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SHORT COMMUNICATION

Short-range clinal variation in the prevalence of a sexually transmitted fungus associated with urbanisation

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Introduction

Sexually transmitted pathogens and parasites have attracted much recent attention by virtue of differences in epidemiology and biology from classical pathogens and parasites (Getz & Pickering, 1983; Anderson & May, 1991; Lockhart *et al.*, 1996). Epidemiologically, the rate of transmission of a sexually transmitted parasite is likely to be less dependent on host density than for ordinary infectious diseases, copulation frequency being generally less affected by density than by gross contact rate (Anderson & May, 1991). While work on the dynamics of sexually transmitted diseases in mammals, especially humans, has been intense (Anderson & May, 1991), study of these diseases in insects is rare. Where studies have been carried out, they have gone little beyond the observation that certain parasites and pathogens of insects are sexually transmitted, with some note of their virulence but little or no information about the dynamics of infection (e.g. Hurst *et al.*, 1995). This is unfortunate, as the dynamics of these diseases in insects are likely to differ from those of their better understood mammalian analogues because of the short lifespan of most adult insects and the voltinism of many insect populations.

Study of the factors affecting the dynamics, and therefore prevalence and incidence, of sexually transmitted disease in insects is therefore timely. One interaction that bears inspection is that between Laboulbeniales fungi and their hosts. The Laboulbeniales is a group of Ascomycete fungi where species grow on the cuticle of their host, are generally specialised on one or two host species, and cause little pathology beyond increased melanisation of the cuticle around the point of infection (Weir & Beakes, 1995). They are frequently, but not exclusively, transmitted during sexual contact (Benjamin & Shalor, 1952; Whisler, 1968; Strandberg & Tucker, 1974; De Kesel, 1995). The interaction between a coccinellid beetle, *Adalia bipunctata*, and the laboubenialian fungus *Hesperomyces virescens* presents a potentially useful case study for aiding understanding of disease dynamics, where incidence appears to vary over fairly short distances. The fungus was originally described on *A. bipunctata* in the London area (Weir & Beakes, 1996) but earlier examination of the host outside London failed to reveal the presence of infection (G. D. D. Hurst, pers. obs.), suggesting an effect of urbanisation on disease incidence and dynamics.

The work reported here had two objectives: (1) To examine whether transmission during host copulation is important for this parasite. This was attained by testing whether males were more likely to be infected on their ventral surface and females on their dorsum, paralleling the position of the sexes during copulation, and echoing observations of other sexually transmitted fungi (Benjamin & Shalor, 1952; Whisler, 1968; De Kesel, 1995). (2) To quantify systematically the extent of variation in prevalence over space, the extent to which this variation altered with time, and the potential role of variation in host phenology in producing the observed changes. Extreme variation in prevalence over a remarkably short distance was observed, with high prevalence in urban areas of London but absence outside these areas. The possible causes of this short-range variation in prevalence in terms of the effect of urbanisation on host phenology are discussed.

Methods

Evidence of transmission during copulation

Adalia bipunctata adults were collected from two sites in London: from *Tilia* x *europaea* in the centre of London, and

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from *Cirsium arvense* and *Urtica dioica* on Hampstead Heath, 6 km north of central London, in June 1999. Adult ladybirds were sexed using the criteria of Randall *et al.* (1992), and the ventral and dorsal surfaces were scored for infection with thalli of Laboulbeniales using a binocular microscope.

Prevalence of the infection in London

The prevalence of infection (proportion of adults infected) was assessed at various points along two transects through London, one running north–south (11 locations between Potters Bar, 25 km north of central London, and Dorking, 25 km to the south), the other east–west (13 locations between Laindon, 40 km east of central London, and Iver, 26 km west of central London). The north–south transect was surveyed during the periods 18–25 May 1998, 13–21 July 1998, 13–20 September 1998, and 15–17 May 1999; the east–west transect was surveyed between 22 and 25 May 1999. To this end, samples of *A. bipunctata* were collected from *Tilia* x *europaea* on these transects, by beating the trees in 1999, and by eye in 1998. These samples were augmented with collections from herbaceous plants, although *A. bipunctata* are not commonly found off *Tilia* x *europaea* in the London area. Samples in excess of 30 were obtained where possible. The beetles were scored for infection in the field and returned to the host plant.

The phenology of the ladybird was monitored over time during 1998 at two sites in London, one in the centre, one 11 km north of the centre. At each site, adult *A. bipunctata* were scored as to the cohort from which they derived (overwintered or new season) and for the presence of infection. Age was differentiated through elytral colour, old ladybirds being deep red and young ladybirds orange–red.

