.* 2008). Post-mortem

examination and culture of infected tissue samples from cross-sectional surveys indicated prevalence over twice that based solely on external signs, with up to 50% of voles infected in some sites (Cavanagh *et al.* 2002; Burthe, Bennett, *et al.* 2008). Prevalence of infection increased with vole mass (a proxy for age) and hence prevalence was highest in spring when the population was mainly comprised of older individuals (Burthe, Bennett, *et al.* 2008).

Anaplasma phagocytophilum

Anaplasma phagocytophilum is a an obligate intracellular bacterial parasite of granulocytes, which is historically associated with causing tick-borne fever in sheep and other livestock (Foggie 1949; Hudson 1950). In the 1990s, the zoonotic potential of *A. phagocytophilum* was realised (Chen *et al.* 1994), although different genetic variants appear to have restricted host ranges (Massung *et al.* 2003; Bown *et al.* 2009). Whilst little is known regarding the effects of *A. phagocytophilum* on rodents, it has well established immunosuppressive effects on livestock (Woldehiwet 2010). Rodents demonstrate no obvious clinical signs of infection and longitudinal studies indicate that infection is short-lived, with the majority of individuals testing positive by PCR for only a single month (Bown *et al.* 2003, 2008). Infection prevalence in field voles may reach 12% in late summer but disappears overwinter when no nymph or adult ticks are feeding (Bown *et al.* 2009).

Of the two most commonly found ticks in Kielder (see below), transmission in small mammals appears to be via *Ixodes trianguliceps* rather than *I. ricinus*, as the absence of *I. ricinus* had no significant effect on infection prevalence in field voles (Bown *et al.* 2008). Similarly, infection in both field voles and common shrews

follows the seasonal dynamics of *I. trianguliceps* nymphs (Bown *et al.* 2003, 2009, 2011).

Bartonella spp.

The bartonellae are gram-negative bacteria and facultative intraerythrocytic parasites of a wide range of mammalian species. Transmission mechanisms are not yet fully understood, but arthropods, often fleas, are important vectors (Birtles 2005a). Several *Bartonella* species are associated with disease in humans or animals (Anderson & Neuman 1997; Breitschwerdt & Kordick 2000). Well-known human infections include the body-lice-mediated *Bartonella quintana*, the causative agent of trench fever in World Wars I and II, and *B. henselae* which is associated with lymphadenopathy (cat scratch disease), ocular infections and other manifestations and has become the most medically important member of the genus (Chomel *et al.* 2004; Birtles 2005b).

Up to five species of *Bartonella* circulate concurrently in woodland rodent communities in the UK (Birtles *et al.* 2001; Telfer, Begon, *et al.* 2007; Telfer, Clough, *et al.* 2007). Although small mammals have demonstrated a high (40-60%) *Bartonella* prevalence (Birtles *et al.* 1994; Kosoy *et al.* 1997), infections are self-limiting and do not usually result in clinical disease (Telfer *et al.* 2008, 2010). In Kielder, contrasting dynamics of three *Bartonella* species have been recorded, with only *B. grahamii* exhibiting a distinct seasonal pattern and the three species also differing in their likelihood of infecting young or mature hosts (Telfer, Begon, *et al.* 2007). Interestingly, all species in general exhibited stronger correlations with host dynamics than those of their vectors, supporting the assertion that flea-borne microparasites can often be incorporated effectively

into epidemiological models as directly-transmitted pathogens (Dye & Williams 1995).

Babesia microti

Babesia microti is an intraerythrocytic protozoan parasite infecting wild rodents and the major causative agent of human babesiosis in the USA, a potentially fatal tick-borne zoonosis.

In common with other members of the *Babesia* genus, *B. microti* requires an ixodid tick vector for the sexual stage of its life cycle. In the UK, this has been identified as *Ixodes trianguliceps* (Randolph 1991). The great host-specificity of this nest-dwelling tick, which does not readily bite humans, may explain the lack of human *B. microti* infections in Europe. However, the human-biting tick *I. ricinus* is sympatric with *I. trianguliceps* in many areas, including Kielder (see below) and may provide a route for transmission to humans.

Babesia microti infections in field voles are usually sub-clinical and persistent, with longitudinal studies demonstrating that individuals testing PCR positive remain so for all subsequent captures (Telfer *et al.* 2008; Bown *et al.* 2008). Interestingly, whilst infections are chronic, laboratory studies indicate that sufficient parasitaemia for transmission to ticks to occur is restricted to a window of only 1-4 days post infection (Randolph 1995). Infection prevalence may reach over 40% in Kielder field vole populations (Telfer *et al.* 2008; Bown *et al.* 2008) and probability of infection has a polynomial relationship with weight, with individuals of 20g being at highest risk of becoming infected (Smith *et al.* 2008).

There is at present some controversy over the taxonomic status of *B. microti*. Classical taxonomic criteria would suggest it is part of the *Theileria* genus (Uilenberg 2006) rather than *Babesia*, whereas molecular evidence suggests that this parasite differs from both *Babesia* and *Theileria* and that a new genus may be required (Uilenberg 2006; Nakajima et al. 2009).

Trypanosoma (Herpestoma) microti

T. microti is a stercorarian trypanosome specific to voles (Noyes *et al.* 2002). Trypanosome infections in rodents are generally considered to be of low pathogenicity, but there is some evidence that trypanosomes can cause anaemia in microtine rodents or detrimentally affect female reproduction (Wiger 1977). In Kielder, *T. microti* prevalence is highly seasonal, being highest in late summer/autumn and lowest in spring (Smith *et al.* 2005). *Trypanosoma microti* is transmitted by fleas and a positive association between trypanosome prevalence and flea infestation in the previous 1-3 months has been observed in Kielder (Smith *et al.* 2005). Following ingestion of the parasite during a blood meal, it develops in the flea hindgut before being shed in the faeces. Infection of a new vole host can then occur via faecal contamination of the skin, or through accidental ingestion of fleas or their faeces (Albright & Albright 1991). However, a study by Smith et al. (2006a), in which flea prevalence was experimentally manipulated, demonstrated that vector-independent transmission of *T. microti*, most likely though mechanical transmission as result of increased aggressive behaviours, is also of epidemiological significance in Kielder (Smith *et al.* 2006).

Ticks (Ixodida)

Ticks are amongst the most important arthropod vectors and, as described above, are responsible for *Babesia microti* and *Anaplasma phagocytophilum* transmission among Kielder voles. In the UK, at least five species of tick may feed on rodents (Snow 1979) of which two, *Ixodes ricinus* and *I. trianguliceps*, are frequently encountered at Kielder (Bown *et al.* 2006, 2008, 2009). Whilst all three stages of *I. trianguliceps* feed upon small mammals (Randolph 1975), *I. ricinus* is more catholic in its feeding behaviour, feeding on a wide variety of hosts including reptiles, birds and mammals (Arthur 1963). As such, the exclusion of deer from an area significantly reduced the abundance of *I. ricinus* but no effect on *I. trianguliceps* was detected (Bown *et al.* 2008).

