Prevalence, transmission and intensity of infection by a microsporidian sex ratio distorter in natural *Gammarus duebeni* populations

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SUMMARY

This is a report of the prevalence, transmission and intensity of infection of a microsporidian sex ratio distorter in natural populations of its crustacean host *Gammarus duebeni*. Prevalence in the adult host population reflects differences in the intensity of infection in transovarially infected embryos and in adult gonadal tissue. The efficiency of transovarial parasite transmission to young also differs between populations, but this alone is insufficient to explain observed patterns of prevalence. Infection intensity may be important in determining future infection of target tissue in the adult and subsequent transmission to future host generations. We consider patterns of parasite infection in terms of selection on transmission and virulence.

Key words: transovarial transmission, parasite burden, microsporidian, sex ratio distorter.

INTRODUCTION

Parasitic sex ratio distorters have important implications for the ecology and evolution of their host populations. They have the potential to drive the evolution of the host population sex ratio (Werren, 1987; Taylor, 1990; Hatcher & Dunn, 1995), impose selective pressures on host sex determination (Bull, 1983; Rigaud, Mocquard & Juchault, 1992; Juchault, Rigaud & Mocquard, 1992; Hatcher & Dunn, 1995; Dunn *et al*. 1995) and may affect host population size, stability and extinction (Werren, 1987; Werren & Beukeboom, 1993). In this paper we examine prevalence, transmission and intensity of infection by a microsporidian sex ratio distorter infecting the crustacean *Gammarus duebeni*.

Vertically transmitted parasites are transmitted from parent to offspring via the host's gametes. If these parasites are uniparentally inherited, selection favours parasites which bias the host sex ratio towards the transmitting sex (Lewis, 1941; Howard, 1942; Hamilton, 1967). Vertically transmitted microsporidia have been found to distort host sex ratio through killing male hosts which releases spores for horizontal transmission (Kellen & Wills, 1962; Kellen *et al*. 1965; Hazard & Weiser, 1968) and through feminization in which genetic males are converted into phenotypic females, so directly increasing the relative frequency of the transmitting sex (Smith & Dunn, 1991; Dunn, Adams & Smith, 1993).

G. duebeni is host to a microsporidian sex ratio distorter described as *Octosporea effeminans* (Bulnheim & Vavra, 1968). The microsporidian is transovarially transmitted in the cytoplasm of the ova and is not passed on through males. There is no evidence of horizontal transmission (Bulnheim, 1978; Dunn *et al*. 1993). The parasite feminizes its host by converting putative males into functional females capable of transmitting the parasite to future generations (Smith & Dunn, 1991; Dunn *et al*. 1993). Hence, parasite-induced feminization boosts the effective transmission base of the parasite, enabling parasite invasion and maintenance at higher prevalences in host populations (Hatcher & Dunn, 1995).

Theoretical analyses (Werren, 1987; Taylor, 1990; Hatcher & Dunn, 1995) have shown that invasion and equilibrium prevalence of parasitic sex ratio distorters in host populations depend upon the efficiency of parasite transmission, the efficiency of parasite-induced feminization of the host, the relative fitness of infected hosts and the underlying host population sex ratio. Although parasite prevalence has been shown to be sensitive to parasite transmission efficiency (Werren, 1987; Hatcher & Dunn, 1995), parasite burden and its relationship with transmission and prevalence has not been explicitly considered. Here we examine parasite prevalence, parasite transmission efficiency and parasite burden in 3 *G. duebeni* field populations.

MATERIALS AND METHODS

During the breeding season of 1995 we collected random samples of adult animals from 3 *Gammarus duebeni* field populations: Budle Bay, Northumber-

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land, UK, Totton Marsh, Hampshire UK, and Douarnenez, Finestere, France. Parasite prevalence and the intensity of infection in adult females were estimated by light microscopy. Individuals were fixed in 10% formalin, dehydrated and embedded in paraffin wax. Previous studies indicated that the microsporidian is restricted to the gonadal tissue of infected females where spore (Dunn *et al*. 1993) and vegetative stages (Dunn, unpublished observations) have been observed. Therefore, serial transverse sections through the thorax (the site of the gonad) were stained with Giemsa's stain and examined for the microsporidian and measurements taken of the number of parasites infecting the mature oocytes of infected females.

