Measuring the transmission dynamics of a sexually transmitted disease

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Sexually transmitted diseases (STDs) occur throughout the animal kingdom and are generally thought to affect host population dynamics and evolution very differently from other directly transmitted infectious diseases. In particular, STDs are not thought to have threshold densities for persistence or to be able to regulate host population density independently; they may also have the potential to cause host extinction. However, these expectations follow from a theory that assumes that the rate of STD spread depends on the proportion (rather than the density) of individuals infected in a population. We show here that this key assumption (''frequency dependence''), which has not previously been tested in an animal STD system, is invalid in a simple and general experimental model. Transmission of an STD in the two-spot ladybird depended more on the density of infected individuals in the study population than on their frequency. We argue that, in this system, and in many other animal STDs in which population density affects sexual contact rate, population dynamics may exhibit some characteristics that are normally reserved for diseases with density-dependent transmission.

density dependence | frequency dependence | population dynamics | parasite | infection

Understanding transmission dynamics is fundamental to understanding the ecology and evolution of host–parasite interactions. The transmission of most types of directly transmitted infectious disease is generally characterized by using a ''density-dependent'' function (refs. 1–4, but see ref. 5), whereby the rate of production of infected hosts per unit of area per unit of time (dI/dt) is described by βSI, where β is the transmission coefficient (which corresponds to both the rate of contact between infectious and susceptible individuals and the probability of successful transmission per contact), S is the density of susceptible individuals, and I is the density of infectious hosts. In models based on this function, the rate at which susceptible individuals become infected is proportional to the density of infectious hosts, because contacts between susceptible and infectious individuals increase with population density. In contrast, classic sexually transmitted disease (STD) models assume that the sexual contact rate is independent of population density (6–9). The transmission rate is instead thought to depend on the frequency (not the density) of infectious hosts (I/N, where N is the total population size, $S + I$) (4, 6–14) and ''frequency-dependent'' transmission therefore takes the form $\beta SI/N$. Although several theoretical studies (4, 15–17) have argued that disease transmission is unlikely to be either purely frequency- or density-dependent in the real world, for simplicity most studies of STD dynamics assume that transmission will follow the standard frequency-dependent ($\beta SI/N$) term (10–14).

This distinction is fundamentally important because frequency dependence is widely understood to lead to specific epidemiological characteristics. Unlike other types of directly transmitted infectious disease, STDs are not expected to have threshold densities for persistence, they may cause deterministic extinction, and it is thought that they cannot regulate their host's population size alone (6–8). Critically, even though STDs are now known to occur throughout the animal kingdom (16, 18), the only experimental assessment of STD transmission dynamics to date was in a plant. That study (19–21) provided evidence of frequency-dependent transmission of a pollinator-mediated ''STD'' in the plant system (anther-smut fungus on *Silene alba*), but there have been no experimental tests of whether transmission is frequency-dependent for animal (or human) STDs. Some of the better-known examples of STDs (i.e., high-profile human diseases) may be adequately modeled by a frequency-dependent function (e.g., ref. 14). But animal STD systems are extraordinarily diverse, and the dynamics of many are likely to be affected by population density. In many invertebrates, for example, the mating system of the host may show marked density-dependent variation in sexual contact rate (see the supporting information, which is published on the PNAS web site). STDs are known to be widespread in these taxa (16, 18), and many are of huge importance in ecological, socioeconomic, and medical terms. Two major arboviruses, for example, are sexually transmitted within their arthropod vectors: dengue in *Aedes albopictus* mosquitoes (22), and Crimean-Congo hemorrhagic fever virus in *Hyalomma truncatum* ticks (23). Therefore, given the importance of animal STDs and the implications of frequency-dependent transmission, it is vital that this key assumption is tested experimentally in suitable models. This research will enable the relative importance of frequency and density dependence to be established and facilitate the development of more realistic theoretical models.