Longitudinal studies indicate that the majority of larvae recorded on field voles were *I. ricinus* (Bown *et al.* 2009) whilst adult ticks were almost exclusively *I. trianguliceps* (Bown *et al.* 2009). Seasonal fluctuations in the abundance of ticks feeding on voles were apparent, with peaks of *I. ricinus* larvae in late spring/early summer, whilst *I. trianguliceps* larvae peak abundance occurs in late autumn (Bown *et al.* 2009). Nymph and adult ticks were recorded in much lower numbers with no obvious peak, but were largely absent between November and April (Bown *et al.* 2009). Male voles were more likely to be infested with nymphal or adult ticks, and mature males were more likely to be infested with larvae of either tick species (Bown *et al.* 2008). The presence of larvae increased the probability of nymphs or adults on a vole and vice-versa (Bown *et al.* 2008).

Fleas (Siphonaptera)

A number of rodent-specific and generalist flea species are known to inhabit Kielder Forest, including *Peromyscopsylla spectabilis*, *Ctenophthalmus nobilis*

vulgaris, *Megabothris walkeri*, *Malaraeus penicilliger*, *Rhadinopsylla pentacanthi* and the largest British species, the mole flea (*Hystrichopsylla talpae talpae*) (Smith *et al.* 2005; Turner *et al.* 2011; Jackson et al., submitted). Fleas which commonly infest field voles exhibit seasonal dynamics and are known to peak in mid-late summer (Smith *et al.* 2005). Within Kielder, to date these species have primarily been studied only in the context of their transmission of *Bartonella* spp. and *Trypanosoma microti* (see preceding sections). However, Telfer et al. (2007) demonstrated that probability of flea infestation is density- and delayed density-dependent; voles from clearcut sites with high densities the preceding autumn were more likely to be infested. Conversely, field voles were less likely to be infested if found in a currently or recently high-density population, suggesting a dilution effect whereby the flea population is divided among a greater number of hosts.

Others

Many other endemic pathogens and parasites of the Kielder field voles are known but have not yet been studied in great detail (see Table 2 for a summary of known macroparasites). However, genetic associations with resistance to nematodes and cestodes have been examined (**see Genetics section**). Research as to the impact of these less well-studied pathogens is on-going (Jackson et al., submitted), and there will undoubtedly be currently undiagnosed pathogens circulating within the field vole populations, particularly microparasites, which will warrant further study.

TRANSMISSION DYNAMICS

400 Our work on transmission dynamics began on the Wirral time series, on which
401 we initially performed a rather unsophisticated analysis of numbers of infected
402 and susceptible hosts to examine the transmission dynamics of cowpox virus
403 (Begon *et al.* 1998, 1999). We examined dynamics within both wood mice and
404 bank voles to ask, first, whether the density-dependent mode of transmission
405 conventionally assumed - especially in modelling studies - for directly- but not
406 sexually-transmitted infections, was in fact appropriate (as opposed, for
407 example, to frequency-dependence, where the contact rate between hosts is
408 assumed to remain constant irrespective of density). We also compared
409 transmission rates within and between species. This is important for two
410 reasons: first, for the insights it provides on whether coexisting wildlife hosts
411 should be considered joint or independent reservoirs of infection, and hence
412 whether dilution or amplification effects are possible (Begon 2008). Second, it
413 allows an assessment to be made from field data of the strength of 'apparent
414 competition' in a host–host–pathogen system, whereas previously this has
415 largely been the subject of theoretical analysis (e.g. Begon & Bowers 1994).
416 Aspects of the same questions were also examined by the analysis of
417 spatiotemporal cowpox data to assess, first within species, the spatial and
418 temporal scales over which an infectious individual poses a risk of infection to a
419 susceptible one (Carslake *et al.* 2005). The same technique was then performed
420 between species (Carslake *et al.* 2006), in both cases asking, in essence, 'who
421 acquires infection from whom?'.
422
423 The results called into serious question the assumption that susceptible and
424 infectious hosts mix at random and hence that transmission of cowpox virus is

425 'density-dependent'. Our time series analysis, for each species in isolation,
426 indicated that frequency-dependent transmission (conventionally assumed to
427 apply to sexually-transmitted diseases) was superior to density-dependent
428 transmission as a descriptor of the dynamics (Begon *et al.* 1998). A *K*-function
429 analysis confirmed that an infectious individual posed a measurable risk of
430 infection for a period roughly equal to the infectious period itself, about four
431 weeks. It also indicated that this risk was detectable only at spatial scales within
432 the species' known home ranges (Carslake *et al.* 2005). These results therefore
433 suggest a rather general conclusion, namely that random mixing may have been
434 too readily assumed, and that many diseases that are not sexually transmitted
435 may nonetheless be socially transmitted, with essentially the same transmission
436 dynamics.
437
438 Moreover, the time series analysis of the two species together indicated that
439 between-species transmission was rare, in spite of the species occupying not
440 only the same general habitat but often even sharing burrows (Begon *et al.*
441 1999). The *K*-function analysis confirmed this, and suggested further that in
442 wood mice most transmission was between sexes, whereas in bank voles
443 infected females may pose the greatest risk of infection to both sexes (Carslake *et*
444 *al.* 2006). Thus, for cowpox virus at least, bank voles and wood mice do not
445 'combine' to any significant extent: the between-species coefficients are too low.
446 Each species acts as an effectively-independent reservoir. Similarly, the results
447 indicated that while the potential for apparent competition between bank voles
448 and wood mice mediated by cowpox virus undoubtedly exists, since the virus
449 depresses the birth rate and possibly the survival of both host species (see

450 **Fitness Effects** section), it is likely to be insignificant in practice because the
451 pathogen is so rarely transmitted from one species to the other.
452 A further study connected to these was carried out by Telfer et al. (2005) using
453 the natural experiment established by the recent invasion of bank voles into the
454 south of Ireland to examine the interaction between bank voles and wood mice
455 and two of their shared pathogens, *Bartonella birtlesii* and *B. taylorii*. The
456 prevalence of both, which occur only in wood mice in Ireland, declined
457 significantly with bank vole density. Results were therefore consistent with there
458 being a dilution effect (Norman *et al.* 1999), a phenomenon which despite its
459 high profile and the recent controversy it has attracted (e.g. Randolph & Dobson
460 2012) is still short of good case studies (see **Fleas** section for example of another
461 possible dilution effect in Kielder).
462
463 Although the Kielder time series, focussing on a single species, could not further
464 our understanding of between-species dynamics, it was possible to use the field
465 vole data to examine much more thoroughly the question of the nature of the
466 transmission function itself (Smith *et al.* 2009). Rather than simply comparing
467 density- and frequency-dependent transmission, the analysis asked where on the
468 spectrum between density- and frequency-dependence the true function might
469 be, and also whether that functional form, or indeed the strength of transmission
470 itself, might vary seasonally. In fact, results showed that overall, transmission of
471 cowpox virus amongst field voles was neither frequency- nor density-dependent.
472 On a scale encompassing zero (density-dependence) and one (frequency-
473 dependence), the observed value was 0.62, significantly different from either
474 (credibility interval 0.49-0.74), appropriate for a transmission function that

475 increases linearly with host density at lower densities (density-dependence) but
476 tends to saturate as density increases further (approaching frequency-
477 dependence).
478
479 Furthermore, when models were examined that allowed parameters to vary
480 seasonally, it appeared, first, that transmission was more readily achieved in
481 winter, perhaps because susceptibility to infection is greatest then. Secondly, the
482 overall picture of transmission lying between density- and frequency-
483 dependence seemed to be hiding a pattern in which transmission was closer to
484 density-dependence in the winter and closer to frequency-dependence in the
485 summer. This is plausible insofar as field voles defend territories much more
486 actively in the breeding season (summer), such that contact will be with
487 neighbours and hence relatively independent of overall density. In winter,
488 mixing is not so constrained and hence contact rates can indeed be expected to
489 increase with density. Repeatedly, therefore, these transmission studies, whether
490 within or between species, have emphasized that once data are collected from
491 natural populations, conventional, widely-held assumptions may be found
492 wanting.
493
494 FITNESS EFFECTS OF INFECTION
495 The impact of endemic infections on the fitness of hosts in the wild is poorly
496 understood, with studies tending to be cross-sectional or to focus on epidemic or
497 emerging infections. Changes in host population dynamics may arise through
498 impacts on host survival and/or fecundity rates. Longitudinal, experimental and
499 modelling work investigating the prevalence of a suite of pathogens in field voles

at Kielder has greatly advanced our understanding of the impacts of endemic infections, indicating that negative fitness costs can be significant.