The efficiency of transovarial parasite transmission to young was estimated by examining early stage embryos produced by infected mothers for the presence or absence of infection. Parasite burden was measured by counting total parasite load in these embryos. To determine the infection status of females from the field it is necessary to kill them. Therefore, we collected embryos from a random sample of infected and uninfected females from each site and screened the mother and her embryos for infection after breeding. *G. duebeni* form precopula pairs a few days before they mate. Pairs of animals were set up in individual containers in brackish water (specific gravity 1005°, corresponding to field salinity) at 12 °C and examined daily. Females mate and lay their eggs when they moult, approximately every 3 weeks. Eggs are laid into a marsupium formed from a series of overlapping plates (oostegites) on the ventral surface of the thorax and are brooded for 3–4 weeks. Within 24 h of fertilization, early-stage embryos (1–128 cells) were flushed from the marsupium using a syringe filled with brackish water. Clutch size and blotted wet weight of the female were recorded. Embryos were freeze fractured, fixed and stained with DAPI (4,6-diamidino-2-phenyl-indole), a fluorescent dye for DNA, and examined using a Zeiss Axiovert 10 fluorescent microscope. This enabled us to see host nuclei and the nuclei of parasites lying in the cytoplasm of the embryo cells (Dunn *et al*. 1995). Under fluorescence microscopy, a parasite nucleus can sometime be resolved into 2 nuclei, although it is not possible to determine whether this is a diplokaryon or a dividing nucleus. However, the increase in parasite number during progressive stages of host embryogenesis (Dunn *et al*. 1995) suggests that these are vegetative stages of the microspordian. Each embryo was scored for the presence or absence of the parasite. Parasite burden was measured by counting total parasite load in embryos at the 64 and 128 cell stage of development.

Data were analysed using the generalized linear modelling package GLIM (GLIM 3.77, Numerical Algorithms Group, Oxford 1985). Significance was assessed by looking at the reduction in deviance caused by deletion of a term from the maximal model. Data for parasite load and for clutch size are count data, therefore we specified a Poisson error distribution. We corrected for overdispersion with a heterogeneity factor (H_f = Pearson's χ^2/D.F.; Crawley, 1993). Changes in deviance caused by removing a factor from the model were compared with χ^2 tables. Data for parasite transmission efficiency were proportion data (number of infected eggs/total number of eggs) and were analysed specifying a binomial error structure, taking the total number of eggs as the binomial denominator. A heterogeneity factor was used to correct for overdispersion and changes in deviance caused by removing a factor from the model were assessed using an F-test.

RESULTS

Parasite prevalence

Parasite prevalence differed significantly between the field sites (χ^2, 2 D.F. = 28·74, $P < 0{\cdot}001$). The highest level of infection was at Budle Bay where 30% of females were infected ($N = 70$), at Douarnenez 15·6% ($N = 64$) were infected and the lowest level of infection, 6·3% ($N = 127$), was found at Totton Marsh (Table 1).

Parasite burden in adults

Parasite burden in the oocytes of adult females also differed between the 3 field sites and the pattern of parasite load was in accord with differences in prevalence: mean parasite load per oocyte was highest at Budle Bay, and lowest at Totton Marsh (Table 1). From Fig. 1, in infected Totton Marsh females most (80%) oocytes had a very low parasite burden (0–4 parasites) and the maximum burden recorded was 52 parasites. In contrast, 64% of oocytes in infected Budle Bay females had a burden of more than 10 parasites with 12% having a burden in excess of 90.

Parasite load differed significantly between the sites (χ^2, 2 D.F. = 45·2, $P < 0{\cdot}001$). In addition, within a population, parasite burden differed between families. Taking the brood of an individual mother to represent a family we found that incorporation into the model of a (site.mother) interaction term caused a significant reduction in the deviance of the data from the model (χ^2, 30 D.F. = 57, $P < 0{\cdot}05$).

Parasite transmission to eggs

The efficiency of transovarial parasite transmission to eggs also differed between sites ($F_{(2,40)} = 3{\cdot}95$, $P < 0{\cdot}05$). However, there did not appear to be a simple relationship between the efficiency of parasite trans-

Table 1. Prevalence, intensity of infection and the efficiency of transovarial transmission of the microsporidian sex ratio distorter in *Gammarus duebeni* from 3 field sites: Budle Bay (BB), Totton Marsh (TM) and Douarnenez (Dou)

(Parasite prevalence: percentage of adult females infected in the population. Parasite burden: mean number of parasites in mature oocyte ±standard error; mean parasite number in 64 cell embryo ±S.E.; mean parasite number in 128 cell embryo ±S.E. Transmission efficiency: mean proportion of infected eggs in the brood of infected mothers ±S.E.)