We used the two-spot ladybird, *Adalia bipunctata*, and its sexually transmitted parasitic mite, *Coccipolipus hippodamiae* (24, 25), to carry out an experimental test of the frequency dependence assumption. This system provides an excellent model because extensive prior field and laboratory studies of the system's dynamics enable the design of ecologically realistic experiments. It is also simple enough to enable generalizations to be extended to a wide variety of other animal STD systems. The mating system of *A. bipunctata* is defined by scramble competition and promiscuity. These features characterize the mating systems of a wide range of animal taxa, particularly among the invertebrates (see supporting information). Thus, not withstanding the differences associated with particular animal mating systems, the ladybird–mite system provides a basic model in which the effect of population density on STD dynamics can be quantified experimentally.

Materials and Methods

Experimental System. Previous work has established that *A. bipunctata* mate approximately once every 2 or 3 days at the height

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Abbreviation: STD, sexually transmitted disease.

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Table 1. The density of males (m), females (f), and infected females (numbers in brackets) in each treatment

	Total density*			
Prevalence†	6	12	18	24
0.33	3m, 3f (1)	6m, 6f (2)	9m, 9f (3)	12m, 12f (4)
0.67	3m, 3f (2)	6m, 6f (4)	9m, 9f (6)	12m, 12f (8)

*Total densities correspond to 2.4, 4.8, 7.2, and 9.6 ladybirds per m^2 of mating arena, respectively.
†Proportion of females infected.

of reproductive activity during the summer months (26). Adult *C. hippodamiae* are sessile and inhabit the protected, ventral surface of the adult ladybird's elytra, where they feed on the hemolymph. Transmission occurs when motile, infective larval mites transfer to an uninfected ladybird during copulation and is almost exclusively sexual; nonsexual transmission is possible only when adult beetles are maintained in unusually close proximity and then occurs only at low levels (24, 27). Prevalence does not differ significantly between male and female *A. bipunctata* in the field, neither of which show any resistance to the mites, and recovery from infection occurs only rarely. Transmission efficiency is very high in both sexes (26). Females are sterilized within 2–3 weeks of becoming infected by *C. hippodamiae* (24), and infected males (and possibly females) also suffer an increase in over-wintering mortality in the field (27). Laboratory studies have also established that the survivorship of both sexes is further reduced by infection when the beetles are nutritionally stressed (J.J.R., J. Hathway, and R.J.K., unpublished data). Perhaps surprisingly, neither sex seems to differentiate between infected and uninfected partners, and infection status does not affect mating success (28).

Infection Procedures. Infected and uninfected *A. bipunctata* were collected from various locations in Berlin in August 2002 and 2003 and used to rear laboratory cultures in London. All ladybirds were maintained at 23°C in a constant-temperature room with a 16-h light:8-h darkness cycle. Larvae and adults were housed separately in 9-cm-diameter Petri dishes in humidified tanks and reared on a daily diet of fresh pea aphids, *Acyrthosiphon pisum.* Separate stocks of infected and uninfected ladybirds were maintained throughout. Experimental ladybirds were artificially infected by using a fine piece of nylon to transfer infective larval mites and/or mite eggs to the undersides of their elytra, between 12 and 20 days after emergence from the pupal stage. Control ladybirds in each replicate were given a sham infection, which involved manipulating their elytra in the same way and at the same time as the infected ladybirds. Newly infected ladybirds became infectious within 15 days and were used in the mating experiments within a further 15 days. Before the infected beetles became infectious, all ladybirds were maintained with individuals of the opposite sex and allowed to mate for between 3 and 7 days; otherwise, beetles were kept in single-sex groups.

Transmission Experiment. To measure the effect of density and prevalence on transmission, we manipulated both the density and frequency (prevalence) of infectious ladybirds within enclosed mating arenas. Our design allowed us to manipulate density while holding prevalence constant at either 33% or 67% (Table 1). The arenas consisted of large glass aquaria ($24 \times 24 \times 44$ cm) filled with stacks of Petri dishes glued together into a layered arrangement. The dishes provided a good substrate for ladybird locomotion and increased the available surface area within each tank to 2.49 m^2. This design enabled us to reproduce densities typically experienced by field populations (K.M.W., V. Isham, and G. D. D. Hurst, unpublished data; see Table 1). The arenas were evenly lit to prevent the ladybirds' aggregating around any one light source, and fresh aphids were added in excess before the start of each replicate. Females were used as the infectious sex, and transmission was measured from females to males. We did not use the reciprocal male-to-female design because host sex does not affect transmission efficiency in this system (26) and infection status does not affect host mating behavior (28).