Evidence from the Kielder field voles suggests that endemic infections can negatively impact field vole survival. Individuals infected with cowpox virus had a 22% lower probability of survival than uninfected individuals and, at the population level survival rates were negatively correlated with cowpox prevalence (Burthe, Telfer, *et al.* 2008). There is also some suggestion that TB has a negative impact on survival (Burthe, Bennett, *et al.* 2008). While not statistically significant, survival of voles following the appearance of an external lesion characteristic of advanced vole tuberculosis was lower than for voles without lesions. As discussed earlier, diagnosis of disease based on lesions underestimates the prevalence of infection and hence the negative impact of late stage TB would be underestimated due to individuals dying before presenting overt late-stage disease symptoms. A significant decline in body condition of individuals at the time of appearance of the first external lesion further suggests that TB may potentially impact individual fitness. Further effects of infection on host condition are discussed in the next section.

Impacts on host reproduction by pathogens have proven difficult to evaluate due to difficulties in assigning juveniles to parents and measuring reproductive success. However, prevalence of trypanosomes was found to be highest in heavier (older) animals at first capture compared to heavier recaptured animals suggesting that infected animals may be less likely to become territory holders and therefore less likely to breed (Smith *et al.* 2005).

In related work on bank voles and wood mice in the Liverpool populations, cowpox virus appeared to have both positive and negative survival effects, depending on the season; survival rates increased with cowpox prevalence in the summer but decreased during the winter (Telfer *et al.* 2002). This may be related to subtle interactions with effects of cowpox virus on reproduction in these species. Female bank voles and wood mice infected with cowpox virus have been shown to delay maturation, and therefore reproduction, often until the following year (Telfer, Bennett, *et al.* 2005), a response seen also in the laboratory (Feore *et al.* 1997). This delay in reproduction, and the associated energetic costs saved may be the reason for the increased survival rates for cowpox-infected compared to non-infected individuals in summer.

Modelling work suggests that theoretically reduced or delayed fecundity following recovery from infection can influence host population dynamics and induce multi-year cycles (Smith *et al.* 2008). However, empirical investigation of parameters such as variation in the onset of maturity in infected hosts relative to uninfected hosts would be necessary to support this theoretical prediction.

As discussed in more detail below (see **Coinfection**), the field vole data indicate clearly that infection with one pathogen may frequently imply coinfection with others. The fitness consequences of infection with, say, cowpox virus, may therefore, in practice, be the fitness consequences of infection with cowpox virus and all the other parasites that are consequently more likely to be found in the same host. This sets limits on the relevance of controlled experiments in the animal house on the effects of parasites on host fitness. It also emphasizes that

there may often be no clear link between the clinical effects of an individual parasite species and the effects it has on host fitness in statistical analyses carried out at the population level.

HAEMATOLOGY AND MEASURES OF HOST CONDITION

Variation among individuals and populations in health status and immunocompetence may influence parasite dynamics, as a result of variable susceptibility to infection (see **Immunology** and **Genetics** sections for studies on the immunological and genetic basis of this variation). In human and veterinary medicine the health status of individuals is routinely monitored by measuring selected physiological indices, and haematological parameters are among the indices most extensively used. Nonetheless, the wealth of information they can provide has only rarely been exploited in wild populations.

The cellular component of the blood consists of various cell types, each of which has a different function and responds distinctively to infection, stress, nutritional deficit, etc. Although the interpretation of these parameters requires caution, in general, red blood cells (erythrocytes, RBCs) and lymphocytes are important indicators of fitness and condition, while the other white blood cells (WBCs) are components of different types of immune responses (Tizard 2004). Low concentrations of RBCs, caused by blood loss, haemolysis or decreased erythrocyte production, result mainly from deficient nourishment and infection or parasitism (Stockham and Scott, 2002). Lymphocytes, the effectors of acquired immunity, proliferate in response to antigenic stimuli and have a long life span in blood, while their numbers decrease (lymphocytopenia) during

575 immunosuppression by glucocorticoids or immunosuppressive infections
576 (Feldman *et al.* 2000; Stockham & Scott 2002). Therefore, circulating levels of
577 lymphocytes may be useful indicators of immunological investment. Of the
578 remaining WBCs, blood concentrations of neutrophils increase rapidly as a
579 response to cytokines released during tissue injury and bacterial infection
580 (Tizard 2004). They are useful proxies for acute inflammatory response, as their
581 levels return to normal soon after antigenic stimulation ceases. Monocytes are
582 found in high concentrations in subacute and chronic inflammatory response
583 caused by bacterial or protozoan infections (Feldman *et al.* 2000; Tizard 2004).
584
585 By evaluating indices of health in wild populations, our knowledge of the
586 dynamics of health and infection may be understood more clearly. Beldomenico
587 et al. (2008b) investigated haematological dynamics within the Kielder field
588 voles, to determine environmental and host factors associated with indicators of
589 inflammatory response (counts of monocytes and neutrophils) and of condition
590 (lymphocyte counts and red blood cell counts). Individuals from three field vole
591 populations were sampled monthly for two years. Comparisons with individuals
592 kept under controlled conditions facilitated interpretation of field data
593 (Beldomenico *et al.* 2008b).
594
595 Unlike in humans and domestic animals, which maintain their haematological
596 parameters within constant 'normal' ranges while in health, these parameters
597 appeared to be highly variable in wild field voles. There was a strong seasonal
598 variation that persisted even after environmental and host factors usually
599 associated with blood cell count variation were considered in the analysis. There

were three well-characterized 'physiological' seasons. The immunological investment appeared lowest in winter (lowest lymphocyte counts), but red blood cells were at their highest levels and indices of inflammatory response at their lowest, indicating a low infection risk during this period. Spring was characterized by dramatic changes, with a steep fall in red blood cell counts and peaks in indicators of inflammatory response. During the course of summer-autumn, the parameters gradually returned to their previous levels: red blood cell counts recovered and the indicators of inflammatory response decreased, while the immunological investment increased.

All the haematological parameters were affected adversely by poor body condition and preceding high population densities. Moreover, the first pregnant females of the year were those in better condition, emphasizing the predominant role of energetics in population dynamics. Indeed, even when RBC counts were 'high' in the field, they were lower than in the near-optimal conditions of the animal house (abundant food and low parasitism/infection), suggesting that voles in the natural populations were generally resource and/or energy-limited, and they could therefore only increase their investment in, for instance, neutrophils by a compensatory decrease in their investment in other functions (e.g. the production of RBCs).