Site	Prevalence (%)	Burden in oocytes	Burden in 64 cell embryos	Burden in 128 cell embryos	Transmission efficiency
BB	30·0 ($N = 70$)	24·4 ± 6·2 ($N = 29$)	68·3 ± 5·6 ($N = 88$)	55·9 ± 12·8 ($N = 6$)	0·95 ± 0·033 ($N = 161$)
Dou	15·6 ($N = 64$)	8·0 ± 1·7 ($N = 49$)	35·8 ± 4·9 ($N = 14$)		0·77 ± 0·072 ($N = 180$)
TM	6·3 ($N = 127$)	4·5 ± 1·0 ($N = 74$)		11·8 ± 3·2 ($N = 8$)	0·87 ± 0·097 ($N = 92$)

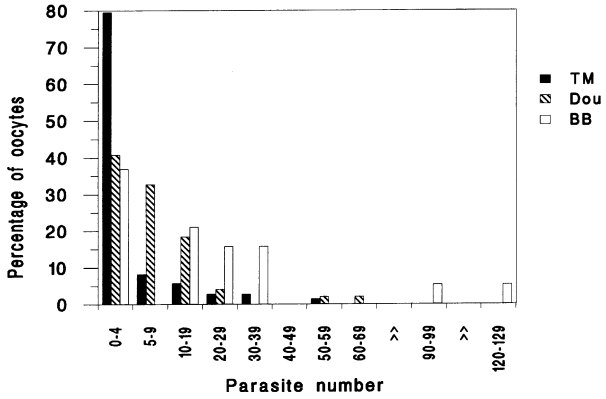

Fig. 1. Frequency distribution for parasite burden in mature oocytes from female *Gammarus duebeni* infected with the microsporidian sex ratio distorter. BB, Budle Bay, Northumberland, UK; Dou, Douarnenez, Finestere, France; TM, Totton Marsh, Hampshire, UK.

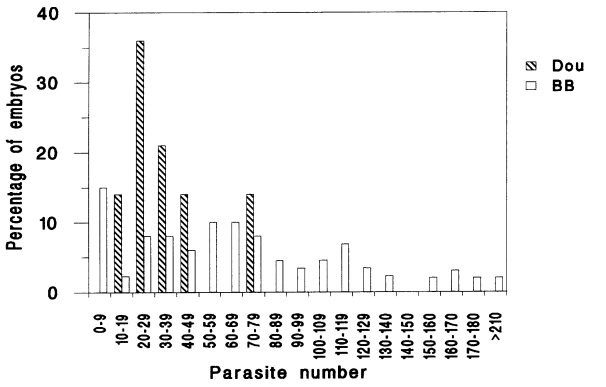

Fig. 2. Frequency distribution for parasite burden in embryos infected with the microsporidian sex ratio distorter. Data for 64 cell stage embryos from 2 field sites: Budle Bay (BB) and Douarnenez (Dou).

mission from mother to eggs, and parasite prevalence in the population. Although the proportion of the young of infected mothers which inherited the infection was highest at Budle Bay (where prevalence is highest), an intermediate proportion was recorded at Totton Marsh, with Douarnenez females showing the lowest transmission efficiency (Table 1).

Parasite burden in embryos

The pattern of parasite burden in 64 cell-stage embryos from Budle Bay and Douarnenez is illustrated in Fig. 2 and Table 1. Low parasite prevalence precluded collection of sufficient data from Totton Marsh embryos to include in the analysis. From Fig. 2, 71% of Douarnenez embryos had a parasite burden of less than 40 and parasite burdens ranged from 14 to 72. In contrast, the variance was greater in Budle Bay embryos (Budle Bay mean burden = 68·3, variance = 2732, Douarnenez mean burden = 35·8, variance = 335) and parasite burden ranged from 1 to 240.

Parasite burden was significantly higher in embryos from Budle Bay than in Douarnenez embryos (χ^2, 1 D.F = 7·6, $P < 0{\cdot}01$). This is in accord with the pattern of prevalence seen in the field. There was also a significant difference between families within the two sites: incorporation in the model of a (site.mother) interaction term caused a significant reduction in the deviance of the data from the model (χ^2, 12 D.F. = 31·9, $P < 0{\cdot}01$); it appears that parasite load is higher in some host families than others.