Within each replicate, ladybirds were allocated randomly to treatments and treatments were carried out in a randomized order. Female ladybirds were placed in the arenas first; then the males were added, and the lids of the tanks were put in place. All ladybirds were observed carefully for a period of 1 h, and the number of copulating pairs that formed was recorded. We used a 1-h interval because this is equal to the approximate minimum copulation duration for ladybirds of equivalent age in our laboratory population (mean duration, 86 min; minimum, 60 min; maximum, 108 min; $n = 15$). Thus, we allowed a single round of infection to occur without permitting any secondary transmission from newly infected ladybirds. The arenas were then disassembled, and both single and paired ladybirds were removed and maintained in individual sample tubes. Copulating pairs were handled carefully so as not to terminate copulation prematurely and were allowed to separate of their own accord. The infection status of all ladybirds in a replicate was checked, and each was assigned to the appropriate category: newly infected male, uninfected male, infectious female, or uninfected female. This check was carried out 24 h after the end of each replicate so that any males infected with mite larvae that failed to attach to the elytra could be discounted (a small proportion of new infections fail).

The number of ladybirds per replicate is as shown in Table 1. Eleven replicates were carried out in total, three of which used ladybirds collected as pupae from Mile End, London, in May 2004. These ladybirds were collected in the pupal stage, which enabled us to control their mating history using the same method as for the German ladybirds. There was no difference in the development of the mites on English compared with German ladybirds, and transmission efficiency was extremely high in both cases. All statistical analyses were carried out with R 2.0.1 for Mac OSX.

Results

Under the frequency-dependent model there should be no change in the individual mating rate when density is varied, such that the proportion of males that become infected changes with prevalence but not with density. However, we found a significant effect of density on the proportion of females that mated, with ≈25% mating at the lowest density and 45% mating at the highest density (Fig. 1). This relationship led to a significant increase in the proportion of males that were infected at the end of the experiment as density increased (Fig. 2). The null hypothesis of pure frequency dependence was therefore rejected. The prevalence of infectious females was also related to the proportion of males becoming infected (Fig. 2) but did not significantly affect mating rate, confirming that infection status did not alter the probability of mating (28).

To determine the relative contributions of frequency- and density-dependent transmission, we calculated transmission rates as $[\ln(S_0/S_t)]S_0$, where S_0 is the number of males present at the start of the experiment and S_t is the number of uninfected males remaining at the end (29), and we examined the fit of a combined frequency- and density-dependent function to the data. This function has the form $\beta_1 SI/N + \beta_2 SI$, where S is the density of males, I is the density of infectious females, N is the total density of females, and β_1 and β_2 are frequency- and density-dependent transmission coefficients, respectively. [Note

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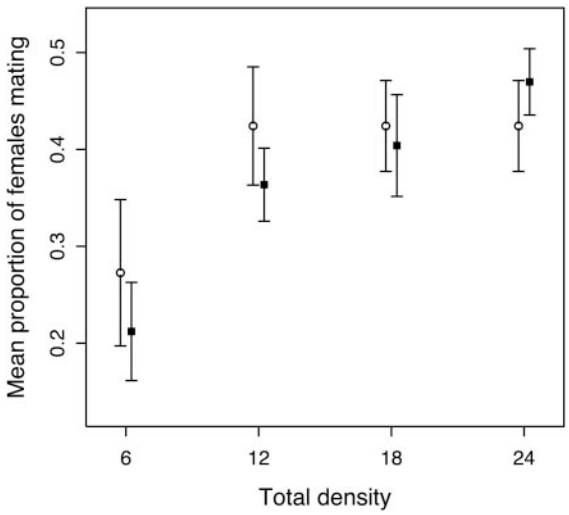