Azurocytes (AZ) are a blood cell type specific to microtine rodents, particularly common in late pregnancy and inducible by progestins both in males and females (Mihok *et al.*; Mihok & Schwartz 1991). Beldomenico *et al.* (2008c) found that indeed the counts of AZ were much higher in pregnant females, and that

these counts were positively correlated with past vole density, suggesting that these cells may have a role in inducing abortion when conditions are not favourable. Males had low prevalences and counts, both for breeding and nonbreeding individuals, but they showed a seasonality that varied with age, body condition, and current and past vole density. Also, the occurrence of AZ in males was more likely after they had had low levels of indicators of condition (**see subsequent section**), suggesting that in males these cells predominantly result from a response to infection.

The strong seasonal variation in health dynamics pinpoints the spring as a period of increased vulnerability, both to infection and other causes of mortality. When preceding densities are low and body condition is good, female field voles begin reproducing early, while high densities are followed by a negative impact on all blood cell types, except for AZ in females, whose rise might indicate spontaneous abortions. Host condition in spring may not only reflect but also determine, in part, whether a year will be in an increase or a decrease phase of the abundance cycle. Poor condition in over-wintering field voles is often a consequence of past densities (also suggested by Huitu et al. 2007), but it could also be caused by unusual increases of metabolic demands during spring, or poor resources bequeathed by a severe winter. This may help to explain why field vole population cycles do not appear entirely regular.

VICIOUS CIRCLES: SYNERGY BETWEEN CONDITION AND INFECTION

The previous section discussed effects of infection on host fitness, including their condition. But equally, a host's condition may affect its propensity to become and

650 to remain infected. Contact between susceptible hosts and infectious hosts,
651 vectors or environmental reservoirs is crucial in determining infection risk.
652 However, following exposure to a pathogen, a continuum of outcomes might be
653 seen, ranging from failure of the infection to progress to overwhelmingly high
654 infection intensity. The outcome may depend on characteristics of the pathogen
655 (e.g. strain, infective dose) or of the host (e.g. genotype, condition).
656
657 As discussed earlier, the Kielder field voles exhibit characteristic periodic peaks
658 followed by declines, and these dynamics are associated with food shortage and
659 poor condition (Huitu *et al.* 2007). To test the hypothesis that poor host
660 condition increases infection risk, Beldomenico et al. (2008a) used longitudinal
661 data from replicated wild field vole populations to evaluate whether individuals
662 with reduced indicators of condition were more likely to become infected.
663 Because the community of obligate and facultative parasites to which field voles
664 are exposed is highly diverse, exhaustively testing for all infections is impossible.
665 To overcome this problem, initially generic indices that capture the physiological
666 response to infection were used. Elevated neutrophil counts (neutrophilia) are
667 an indication of acute inflammatory response associated with bacterial infection,
668 and high monocyte counts (monocytosis) are expected in subacute and chronic
669 inflammatory response caused by infections with bacteria or protozoans
670 (Feldman *et al.* 2000). In addition, low peripheral lymphocyte counts
671 (lympocytopenia, an indication of immunosuppression or poor immunological
672 investment) or low red blood cell (RBC) counts (anaemia, an indication of poor
673 aerobic capacity) were used as haematological indicators of condition (see
674 **Haematology**). Results showed that poor condition increases the probability of

675 infection: individuals with anaemia and lympocytopenia had increased
676 probabilities of developing monocytosis and higher increments in neutrophils
677 when re-sampled 4 weeks later (Beldomenico *et al.* 2008a).
678
679 The results above provide evidence supporting Lochmiller's hypothesis
680 (Lochmiller 1996), which states that opportunistic pathogens take advantage of
681 altered host immunocompetence (see **Immunology** section for further
682 discussion on the concept of immunocompetence) during stress periods,
683 consequently regulating wild animal populations. To test this hypothesis in the
684 Kielder system, Beldomenico et al. (2009a) carried out a nested case-control
685 study that assessed whether susceptible individuals with poorer condition had
686 higher probabilities of contracting cowpox over a four week period. The results
687 were particularly striking for males. For males caught at the same time, a
688 susceptible individual with poor body condition (low degree of fat and muscle
689 cover) was twice as likely to contract cowpox as a susceptible male with good
690 body condition; if this individual was also anaemic, the chances were almost
691 quadrupled (Figure x). This result not only supported Lochmiller's hypothesis,
692 but it showed that it holds not only for opportunistic pathogens, but here for an
693 endemic virus.
694
695 If this condition-dependent infection risk originates from a reduced resistance of
696 the host, it will not only result in greater proneness to becoming infected of those
697 that are in poorer condition; it may also cause infections of higher intensity, thus
698 resulting in individuals that suffer a more severe disease and are a more
699 significant source of infection. Beldomenico et al. (2009b) assessed this

700 hypothesis by investigating the temporal relationship between host condition
701 and intensity of infection by the protozoan *Trypanosoma* (*Herpestoma*) *microti* in
702 wild field vole populations. The individuals that developed high levels of
703 parasitaemia were those that previously had very low lymphocyte counts (Figure
704 z).
705
706 As noted above, not only can poor host condition predispose individuals to
707 infection; infection itself can have a detrimental effect on condition. Besides their
708 specific pathogenic effects, parasites extract host resources and induce a
709 nutritionally demanding immune response (Sheldon & Verhulst 1996;
710 Lochmiller & Deerenberg 2000). There is a clear potential for synergy: poor
711 condition predisposes individuals to infections, which further reduces the
712 condition of the host, which further predisposes the host to infection, and so on.
713 Thus, as previously noted, at the individual level, low haematological indicators
714 of condition precede elevated levels of haematological indicators of infection in
715 wild field voles. However, those individuals with high indicators of infection
716 subsequently experience a decline in their indicators of condition (Beldomenico
717 *et al.* 2008a). Furthermore, because individuals in poorer condition are expected
718 to have infections of greater intensity, the resulting deterioration in condition is
719 likely to be even more marked for infections in individuals with a preceding
720 impoverished condition. This was supported by our study on trypanosome
721 dynamics: field voles with decreased indicators of immunological investment
722 developed high intensities of *T. microti* parasitaemia, and subsequently, further
723 declines of these indicators were observed (Beldomenicoi *et al.* 2009b).

724 The above suggests that small initial differences in host condition caused by
725 resource shortage, competition, climate change, etc., can become exaggerated
726 and populations might become 'polarised' into the weak and the strong
727 (Beldomenico & Begon 2010). Vicious circles emerge, whereby an individual
728 with an impoverished condition is more prone to developing infections, which
729 are also more likely to be severe; in turn, this results in further deterioration in
730 condition that can eventually and substantially affect its performance and
731 survival. At the population level, a great proportion of individuals in poor
732 condition will cause both a large number of infections and more severe
733 infections, resulting in pathogen exposure dose being greater, with a
734 consequential greater impact on host dynamics (Beldomenico & Begon 2010).
735 Similar results have been reported in other systems including an observational
736 study on fish (Blanchet *et al.* 2009) and a field-experimental study on mice
737 (Pedersen & Greives 2008).
738
739 These reciprocal effects between host condition and infection might be the
740 mechanism by which parasites exert a control on their host populations, as hosts
741 tend to be more stressed and in poorer condition (thus becoming more
742 vulnerable to their parasites) when their densities are high (Huitu *et al.* 2007;
743 Beldomenico *et al.* 2008b).
744
745 COINFECTION
746 While most of the studies at Kielder have focused, as they have in other systems,
747 on a single species of pathogen (and of host), there is no doubt that most hosts,
748 most of the time, are infected by a multiplicity of parasites and pathogens.