Results and discussion

Evidence of transmission during copulation

Infection was observed more frequently on the ventral surface of male beetles and on the dorsal surface of female beetles. In a sample of 36 males, 27 were infected on the ventrum only, six on the dorsum only, and three on both dorsum and ventrum. In contrast, in a sample of 23 females from the same location, two were infected on the ventrum only, 16 on the dorsum only, and five on both dorsum and ventrum. Ignoring cases of joint dorsal and ventral infection, which previous studies on Laboulbeniales have shown to be the result of auto-infection from an initial infection point rather than multiple infection points (Whisler, 1968), there was a significant association between sex and site of infection ($\chi^2 = 20.96$ with Yates' correction, 1 d.f., $P < 0.001$). Males were more likely to be infected on their ventrum, and females on their dorsum, as predicted by the sexual transmission hypothesis.

In addition to sex differences in the site of infection, there was a difference in the prevalence of infection on male and female hosts in the central London area. Prevalence was 54.7%

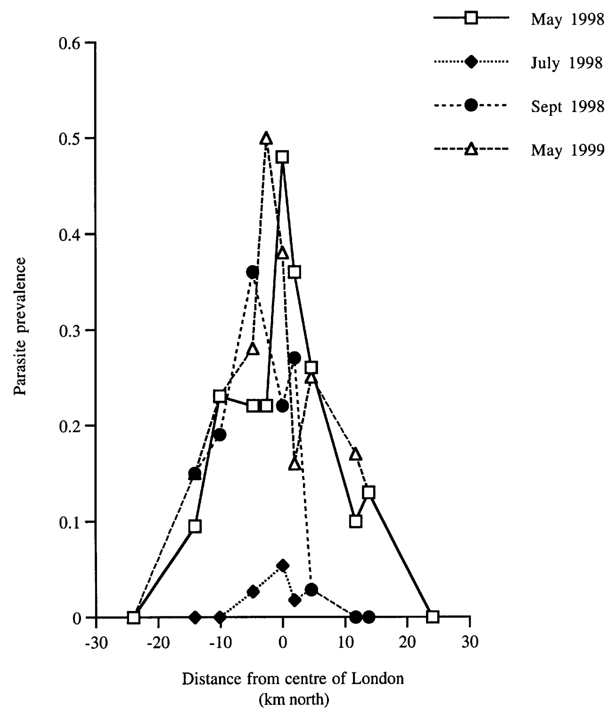

Fig. 1. Prevalence of *Hesperomyces virescens* infection on *Adalia bipunctata* on a north–south transect of London surveyed during 1998 and 1999.

($n = 42$) on males and 30.2% ($n = 63$) on females, with a population sex ratio of 1.5 females : 1 male. The difference in prevalence between the sexes was significant ($\chi^2 = 5.37$ with Yates' correction, 1 d.f., $P < 0.05$), and is consistent with the presence of a sexually transmitted parasite in a sex ratio biased population (for test of population sex ratio vs. 1:1: $\chi^2 = 3.81$ with Yates' correction, 1 d.f., $P = 0.05$).

Prevalence of the infection in London

Surveys of prevalence of the infection in the overwintered generation (May 1998 and May 1999) showed a marked peak in prevalence in the centre of London on both north–south and east–west transects, with lower prevalence at the extremes of the city and the infection absent or rare outside the urban area (Figs 1 and 2). On these transects, there was a negative correlation between distance of the site from central London (taken as Euston station) and the prevalence of infection (Spearman's rank correlation between distance of site from central London and prevalence: north–south transect 1998: $R = -0.94$, $n = 11$, $P < 0.001$; 1999: $R = -0.92$, $n = 11$, $P < 0.01$; east–west transect: $R = -0.94$, $n = 13$, $P < 0.001$).

Prevalence of the parasite decreased between May 1998 and July 1998 at all locations on the north–south transect, with very few observations of infected beetles in July 1998. Consistent with the negative correlation between distance from central London and parasite prevalence observed in the May transects,