Fig. 1. The mean proportion of females that mated at each density. ■, 0.67 females infectious; ○, 0.33 females infectious. There was a significant increase in mating rate with increasing density ($P < 0.01$) but no effect of the prevalence of infectious females ($P = 0.87$; generalized linear model with binomial errors and LOGIT link; prevalence entered as a factor, density a continuous variable). There was no significant interaction between density and prevalence, so the interaction term was dropped from the final model. Error bars show ± 1 standard error.

that, in our experiment, population density and size are equivalent in relation to the derivation of the transmission function, because neither arena size nor available surface area varied within or between treatments (4).] Standard linear modeling was used because both SI and SI/N were known. Of the two estimated transmission coefficients, only β_2 was significantly different from zero (residual standard error = 1.13, d.f. = 86; $\beta_1 = 0.122 \pm 0.115$, $t = 1.059$, $P = 0.29$; $\beta_2 = 0.037 \pm 0.011$, $t = 3.31$, $P = 0.001$), indicating that the frequency-dependent component of the combined function did little to improve its fit to the data. Simplification of the model to the standard density-dependent function (βSI) gave an estimated transmission coefficient that was again significantly different from zero ($\beta = 0.048 \pm 0.003, t = 17.95, P < 0.0001$). The residual standard error was approximately the same (1.13, d.f. = 87), despite the absence of the frequency-dependent term, and a partial F test showed that there was no significant reduction in the ability of the model to explain the data ($F_{1,87} = 1.12$, $P = 0.29$). By contrast, removal of the density-dependent term from the combined model led to a substantial decrease in explanatory power ($F_{1,87} = 10.98$, $P = 0.001$). This analysis indicates that density dependence represents the "minimum adequate model" (30) to explain transmission (see Fig. 2).

Discussion

We found that a density-dependent function provides a superior means of describing STD transmission in our experimental system when compared with the frequency-dependent term that is usually applied to STDs. The mating rates we measured are comparable to field data for a very similar range of population densities (K.M.W., V. Isham, and G. D. D. Hurst, unpublished data). We are therefore confident that this effect is biologically realistic. The mechanistic explanation of our data is that encounter rate was positively associated with the density of ladybirds in the arenas; males at high densities spent less time searching before finding a female than males at low densities, and both contact rate and transmission therefore varied with population density (4).

A number of authors have investigated more complex, nonlinear formulations of the transmission function (e.g., refs. 15 and 17). The combined function ($\beta_1 SI/N + \beta_2 SI$) that we developed allows us to model varying amounts of frequency and density dependence simultaneously, but we cannot rule out the possibility that the transmission function underlying our data was really nonlinear. There is a suggestion in Fig. 2*B*, for example, that transmission rates may have been saturating at high densities for the lower prevalence. It was not possible for us to include more than four experimental densities or any additional infection levels (although our experimental design did reproduce key aspects of the system's ecology in this respect). Therefore, although we found that a similar analysis to the one we have presented, but based on a nonlinear fit, did not have greater explanatory power, further work would be needed to confirm this. However, two lines of argument suggest that the amount of saturation was probably low and that our linear analysis was robust. First, there was no significant interaction between the effects of density and prevalence in the generalized linear model, indicating that density did not affect transmission significantly differently for each prevalence. [That previous work in *A.*