749 Questions naturally arise, therefore, regarding the effects of one infection on
750 another. Indeed, some such effects may also occur when infections are
751 consecutive rather than simultaneous. The probability, intensity and length of
752 one infection may be altered by the presence of, or repercussions from, another,
753 as may any effects on host fitness. The idea of 'vicious circles' (above) carries
754 within it the implicit acknowledgement that individual infections cannot be
755 considered in isolation.
756
757 Experiments in laboratory model systems have demonstrated effects of
758 coinfection on host susceptibility, infection length, and intensity and clinical
759 signs. Studies in wildlife populations and humans, while establishing firmly that
760 positive and negative associations can occur between parasites, have tended to
761 be cross-sectional, with each host providing infection data at one time point only.
762 The time of initial infection is unknown in such studies. There is, therefore,
763 limited scope for determining whether patterns reflect inherent differences
764 between hosts in either susceptibility or exposure to infection, rather than
765 interactions (Telfer *et al.* 2008), or for exploring the impact of infection sequence
766 (Jackson *et al.* 2006). Consequently, in natural populations, the relative
767 importance of interspecific interactions, compared with other factors, in
768 determining the dynamics and structure of parasite communities is only poorly
769 known.
770
771 Telfer *et al.* (2010) used the field vole data set to examine individual infection
772 risks for a community of microparasites consisting of cowpox virus, *B. microti*,

773 the *Bartonella* species taken as a group and *A. phagocytophilum*. Infection risk
774 will depend on both the probability of encountering an infectious dose and the
775 probability of infection given exposure (host susceptibility). The aim was to
776 determine whether susceptibility to infection by one microparasite species was
777 influenced by others. Therefore, for each microparasite, the study investigated
778 whether the other microparasites influenced the probability that a susceptible
779 animal *became* infected at a given time point (t_0). It did so by adding infection
780 status for these other pathogens as explanatory variables to baseline statistical
781 models that accounted for environmental and individual variables (sex, season
782 etc.). As noted earlier, this method guards against detecting spurious
783 associations, which, in reality, reflect correlated exposure risk (e.g., a positive
784 association simply because both parasites are most prevalent in late summer).
785
786 It was apparent that this community of parasites represents an interconnected
787 web of interactions: effects of other infections on infection risk were both strong
788 and widespread, and connectance within the parasite community was
789 exceptionally high, with evidence detected for all possible pair-wise interactions
790 (Fig. 1). Both positive and negative associations were detected, and their
791 magnitude was frequently considerable: up to 5.5-fold increases in risk and
792 reductions in the odds of becoming infected of the order of 15% compared with
793 uninfected individuals (Fig. 1, Fig. 2). Indeed, perhaps most strikingly, in all cases
794 except for cowpox, infection with other parasite species explained more
795 variation in infection risk than factors related to exposure risk and host
796 condition, such as age and season. Moreover, the sizes of the effects of other
797 parasites on infection risk were also similar to, and frequently greater than,

other factors. For example, of all the non-infection variables, season generally had the largest effect on infection risk, with seasonal increases in infection probability ranging from approximately 3-fold (*A. phagocytophilum*) to 15-fold (*B. microti*); but these were broadly matched by the magnitude of infection effects (**Fig. 1, Fig. 2**). These results are not explicable by simple co-occurrence of infections in hosts in poor condition, since for a subset of the data, this was accounted for explicitly through variations in individual host condition indices at the time of infection (body condition and haemotological condition), and there was no evidence of any reduction in the strength of between-parasite interactions.

Several infections increased susceptibility to other microparasites. Jackson *et al.* (2009) have shown previously that naturally-occurring parasites are capable of exerting immunomodulatory effects on wild rodents, and release from effective control by the immune system is perhaps therefore the most likely explanation, especially when supported by experimental studies. For example, laboratory studies have indicated the importance of immunomodulation for host exploitation by pox viruses (Seet *et al.* 2003), which may explain the positive effect of cowpox virus on susceptibility to other parasites. The same immune-mediated mechanisms might also account for an earlier demonstration that cowpox virus increases the length of *Bartonella taylorii* infections (Telfer *et al.* 2008). Thus, mechanisms responsible for increasing susceptibility may also prolong infections in those that do succumb.

822 Strong decreases in susceptibility caused by other infections were also observed.
823 The largest effect overall was reduced susceptibility to *Bartonella* spp. in
824 individuals infected with *B. microti*, and was especially apparent in chronically
825 infected animals, where the odds of infection were 15% of those of uninfected
826 animals (**Fig. 1, Fig. 2B**). Resource depletion may play a role here, as both
827 species target erythrocytes (Table 1). Alternatively, negative effects may reflect
828 up-regulation of mediators of a cross-effective Th_1 response and therefore could
829 represent an example of immunologically driven ecological interference (see
830 **Immunology** and **Genetics** sections, below).
831
832 This study demonstrates, therefore, that communities of microparasites are
833 structured by strong interactions between species, providing the first evidence
834 from natural populations that such interactions can be driven by effects on
835 susceptibility and have as much impact on infection risk as more commonly
836 considered factors such as host age and season. As field voles are also infected by
837 macroparasites, as well as other microparasites, it is likely that the identified
838 relationships represent just one part of an even larger web of interactions. These
839 results also emphasize that the standard practice of classifying individuals in
840 natural populations as infected or uninfected by one parasite alone fails to
841 recognize that much more may be implied by the categorization 'infected'. For
842 example, as we note above, cowpox virus infection has been associated with
843 major reductions in survival and fecundity. However, in the coinfection study,
844 39% of those infected with cowpox virus were also infected with *B. microti*, 65%
845 of the remainder had *Bartonella* spp. infections, and overall, 79% were co-
846 infected with at least one of the three microparasites considered. Clearly, even

847 when significant associations between a given infection and host fitness are
848 detected in a wildlife host, attributing the effect to that parasite alone may be
849 unjustified.
850 A subsequent study applied more sophisticated and novel statistical techniques
851 to the data set from March 2005-March 2007 and dealt separately with three
852 *Bartonella* species, *B. doshiae*, *B. grahamii* and *B. taylorii* (Sherlock *et al.* 2013a).
853 Once again, *B. microti* increased the likelihood of contracting all three *Bartonella*
854 species, whether the *B. microti* infection was acute or chronic. This time,
855 moreover, *B. microti* was also seen to decrease the chances of recovery from all
856 three *Bartonella* infections: that is, *Bartonella* infections were longer when *B.*
857 *microti* was also present. This had previously also been suggested in the case of
858 *B. taylorii* (Telfer *et al.* 2008). It is important to recognise that the consequences
859 of one infection extending the length of another – in terms of the period of time
860 host fitness may be affected and the parasite transmitted – may easily be as
861 profound as those of simply increasing susceptibility. In this subsequent study,
862 however, there was no evidence of the reverse interaction: *Bartonella* increasing
863 susceptibility to *B. microti*. This runs counter to the Telfer *et al.* (2010) study and
864 suggests that the suggestion of an effect there may be a statistical artefact arising
865 from the extremely strong effects of *B. microti* on *Bartonella* (Sherlock *et al.*
866 2013b).
867
868 This study also allowed interactions among different, coinfecting *Bartonella*
869 species to be examined for the first time. Notably, voles that had previously been
870 infected with *B. taylorii* were less likely to contract infections of either *B.*
871 *grahamii* or *B. doshiae*. The suggestion that this positive interaction between the