We also collected data from a small number of 128 cell embryos from Budle Bay and Totton Marsh (Table 1). Parasite burden differed significantly between-sites (χ^2, 1 D.F. = 34, $P < 0{\cdot}001$). However, we treat these data with caution due to the problem of small sample size. In addition, all 8 Totton Marsh embryos were from the same brood so it is not possible to dissociate inter-family differences from between-site differences in the host–parasite relationship.

The data also suggest that the parasite is not replicating during this period of host development (assuming no parasite death). From Table 1, it appears that mean parasite numbers in Budle Bay embryos decrease from the 64 to 128 cell stage. However, variances are high and the sample size for 128 cell embryos is small. There is, in fact, no significant difference in the parasite burden in 64 and 128 cell embryos (χ^2, 1 D.F = 0·41, $P > 0·05$). A previous study of parasitism in Budle Bay embryos also found that parasite replication appeared to be limited during early host embryogenesis (Dunn *et al*. 1995).

Fitness effects of parasitism

Female weight differed significantly between the 3 field sites (mean weight mg ± S.E.: Budle Bay 22·98 ± 0·83; Douarnenez 19·96 ± 0·58; Totton Marsh 26·01 ± 0·80; $F_{(2,129)} = 38·2$, $P < 0·01$). This may reflect differences in temperature or food availability at the different field sites, both of which affect growth. However, parasite burden did not significantly affect female weight ($F_{(1,129)} = 0·23$, $P > 0·05$). In addition, there was no significant effect on weight caused by an interaction between site and parasite burden ($F_{(3,126)} = 0·52$, $P > 0·05$). Hence, there is no evidence that parasite burden affects weight at any site.

Clutch size was significantly related to the weight of the mother (χ^2, 1 D.F. = 67·4, $P < 0·001$; larger females produced more eggs and this is in accord with previous studies (Kolding & Fenchel, 1981; McCabe & Dunn, 1994; Dunn & McCabe, 1995). Taking female weight as a covariate, we found no significant effect of parasite burden on clutch size (χ^2, 1 D.F = 0·23, $P > 0·05$). Clutch size did not differ between the sites (χ^2, 2 D.F. = 4·77, $P > 0·05$). Similarly, there is no significant effect on clutch size of the interaction between site and parasite burden (χ^2, 3 D.F. = 4·4, $P > 0·05$). These data are in accord with a previous study which found no parasite-induced reduction in host fecundity (Dunn *et al*. 1993).

DISCUSSION

Although the transmission route for most microsporidia is horizontal there are a number of microsporidia for which vertical transmission is also an important transmission mechanism (Chapman *et al*. 1966; Canning, 1982) and this may be the sole transmission route for the sex ratio distorter infecting *G. duebeni* (Smith & Dunn, 1991; Dunn *et al*. 1993). Although we cannot discount the possibility of horizontal transmission of the microsporidian in *G. duebeni*, either directly or through some intermediate host (e.g. Chapman *et al*. 1966; Andreadis & Hall, 1979; Sweeney, Hazard & Graham, 1985; Avery & Undeen, 1990), mathematical models predict that, as a result of its feminizing effect, the microsporidian may be maintained in host populations through vertical transmission alone (Hatcher & Dunn, 1995).

This study indicates that the parasite burden and the efficiency of transovarial parasite transmission are important factors underlying the level of infection in natural host populations. Parasite burden also varies between host families within a site. The pattern of prevalence in the field reflects the intensity of infection in oocytes of adults and in developing embryos: parasite burden is highest at Budle Bay where prevalence is also high. Previous studies of vertically transmitted microsporidia record similar parasite loads per ovum (Kellen & Wills, 1962) and also demonstrate a relationship between intensity of infection in the adult female and the efficiency of vertical transmission (Milner & Lutton, 1980; Canning *et al*. 1985).