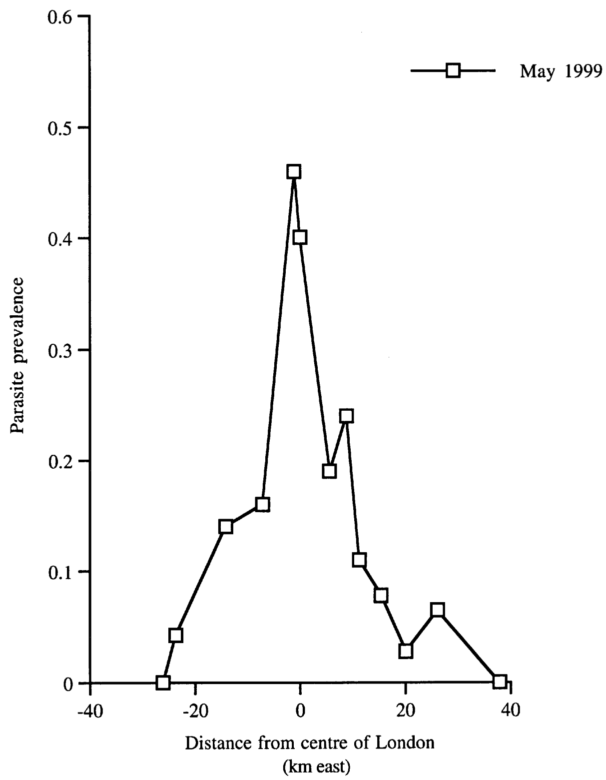

Fig. 2. Prevalence of *Hesperomyces virescens* infection on *Adalia bipunctata* on an east–west transect of London surveyed during May 1999.

infected beetles were only observed in central London. While prevalence had increased by September 1998, however, it was still lower than in the May 1998 and May 1999 samples from the same transect, and there was no observation of infection in samples taken from north London.

Phenology of the host

The decrease in prevalence between May and July 1998 was associated with the emergence of the new (uninfected) cohort of adult beetles that began in early June and the death of the old (infected) cohort. In both north and central London, the two cohorts were present together over a 4-week period in June, during which a few inter-generational matings were observed, matings that would spread the infection from the old to the new cohort. The occurrence of inter-generational matings appeared to be limited by the sexual immaturity of many of the adult beetles from the new cohort during the first 2 weeks in which both cohorts were observed together, then by the paucity of old cohort adult ladybirds, especially in north London, during the last 2 weeks. The ratio of new:old adults was 8:1 in central London and 23:1 in north London during this period.

The rise in the prevalence of infection in the centre of London towards September coincided with cessation in the emergence of the new cohort in central London in late July, and contrasts with the minimal increase in prevalence seen at the periphery, where the emergence of new (uninfected) adults continued into early September.

Discussion

Adalia bipunctata adults in London are parasitised by an ectoparasitic fungus, *H. virescens*. The sexual dimorphism in site of infection of hosts suggests that this parasite, like other species of this family, is transmitted predominantly during copulation, although a low degree of non-sexual contact transmission cannot be ruled out. Prevalence of the parasite varies over time, decreasing sharply with the emergence of the new cohort of adults. This variation over time parallels observations of the dynamics of other Laboulbeniales infections (Scheloske, 1969; Applebaum *et al.*, 1971; De Kesel, 1995), and is probably a fairly general feature of insect sexually transmitted diseases. More interestingly, there is also marked spatial variation in the prevalence of the parasite, with a higher prevalence of infection in central London than at the periphery, and infection rare or absent in the surrounding area.

The short-range variation in prevalence is unusual, with variation in prevalence occurring over distances less than 25 km. While the presence of both east–west and north–south clines indicates an association between urbanisation and disease dynamics, this represents only a superficial explanation of the causes of spatial variation in parasite prevalence, with the elements of urbanisation that impinge on disease dynamics remaining unclear. The most obvious effect of urbanisation is the effect that it has on host phenology. This is mediated in two ways. First, it affects the rate of death of the overwintered cohort, affecting the level of interbreeding between the cohorts. While the period over which old and new cohorts interbreed is the same across London, the proportion of old cohort hosts in the population during the period of overlap is higher in the centre than at the periphery. This will mean that the rate of inter-generational matings is higher in the centre, and this will increase the rate of infection of the new cohort. The second difference lies in the period over which members of the new cohort emerge. New cohort adults emerged for roughly 1 month longer at the periphery than in the centre. The spread of the parasite within the new cohort may be limited at the periphery by recruitment of new, uninfected adults.

How urbanisation produces these changes in host phenology remains unclear. There are two possibilities, which are not mutually exclusive. First, there may be a direct effect of increased temperatures within the urban environment on ladybird phenology. Central London is slightly warmer than its periphery, as seen in thermal images of the city and reflected in data from the U.K. Meteorology Office, which records a one degree difference in mean maximum temperature between central London and the periphery. Warm temperatures will accelerate ladybird development. They may also increase promiscuity and decrease disease latent period, both of which will affect disease dynamics. The second possibility is that changes in ladybird phenology follow the pattern of aphid growth on trees, which is affected by urbanisation. Aphid growth is known to be promoted by both pollution from cars (Przybylski, 1979; Braun & Flückiger, 1984; Bolsinger & Flückiger, 1987; Spencer & Port, 1988) and more generally by urbanisation (Dohmen *et al*., 1984; Dohmen, 1985). Temperature increases may also promote aphid growth. It is notable that aphids are present at high densities on trees over a longer period within London, and the period over which ladybirds are found to be ovipositing is prolonged compared with other parts of the U.K.; however, accurate data are not yet available.