872 species may be the result of cross-immunity is supported by evidence from the
873 analysis of an effective immune response to *Bartonella* infections more generally:
874 voles previously infected with either *B. grahamii* or *B. taylorii* were less likely to
875 re-contract the same infection. This in turn makes the more general point that
876 patterns of coinfection, particularly in longitudinal data, can suggest or even
877 support particular processes giving rise to them, but understanding coinfection
878 is likely, ultimately, to require those processes to be examined directly. One of
879 the most important class of processes, that acting through the immune system, is
880 examined next.
881
882 IMMUNOLOGY
883 Traditionally, research into wildlife immunology has concentrated on broad
884 definitions and single measurements of 'immunocompetence' (such as
885 phytohaemagglutinin-induced swelling), defined as a host's general ability to
886 resist infection. However, as we have discussed in previous sections, host-
887 pathogen interactions are dynamic and context dependent; therefore 'resistance'
888 is unlikely to be accurately represented by a single, simplified immunological
889 measure (Demas *et al.* 2011). Post-genomic technologies now allow us to define
890 immune variability much more precisely in naturally occurring non-model
891 organisms and move beyond this simplified view of immunocompetence. Wild
892 rodents, in particular, represent an exciting model for this expanded 'wild
893 immunology' as researchers can capitalise on the immunological and genetic
894 resources developed for laboratory rodents. Thus, measurements of the
895 expression of genes or gene products underpinning immunological traits may be
896 linked to environmental causes and to life history consequences for the

897 individual. In the Kielder field voles, where responses to infection have been the
898 central interest, we have focussed on variability in the immune system as the
899 possible key to individual variation in the response to infection.
900 Our approach to measurement has therefore made a break from traditional
901 ecological immunology (Bradley & Jackson 2008) by considering the immune
902 system explicitly from the perspective of immunoregulation derived from
903 studies of the laboratory mouse. Thus, the immune system is considered as a
904 multi-faceted defensive apparatus with different arms that drive different types
905 of immune responses. This is exemplified by the different T-helper (Th) cell
906 effector arms: Th1 cells driving responses against intracellular microbes, Th2
907 cells responses against macroparasites, Th17 cells responses against
908 extracellular bacteria, and regulatory Th cells immunosuppressive responses
909 (Reiner 2007). These different arms of the immune system may trade-off with
910 each other and with other life history components for resources and there may
911 also be complex functional cross-talk (cross-regulation) within the immune
912 system itself and between immune system and other traits.
913 The broad aim of the Kielder immunology studies has been to analytically
914 decompose immune system function through measurements of different effector
915 arms and to link these measurements to environmental causes and life history
916 responses. Developing appropriate measurement strategies is a central difficulty,
917 though, in analyzing the immune system in naturally-occurring non-model
918 organisms. In our initial studies we were hampered by the lack of species-
919 specific antibody reagents and by a deficit of genomic information for *M. agrestis*.
920 However, by *de novo* sequencing using traditional PCR methods, we were able to
921 design real-time PCR expression assays for a panel of immunological genes

922 reflecting different immunological pathways (Jackson *et al.* 2011). These
923 measurements were used both on *in vivo* (peripheral blood) samples from
924 repeat-sampled animals and in cultured splenocytes from destructively sampled
925 animals. Culturing of splenocytes allowed stimulation of the cells with defined
926 stimulants (e.g., mitogen, Toll-like receptor agonists), in order to selectively
927 stimulate immunological pathways and cell populations and measure latent (un-
928 deployed) responsiveness (Jackson *et al.* 2011).
929 We combined these measurement approaches with interwoven longitudinal and
930 cross-sectional sampling protocols in replicated habitats (see **Data sets**), in
931 which, in addition to immunological measurements, detailed infection and
932 biometric variables were also recorded. This hybrid study design exploited the
933 respective strengths of the different types of sampling: on the one hand,
934 destructive cross-sectional sampling allowing a wider range of more precise
935 immunological, biometric and infection measurements; on the other hand,
936 longitudinal sampling allows stronger inference of cause and effect (cause
937 typically preceding effect in time series data).
938
939 In a preliminary proof-of-concept study, Jackson *et al*. (2011) reported non-
940 periodic temporal trends in pro-inflammatory and regulatory gene expression,
941 which seem likely to relate to unidentified environmental drivers. Further
942 experimental work may identify the environmental causes and also the
943 consequences of these patterns. We have also found negative associations
944 between the expression of pro-inflammatory mediators and some individual
945 condition indices. It is tempting to think that this may be due to a cost of
946 resistance mediated through inflammation. However, our on-going analyses

947 suggest that the effect may, at least in part, be due to interferon gamma (IFN-γ)-
948 associated resistance to the blood protozoan *Babesia microti*: with high IFN-γ-
949 expressing individuals tending to be uninfected and to lack the organomegaly
950 associated with babesiois (**Jackson(?), unpublished data/in prep?).**
951
952 A key finding of the work so far has related to the immunological basis of disease
953 tolerance to some infection types in wild field voles (Jackson *et al.*, submitted).
954 Disease tolerance is a defence mechanism whereby the host endures infection
955 whilst minimising the damage caused by the pathogen itself or by the host's own
956 immune response (Ayres & Schneider 2012; Medzhitov *et al.* 2012). This is
957 distinct from disease resistance, where the host actively detects and eliminates
958 pathogens, in that tolerance has no obvious effect on pathogen burden.
959 Although the mechanisms of resistance are well understood, there is still
960 relatively little known about the natural ability of animals to tolerate infection
961 (Medzhitov *et al.* 2012; Turner & Paterson 2013). A tolerance-like strategy to
962 macroparasites (both ectoparasites and helminths) was evident in the Kielder
963 field voles, involving overcompensation (i.e., increases) in general body
964 condition as a response to infection. The voles accumulating the most
965 macroparasites were in the best condition (reflected by size-adjusted body and
966 organ weights). This pattern was most marked in mature males and our
967 subsequent analyses in this subset of the population indicated that
968 macroparasite infections are likely to be an indirect trigger (rather than a mere
969 correlate) of the elevated body condition. Thus, a clear immunological signature
970 of the high condition/high macroparasites syndrome was found to be elevated
971 expression of *Gata3*, a transcription factor centrally involved in Th2 cell

development and expressed by activated Th2 cells (Hosoya *et al.* 2010). As might be expected from laboratory infection models, where Th2 responses frequently result from macroparasite infection (Boppana *et al.* 2009), high *Gata3* expression in voles appeared to be triggered by macroparasites (macroparasite exposures preceding elevated *Gata3* expression in time series for individual animals). High *Gata3* expression was in turn causally linked with increased body condition and changes in other fitness components, including individual survival, thus placing the apparent tolerance strategy within a life history context of costs and benefits for different traits. The life history ramifications of the elevated *Gata3* signature were complex, with, in addition to the overcompensation in body condition, an indication of a reduction in reproductive investment and an age-dependent effect on survival. Tolerance mechanisms have been poorly studied in the laboratory and are virtually un-studied in natural populations. By implicating adaptive Th2 immunity in tolerance responses, rather than more conventional regulatory mediators, the results raise fundamental questions about the nature of tolerance strategies in natural populations and the role of Th2 responses in the immune system.