Parasite transmission efficiency is also highest at Budle Bay, although there is no simple relationship between parasite prevalence and transovarial transmission efficiency. Studies of other transovarially transmitted microsporidia reveal a range of transmission efficiencies: e.g. transmission of *Amblyospora* spp. to 90% of the adult mosquito hosts (Andreadis & Hall, 1979); transmission of *Perezia pyraustae* to 50% of the eggs of the European cornborer host (Kramer, 1959); transmission of *Nosema plodiae* to 12% of eggs of the Indian meal moth host (Kellen & Lindegren, 1973). Kellen & Lindegren (1973) also noted considerable interfamily variation in the rate of *Nosema* transmission to host eggs. However, these microsporidia have both vertical and horizontal transmission routes (Kramer, 1959; Kellen & Lindegren, 1973; Sweeney *et al*. 1985) and *Amblyospora* has an indirect life-cycle which involves 2 host species (Sweeney *et al*. 1985). Parasite spread and maintenance in the host population is, therefore, less dependent on transovarial transmission for these microsporidia than it is for *O. effeminans*.

The pattern of parasite burden is recognized as an important factor for the dynamics of horizontally transmitted parasites (Anderson & May, 1978; May & Anderson, 1978; Anderson, 1991). However, parasite burden has not been explicitly considered in many studies of vertically transmitted parasites (Fine, 1975; Taylor, 1990; Werren, 1987; Hatcher & Dunn, 1995). A mismatch in previous studies of the *G. duebeni* system between estimates of transmission from adult to adult host (69% in Dunn *et al*. 1993) and estimates of transmission from adult to egg (96% in Dunn *et al*. 1995) may be accounted for by a relationship between parasite burden, growth rate and infection in the developing host. Low parasite numbers in oocytes may be sufficient to ensure transmission to the embryos of the next host generation but continued transovarial transmission

though generations of hosts is dependent on the presence of the parasite in the gonadal tissue of the breeding female (parasites in other tissues are not transmitted; Dunn *et al*. (1995); Hatcher, Tofts & Dunn (1996)). Initial parasite load and parasite growth may dictate transmission to the target gonadal tissue and feminization of the host, factors which have an impact upon parasite prevalence at the population level. The precise mechanisms of parasite development and transmission within individual hosts are currently under investigation.

Parasite numbers will represent a trade-off between opposing selective forces. On the one hand, selection will favour a high burden, which will maximize the chances of transmission from adult to offspring and of infection of gonadal tissue in the new host (Dunn *et al*. 1995; Hatcher *et al*. 1996). On the other hand, vertically transmitted parasites are dependent on host reproduction for their own transmission and so selection may favour a parasite which minimizes the metabolic burden imposed on the host (Ewald, 1987; Bull & Rice, 1991; Bull, Molineux & Rice, 1991; Smith & Dunn, 1991). Any negative effects on host fitness (reduced reproductive success of infected hosts) must be traded-off against the consequent loss of potential targets for infection.

Furthermore, parasite transmission strategy is likely to reflect a trade-off between initial load in the egg and replication rate within the embryo, if low initial parasite load is associated with increased host survival (Dunn *et al*. 1995). Although a reduction in egg production, egg hatch (Andreadis & Hall, 1979; Hembree & Ryan, 1982) and subsequent survival (Kramer, 1959; Raina *et al*. 1995) is commonly associated with transovarially transmitted microsporidia, we found no evidence of a relationship between parasite burden and fitness in *G. duebeni*. Parasite burden had no effect on clutch size or on adult female weight. However, other fitness parameters which may be affected by parasite burden (e.g. growth rate, onset of sexual maturity, lifetime reproductive output) were not measured. A previous study revealed no reduction in survival or growth rate of infected young (Dunn *et al*. 1993) but infection status rather than precise burden was considered. An increased understanding of transmissibility and virulence in terms of within host processes of infection and parasite growth has been identified as important for determining population-level outcomes for the host (Bull, 1994). Future studies will investigate in more detail the relationship between parasite burden and host fitness in the *G. duebeni* system, and the implications for parasite–host dynamics.

We found between population and between family differences in the parasite–host relationship. Differences in the pattern of infection by the microsporidian sex ratio distorter in natural *G. duebeni* populations may result from differences in host susceptibility or resistance to parasite transmission and growth. The differences may reflect clonal differences between parasite strains in the different populations or may result from the impact of abiotic factors on parasite development (Bulnheim 1978). In further studies we will investigate the importance of these factors for parasite–host dynamics.

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REFERENCES

ANDERSON, R. M. (1991). Populations and infectious diseases: ecology or epidemiology? *Journal of Animal Ecology* **60**, 1–50.