One last point bears note. The association between infection and urbanisation in the London area suggests that it may be worth exploring the utility of the infection as an indicator of urban health. It has the benefit of being a readily scorable infection of a commonly found insect species. The question that remains is whether the effect is particular to London or whether the patterns observed are more general.

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References

Anderson, R.M. & May, R.M. (1991) *Infectious Diseases of Humans: Dynamics and Control*. Oxford University Press, Oxford.

Applebaum, S.W., Keir, R., Gerson, U. & Tadmor, U. (1971) Studies on the summer decline of *Chilocorus bipustulatus* in citrus groves of Israel. *Entomophaga*, **16**, 433–444.

Benjamin, R.K. & Shalor, L. (1952) Sex of host specificity and position specificity of certain species of Laboulbenia on *Bembidion picipes*. *American Journal of Botany*, **39**, 125–131.

Bolsinger, M. & Flückiger, W. (1987) Enhanced aphid infestation at motorways: the role of ambient air pollution. *Entomologia experimentalis et applicata*, **45**, 237–243.

Braun, S. & Flückiger, W. (1984) Increased population of the aphid *Aphis pomi* at a motorway: Part 1 – Field evaluation. *Environmental Pollution Series A*, **33**, 107–120.

De Kesel, A. (1995) Relative importance of direct and indirect infection in the transmission of *Laboulbenia slackensis* (Ascomycetes, Laboulbeniales). *Belgian Journal of Botany*, **128**, 124–130.

Dohmen, G.P. (1985) Indirect effects of air pollutants: changes in plant/parasite interactions. *Environmental Pollution*, **53**, 197–207.

Dohmen, G.P., McNeill, S. & Bell, J.N. (1984) Air pollution increases *Aphis fabae* pest potential. *Nature*, **307**, 52–53.

Getz, W.M. & Pickering, J. (1983) Epidemic models: thresholds and population regulation. *American Naturalist*, **121**, 892–898.

Hurst, G.D.D., Sharpe, R.G., Broomfield, A.H., Walker, L.E., Majerus, T.M.O., Zakharov, I.A. *et al*. (1995) Sexually transmitted disease in a promiscuous insect, *Adalia bipunctata*. *Ecological Entomology*, **20**, 230–236.

Lockhart, A.B., Thrall, P.H. & Antonovics, J. (1996) Sexually transmitted diseases in animals: ecological and evolutionary implications. *Biological Reviews*, **71**, 415–471.

Przybylski, Z. (1979) The effects of automobile exhaust gases on the arthropods of cultivated plants, meadows and orchards. *Environmental Pollution*, **19**, 157–161.

Randall, K., Majerus, M.E.N. & Forge, H.E. (1992) Characteristics for sex determination in British ladybirds, Coleoptera: Coccinellidae. *Entomologist*, **111**, 109–122.

Scheloske, H.W. (1969) Beiträge zur biologie, ökologie und systematik der Laboulbeniales (Ascomycetes) unter besonder berücksichtigung des parasit-Wirt-Vergältnisses. *Parasitogische Schriftenreihe*, **19**, 1–176.

Spencer, H.J. & Port, G.R. (1988) Effects of roadside conditions on plants and insects. II. Soil conditions. *Journal of Applied Ecology*, **25**, 709–715.

Strandberg, J.O. & Tucker, L.C. (1974) *Filariomyces forficulae*: occurrence and effects on the predatory earwig, *Labidura riparia. Journal of Invertebrate Pathology*, **24**, 357–364.

Weir, A. & Beakes, G.W. (1995) An introduction to the Laboulbeniales: a fascinating group of entomogenous fungi. *Mycologist*, **9**, 6–10.

Weir, A. & Beakes, G.W. (1996) Correlative light- and scanning electron microscope studies on the developmental morphology of *Hesperomyces virescens* Thaxt. *Mycologia*, **88**, 677–693.

Whisler, H.C. (1968) Experimental studies with a new species of *Stigmatomyces* (Laboulbeniales). *Mycologia*, **60**, 65–75.

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