The results briefly described in this section have then begun to identify important processes in host-parasite relationships that result from variation in immune function and were not immediately apparent from a simpler focus on just the parasites and the hosts themselves. In the future, continuing development of immunological methods for *M. agrestis* and the possibility to monitor the expression of many more genes using RNAseq, very high throughput

Q-PCR and bioplexing are likely to further extend our understanding of the strategic role of the immune system within life history variation.

GENETICS, SELECTION AND DISEASE SUSCEPTIBILITY

It is now well established that genetic diversity underlies a substantial component of the variation in susceptibility to infectious disease observed in natural populations. As they have for immunological studies, laboratory rodents have proved an invaluable resource in the discovery and functional annotation of genes involved in immunity to infection. However, although these animals are well-established functional genetic models, they differ from natural populations, including those of humans, in several important ways (Turner & Paterson 2013): laboratory rodents are generally isogenic and therefore lack the genetic diversity of natural populations; genetic variation between laboratory strains is driven by selective breeding and deliberate mutations of the genome, rather than natural selection and genetic drift in the wild; laboratories provide homogenous, comfortable and largely sterile environments with none of the pressures of the natural environment (for example, suboptimal nutrition, fluctuating climate, predation, competition etc.); and finally, laboratory infection experiments have tended to concentrate on single infections, whereas wild individuals are likely to experience multiple simultaneous or sequential infections from a variety of taxa. Because of this lack of ecological validity, functional laboratory studies offer few insights into the causes and consequences of natural genetic diversity or the role of natural selection on the maintenance of variation in susceptibility to disease. Wild rodents are related to well-established laboratory model species and yet provide a much more realistic ecological model of human and other natural

1021 populations. They have therefore been put forward as a novel model to build on
1022 and utilise the genetic resources gained from their laboratory cousins, thus
1023 providing biomedically-relevant yet ecologically valid insights into the genetic
1024 determinants of infectious disease resistance (Turner & Paterson 2013).
1025
1026 In an attempt to broaden the immunogenetic research traditionally conducted on
1027 laboratory rodents to natural populations, Turner et al. (2011) used the
1028 longitudinal and cross-sectional system of Jackson *et al*. (2011) (see
1029 **Immunology** and **Data sets**) to examine the genetic diversity within a number
1030 of Kielder field vole immune genes, concentrating primarily on cytokines.
1031 Cytokines are signalling molecules that facilitate communication between
1032 immune cells and are crucial in the induction and polarisation of immune
1033 responses. Despite the breadth of genetic research into cytokines in human and
1034 laboratory studies, there have thus far been few studies on wildlife, where the
1035 overwhelming majority of immunogenetic studies have concentrated solely on
1036 genes of the major histocompatibility complex (MHC). In their study, Turner et
1037 al. (2011) utilised multiple regression methods to first control for confounding
1038 non-genetic factors, many of which were identified by Jackson *et al*. (2011).
1039 Subsequently, they demonstrated strong associations between genetic
1040 polymorphism within three cytokines (*Interleukin 1 beta* [*Il1b*], *Il2* and *Il12b*)
1041 and individual variation in immune responses, as measured through expression
1042 levels of multiple immune genes. Following this, the authors hypothesised that if
1043 this genetic variation at cytokine loci affects immune responses, it would also
1044 impact upon pathogen resistance. To test this, Turner *et al*. again first controlled
1045 for possible confounding factors, and found that the same three genes associated

with variation in immune responses – *Il1b, Il2* and *Il12b* - were also strongly associated with variation in susceptibility to a number of endemic and pathogens. The magnitude of the genetic effects observed were of comparable size to non-genetic factors such as age and sex, which are commonly acknowledged as important in natural studies of infection. Importantly, given the importance of simultaneous infections (see above), all genetic effects remained after addition of coinfecting parasites to the models as explanatory variables. Moreover, the fact that these genes were associated with resistance to a taxonomically diverse range of natural pathogens (bacteria, protozoa, helminths and arthropod ectoparasites) demonstrates the value of examining such genetic relationships in the wild, in contrast to laboratory studies that typically focus on single experimental infections (Turner *et al.* 2011). For example, apparently antagonistic and pleiotropic effects of genetic variation were noted, with genetic variants simultaneously associated with an increased likelihood of infection with one parasite and a decreased chance of infection of another. This suggests that that the advantage to the host of a 'protective' genotype against one pathogen depends greatly on the context of the local pathogen community.

In a complementary study, Turner et al. (2012) examined the evidence for natural selection acting on field vole immune genes, hypothesising that those genes identified as being associated with disease resistance may be shaped by pathogen-mediated selection. Using a range of population genetic techniques they indeed found signatures of natural selection acting on several cytokine and Toll-like receptor (TLR) genes. Of particular note was that high genetic diversity observed within *Il1b* and *Il2* genes, both of which were strongly associated with

variation in immune function and pathogen susceptibility, appears to have been maintained via balancing selection (a term encompassing any type of natural selection which acts to maintain genetic polymorphism). As pathogen abundances vary spatiotemporally in Kielder Forest, fluctuating, pathogen-specific and often antagonistic selection pressures perhaps represent the most likely mechanism driving the maintenance of polymorphism at these loci. Integration of the findings of the two studies provides robust, corroborative evidence that genetic diversity within several field vole cytokine loci has a discernible effect on susceptibility to a number of infectious diseases, via cytokine-mediated modulation of host immune phenotypes. In turn, as has been commonly reported for genes within the MHC (Spurgin & Richardson 2010), cytokine genetic diversity is then maintained through the action of pathogen-mediated balancing selection (Figure XXX).

CONCLUSION

Studies of infectious diseases in wild rodent populations have traditionally been driven by perhaps two major motivations (Begon 2003). First, a fundamental desire to understand the ecological importance of the interactions between hosts and their parasites. Second, a more applied goal of understanding the dynamics of rodent reservoirs and their pathogens in order to practice disease control. With the advent of genomic technologies and the continued rise of ecological ('wild') immunology, a third motivation has now emerged: to expand traditional genetic and immunological research beyond laboratory models and into the natural world. The continued integration of the mechanistic knowledge garnered from laboratory rodents with the understanding of the ecology,

1096 population dynamics and – more recently – immunology and genetics of wild
1097 rodents will provide fresh insights relevant not only to evolutionary biology and
1098 ecology, but also to conservation biology and biomedical science (Turner &
1099 Paterson 2013).
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1103

Table 1: Summary of microparasites studied in Kielder field voles. Adapted and expanded from Telfer *et al*. (2010).

Species	Type	Transmission mode	Primary site of infection	Infection length	Clinical signs/effec fitness
Cowpox virus (CPXV)	Virus	Direct	Respiratory tract and lymphoid tissues (monocytes and macrophages)	Self-limiting (four weeks)	No apparent clinica signs, but reduction fecundity and survi
Mycobacterium microti (vole tuberculosis)	Bacterium	Direct	Unknown but cutaneous, respiratory tract and lymphoid tissues likely	Unknown but most likely chronic (lifelong)	Characteristic skin
Anaplasma phagocytophilum	Bacterium	Vector-borne (ticks)	Granulocytes	Self-limiting (four to eight weeks)	Transient cytopeni
Bartonella spp.	Bacterium	Vector-borne (fleas)	Erythrocytes	Self-limiting (four to eight weeks)	No apparent clinica
Babesia microti	Protozoan	Vector-borne (ticks)	Erythrocytes	Chronic (lifelong)	Haemolytic anaemi generally subclinica
Trypanosoma (Herpestoma) microti	Protozoan	Vector-borne (fleas)	Blood	**??**	**??**

1112

1113 Table 2: Other macroparasites observed in Kielder Forest field voles

Nematodes	*Syphacia nigeriana*
	Trichuris arvicolae
	Heligmosomoides laevis
Cestodes	*Taenia polyacantha*
	Taenia taeniaeformis
	Anoplocephaloides dentata aff.
	Paranoplocephala sp.
	Rodentolepis asymmetrica
	*Arostrilepis horrid*a
	Taenia mustelae
Mites	Laelapidae
	Listrophoridae
	Myobiidae
	Ear mites
Lice	*Hoplopluera acanthopus*

1114

1115

Figure x: Modified from Beldomenico et al. (2009b) Predicted probability of seroconverting as simulated by GLMM for male field voles from Kielder. Variation by month, body condition score (4 = black lines; 8 = grey lines) and RBCs (past density fixed at 50). In the simulation, anaemic (dashed lines) represents individuals with 3 million RBCs/ml, and normocytic (solid lines) represents voles with 8 million RBCs/ml.