ANDERSON, R. M & MAY, R. M. (1978). Regulation and stability of host-parasite population interactions I. Regulatory processes. *Journal of Animal Ecology* **47**, 219–247.

ANDREADIS, T. G. & HALL, D. W. (1979). Significance of transovarial infections of *Amblyospora* sp. (Microspora: Thelohaniidae) in relation to parasite maintenance in the mosquito *Culex salinarius*. *Journal of Invertebrate Pathology* **34**, 152–157.

AVERY, S. W. & UNDEEN, A. H. (1990). Horizontal transmission of *Parathelohania anophelis* to the copepod, *Microcyclops varicans*, and the mosquito, *Anopheles quadrimaculatus*. *Journal of Invertebrate Pathology* **56**, 98–105.

BULL, J. J. (1983). *The Evolution of Sex Determining Mechanisms*. Benjamin Cummings, California.

BULL, J. J. (1994). Virulence. *Evolution* **48**, 1423–1437.

BULL, J. J. & RICE, W. R. (1991). Distinguishing mechanisms for the evolution of co-operation. *Journal of Theoretical Biology* **149**, 63–74.

BULL, J. J., MOLINEUX, I. J. & RICE, W. R. (1991). Selection of benevolence in a host-parasite system. *Evolution* **45**, 875–882.

BULNHEIM, H. P (1978). Interaction between genetic, external and parasitic factors in sex determination of the crustacean amphipood *Gammarus duebeni*. *Helgolander wissenschaftliches Meeresunters* **31**, 1–33.

BULNHEIM, H. P. & VAVRA, J. (1968). Infection by the microsporidia *Octosporea effeminans* sp. n., and its sex determining influence in the amphipod *Gammarus duebeni*. *Journal of Parasitology* **54**, 241–248.

CANNING, E. U. (1982). An evaluation of protozoal characteristics in relation to biological control of pests. *Parasitology* **84,** 119–149.

CANNING, E. U., BARKER, R. J., PAGE, A. M. & NICHOLAS, J. P. (1985). Transmission of microsporidia, especially *Orthosoma operophterae* (Canning 1960) between generations of winter moth *Operophtera brumata* (L) (Lepidoptera: Geometridae). *Parasitology* **90,** 11–19.

CHAPMAN, H. C., WOODARD, D. B., KELLEN, W. R. & CLARK, T. B. (1966). Host–parasite relationships of *Thelohania*

associated with mosquitoes in Louisiana (Nosematidae; Microsporidia). *Journal of Invertebrate Pathology* **8,** 452–456.

CRAWLEY, M. J. (1993). *GLIM for Ecologists*. Blackwell Scientific Publications, Oxford.

DUNN, A. M., ADAMS, J. & SMITH, J. E. (1993). Transovarial transmission and sex ratio distortion by a microsporidian parasite in a shrimp. *Journal of Invertebrate Pathology* **61**, 248–252.

DUNN, A. M., HATCHER, M. J., TERRY, R. S. & TOFTS, C. (1995). Evolutionary ecology of vertically transmitted parasites: strategies of transovarial transmission of a microsporidian sex ratio distorter in *Gammarus duebeni*. *Parasitology* **111**, S91-S110.

DUNN, A. M. & MCCABE (1995). Resource allocation to young: seasonal patterns within and between *Gammarus duebeni* populations. *Oikos* **73**, 199–202.

EWALD, P. W. (1987). Transmission modes and the evolution of the parasite-mutualism continuum. *Annals of the New York Academy of Science* **503**, 295–306.

FINE, P. E. M. (1975). Vectors and vertical transmission; an epidemiological perspective. *Annals of the New York Academy of Science* **266**, 173–194.

HAMILTON, W. D. (1967). Extraordinary sex ratios. *Science* **156**, 477–488.

HATCHER, M. J. & DUNN, A. M. (1995). Evolutionary consequences of sex ratio distortion by cytoplasmically inherited feminizing factors. *Philosophical Transactions of the Royal Society, London* B **348**, 445–456.

HATCHER, M. J., TOFTS, C. & DUNN, A. M. (1996). The effect of the embryonic bottleneck on vertically transmitted parasites. *Selected Proceedings of the 1st Conference on Information Processing in Cells* (in the Press).