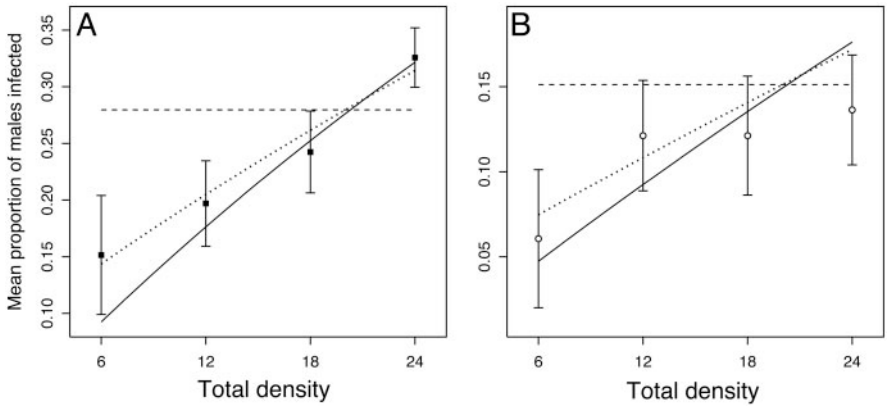

Fig. 2. The mean proportion of males that were infected at the end of the experiment at each density: 0.67 (*A*) and 0.33 (*B*) females infectious. There was a significant increase in the proportion of males that became infected, both as density increased ($P < 0.001$) and as prevalence increased from 0.33 to 0.67 ($P < 0.0001$; generalized linear model with binomial errors and LOGIT link; prevalence entered as a factor, density a continuous variable). There was no significant interaction, and this term was dropped from the final model. In *A* and *B*, the lines show the predicted proportion infected under the fitted density-dependent (solid line), frequency-dependent (dashed line), and combined frequency- and density-dependent (dotted line) models. See the text for a full explanation. Error bars show ± 1 standard error.

bipunctata has failed to find any effect of infection status on mating behavior also suggests that an interaction is unlikely (28).] Second, the fact that the frequency-dependent term dropped out of the minimum adequate model in our analysis suggests that any increase in the relative importance of frequency dependence with increasing density was relatively low.

We also analyzed the theoretical properties of the combined transmission function. This analysis showed that even a small degree of density dependence can radically alter the dynamics of a system (J.J.R., M. Miller, R.J.K., and M.B., unpublished analysis). In particular, any amount of density dependence will result in threshold densities (2) for STD persistence and even a small amount of density dependence can broaden the conditions for host–parasite coexistence (although frequency dependence can still lead to deterministic host extinction) (6). The fact that the density-dependent model provides the best means of explaining transmission in our system is therefore very important, because the theoretical dynamics of the system will differ fundamentally from those predicted under classic STD models. Populations of *A. bipunctata* in central and eastern Europe are subject to recurrent seasonal epidemics of *C. hippodamiae* (26). The density-dependent effect on transmission that we detected may thus help to explain the coexistence of *C. hippodamiae* and *A. bipunctata*, which may be less likely under pure frequency dependence. It is also possible that *C. hippodamiae* may fade out in a given subpopulation if host density drops to a low level, which would not be anticipated under purely frequency-dependent dynamics.

Having tested the frequency dependence assumption experimentally in a model animal STD system, we found that the density-dependent model usually applied to other types of infectious disease provides a superior fit to our data. Critically, the system we focused on is simple enough to enable us to make general inferences in relation to other animal groups. The mating system of *A. bipunctata* is defined by promiscuity and relatively brief sexual interaction. These characteristics are shared by an extremely diverse range of animal taxa, particularly among the invertebrates (31). A review of the published literature (see supporting information) shows that increases in population density are commonly associated with increases in mating rate among insect taxa. A number of reports also indicate that increases in promiscuity with population density may be common in some vertebrate groups, such as birds, which have more structured mating systems. In all of these cases (and, presumably, many others) transmission of any STDs should be at least partly density-dependent. For the reasons we discussed above (in relation to the effect of incorporating even small amounts of density dependence into STD dynamics), this trend will lead to population dynamics that differ markedly from those predicted under purely frequency-dependent models.

Antonovics *et al.* (15) suggested that a continuum of transmission dynamics exists between pure frequency and density dependence. By experimentally quantifying the relative importance of each using a combined transmission function we have effectively placed an animal STD system on this continuum with empirical data for the first time. Future studies of the ecology and evolution of animal STDs should examine the effects of density for a variety of different mating systems, enabling us to determine where on the density–frequency continuum each system lies. The use of a more general transmission term (4, 15), such as the combined function we have suggested here, provides a means of doing so and will help us to explore the dynamical implications.

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