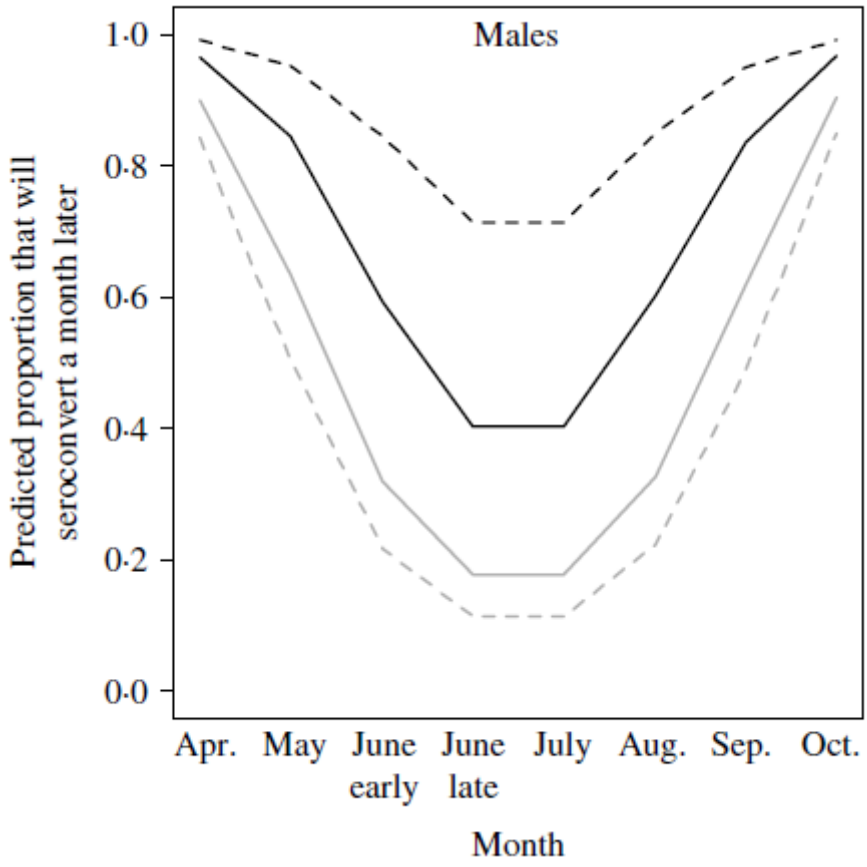


Figure z. From Beldomenico et al. (2009a). Lymphocyte levels before (4 weeks previously) natural infection with *Trypanosoma microti* for field voles that acquired high infection intensities and others that developed lower levels of parasitaemia

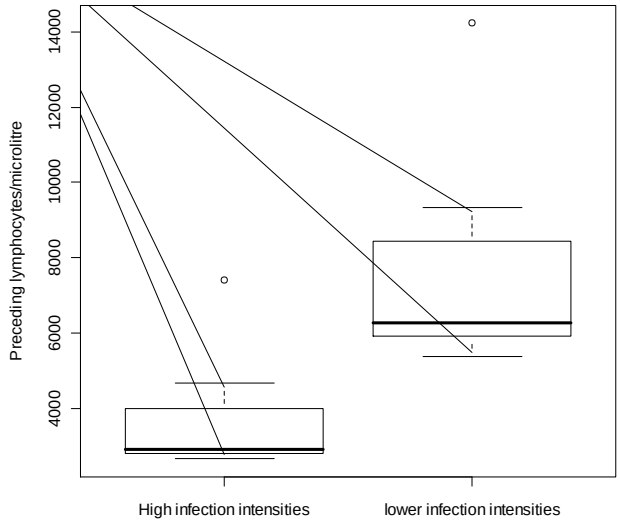
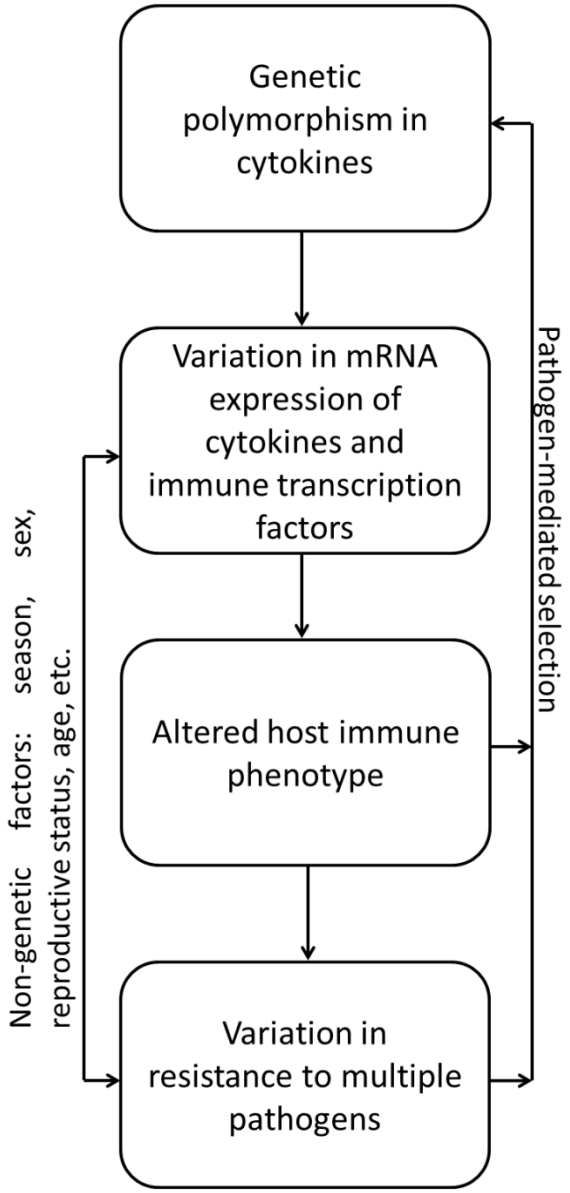


Figure XXX. From Turner and Paterson (2013). Causes and consequences of immunogenetic variation in Kielder voles. Polymorphism within cytokine genes – interacting with non-genetic factors - has a discernible effect on the transcription of immune genes and thus on host immune phenotype. Phenotypic variation in immune responses leads to variation among individuals in resistance to a taxonomically diverse range of endemic pathogens, the selective pressures of which drive the maintenance of cytokine genetic diversity.



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