HAZARD, E. I. & WEISER, J. (1968). Spores of *Thelohania* in adult female Anopheles: development and transovarial transmission, and redescriptions of *T. legeri* Hesse and *T. obea* Kudo. *Journal of Protozoology* **15**, 817–823.

HEMBREE, S. C. & RYAN, J. R. (1982). Observations on the vertical tranmsission of a new microsporidian pathogen of *Aedes aegypti* from Thailand. *Mosquito News* **42**, 49–54.

HOWARD, H. W. (1942). The genetics of *Armadillidium vulgare* Latreille. II. Studies on the inheritance of monogeny and amphogeny. *Journal of Genetics* **44**, 143–159.

JUCHAULT, P., RIGAUD, T. & MOCQUARD, J. P. (1992). Evolution of sex-determining mechanisms in a wild population of *Armadillidium vulgare* Ltr. (Crustacea, Isopoda): competition between two feminizing parasitic sex factors. *Heredity* **69**, 382–390.

KELLEN, W. R., CHAPMAN, H. C., CLARK, T. B. & LINDEGREN, J. E. (1965). Host–parasite relationship of some *Thelohania* from mosquitoes (Nosematidae: Microsporidia). *Journal of Invertebrate Pathology* **7**, 161–166.

KELLEN, W. R. & LINDEGREN, J. E. (1973). Transovarial transmission of *Nosema plodiae* in the Indian-meal moth, *Plodia interpunctella*. *Journal of Invertebrate Pathology* **21**, 248–254.

KELLEN, W. R. & WILLS, W. (1962). The transovarial transmission of *Thelohania californica* Kellen and Lipa in *Culex tarsalis* Coquillett. *Journal of Insect Pathology* **4**, 321–326.

KOLDING, S. & FENCHEL, T. M. (1981). Patterns of reproduction in different populations of five species of the amphipod genus *Gammarus*. *Oikos* **37**, 167–172.

KRAMER, J. P. (1959). Some relationships between *Perezia pyraustae* Paillot (Sporozoa, Nosematidae) and *Pyrausta nubilalis* (Hubner) (Lepidoptera, Pyralidae). *Journal of Insect Pathology* **1**, 25–33.

LEWIS, D. (1941). Male sterility in natural populations of hermaphrodite plants. *New Phytologist* **40**, 50–63.

MAY, R. M. & ANDERSON, R. M. (1978). Regulation and stability of host-parasite population interactions II. Destabilising processes. *Journal of Animal Ecology* **47**, 249–287.

MCCABE, J. & DUNN, A. M. (1994). Is resource partitioning a response to brood sex ratio in an amphipod with environmental sex determination? *Oikos* **69**, 203–206.

MILNER, R. J. & LUTTON, G. G. (1980). Interactions between *Oncopera alboguttata* (Lepidoptera: Hepialidae) and its microsporidian pathogen, *Pleistophora oncoperae* (Protozoa: Microsporida). *Journal of Invertebrate Pathology* **36**, 198–202.

RAINA, S. K., DAS, S., RAI, M. M., & KHURAD, A. M. (1995). Transovarial transmission of *Nosema locustae* (Microsporida: Nosematidae) in the migratory locust *Locusta migratoria migratorioides*. *Parasitology Research* **81**, 38–44.

RIGAUD, T., MOCQUARD, J. P. & JUCHAULT, P. (1992). The spread of parasitic sex factors in populations of *Armadillidium vulgare* Latr. (Crustacea, Oniscidea): effects on sex ratio. *Genetics, Selection, Evolution* **24**, 3–18.

SMITH, J. E. & DUNN, A. M. (1991). Transovarial transmission. *Parasitology Today* **7**, 146–148.

SWEENEY, A. W., HAZARD, E. I. & GRAHAM, M. F. (1985). Intermediate host for an *Amblyospora* sp. (Microspora) infecting the mosquito, *Culex annulirostris*. *Journal of Invertebrate Pathology* **46**, 98–102.

TAYLOR, D. R. (1990). Evolutionary consequences of cytoplasmic sex ratio distorters. *Evolutionary Ecology* **4**, 235–248.

WERREN, J. H. (1987). The coevolution of autosomal and cytoplasmic sex ratio factors. *Journal of Theoretical Biology* **124**, 313–334.

WERREN, J. H. & BEUKEBOOM, L. W. (1993). Population genetics of a parasitic chromosome: theoretical analysis of PSR in subdivided populations. *American Naturalist* **142**, 224–241.