REFERENCES AND NOTES

12. We performed molecular modeling using MACROMODEL 5.5 (Amber force field) (1); F. Mohamadi et al., J. Comput. Chem. 11, 440 (1990). The volumes for the guest and the cavities were obtained from the GRASP program [A. Nicholls, K. A. Sharp, B. Honig, Proteins 11, 281 (1991)].
14. We obtained all measurements by 1H NMR experiments using the integrals for the peaks of the guest inside and outside the capsules. There is an estimated 10% error in these measurements. The equilibrium may be described as follows:

$$K_B = \frac{[H^+G]^B}{[H^+G]^A} = \frac{[H^+G]^B}{[H^+G]^A}$$

The following assumptions were made: (i) the amount of dimer (unified or filled with solvent) present before addition of the guest is negligible; (ii) after addition of the guest, all of the host material not assembled into the capsule is in the aggregate state; and (iii) the association of the guest with itself is negligible.

$$K_A = \frac{[H^+G]^B}{[H^+G]^A} = \frac{[H^+G]^B}{[H^+G]^A}$$

and$$K_B = \frac{[H^+G]^B}{[H^+G]^A} = \frac{[H^+G]^B}{[H^+G]^A}$$

In these equations H is the host; G is the chiral guest; [H^+G]^B and [H^+G]^A are the concentrations of the predominant and the subordinate complexes, respectively; $\Delta G$ is the free energy of formation; T is temperature; and R is the ideal gas constant. $\alpha_0$ is the sum of all the integrals corresponding to the guest (subscript T stands for total); $\alpha_1$ is the integral for the signal of the guest outside the capsule; $\alpha_2$ is the integral for the signal of the guest in complex A; $\alpha_3$ is the integral for the signal of the guest in complex B; $\alpha$ is the amount of guest (in millimoles) of monomer (in millimoles); g is the amount of guest (in millimoles) of complex A; b is the amount of guest (in millimoles) in complex B; and $V$ is the total volume (in milliliters).

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Reports

Chain Reactions Linking Acorns to Gypsy Moth Outbreaks and Lyme Disease Risk

Clive G. Jones,* Richard S. Ostfeld, Michele P. Richard, Eric M. Schauber, Jerry O. Wolff

In eastern U.S. oak forests, defoliation by gypsy moths and the risk of Lyme disease are determined by interactions among acorns, white-footed mice, moths, deer, and ticks. Experimental removal of mice, which eat moth pupae, demonstrated that moth outbreaks are caused by reductions in mouse density that occur when there are no acorns. Experimental acorn addition increased mouse density. Acorn addition also increased densities of black-legged ticks, evidently by attracting deer, which are key tick hosts. Mice are primarily responsible for infecting ticks with the Lyme disease agent. The results have important implications for predicting and managing forest health and human health.

Oak trees (Quercus spp.) produce large annual acorn crops (masting) every 2 to 5 years, producing few or no acorns during intervening years (1–4). Acorns are a critical food for white-footed mice, Peromyscus leucopus (1, 4–6). Mice are important predators of pupae of the gypsy moth, Lymantria dispar (1, 6–10). This introduced insect periodically undergoes outbreaks (11, 12) that defoliate millions of hectares of oak forests, decreasing tree growth, survival, and mast production (13). An abundance of acorns draws white-tailed deer, Odocoileus virginianus, into oak forests (14, 15). Mice and deer are the primary hosts of the black-legged tick, Ixodes scapularis, which is the vector of spirochete bacteria (Borrelia burgdorferi) that cause Lyme disease in humans (16–18). Here we report the results of experimental removal of mice and addition of acorns, which demonstrate how acorn production is connected to gypsy moth outbreaks and Lyme disease risk.

Masting is associated with increased survival and breeding of mice in winter and spring (19), with peak densities occurring the following midsummer (1, 4, 6). High mouse density correlates with high predation rates on moth pupae (1, 6), which may prevent low-density moth populations from increasing (1, 6–8). Conversely, mast crop failure correlates with low mouse densities and low rates of pupal predation the following summer (1, 4, 6), which may initiate moth outbreaks (7, 9).

Moth populations at our research site reached peak densities in 1990, declined by four orders of magnitude to 0.2 egg masses ha$^{-1}$ by 1992, and remained between 6 and 38 egg masses ha$^{-1}$ in 1993–1994 (1). A large red oak (Q. rubra) acorn crop in autumn 1994 led to high mouse densities in summer 1995 (1). We took advantage of low moth and high mouse densities to remove mice during moth pupation, testing the chain of interactions linking acorns to mice to moths. Mice were removed from three grids of approximately 2.7 ha but were left unmanipulated on three control grids (20). Mouse densities did not differ between control and experimental grids in June 1995, just before mouse removal (Fig. 1; $P = 0.18$, paired t test) (21). Continuous live trapping reduced mouse densities on experimental grids to less than half those on control grids by the midpoint of a 32-day removal period in June–July coincident with female moth pupation (Fig. 1; $P =$...
Densities of late-stage moth larvae (22) did not differ between treatments at the start of the experiment (Fig. 2A). Predation on female pupae was estimated by monitoring survival of the native population and by recording attacks on freeze-dried pupae (23). On control grids with high mouse densities, no living female pupae were found, and 100% of freeze-dried pupae were attacked by predators in 2 to 4 days, which is much less than the 13 days required for eclosion to the adult stage. Over 99% of attacks on freeze-dried pupae that could be attributed to vertebrates or invertebrates were caused by vertebrates, and 97% of vertebrate attacks where the predator species was identifiable were made by mice. In contrast, on experimental grids, 42% of native female pupae survived for 13 or more days, and 22% of freeze-dried pupae were unattacked at 14 days; 77% of these attacks were caused by vertebrates, with 89% being mouse attacks. The number of successfully eclosed female pupae and resulting egg masses on trees (24) was respectively 45-fold (Fig. 2B) and 43-fold higher (Fig. 2C) on experimental than on control grids. Comparison of control grids in 1995 and 1994 showed that oak masting in 1994 led to a 15-fold increase in July mouse densities, a 34-fold increase in mouse predation on freeze-dried pupae, and a decrease by a factor of 26 in moth egg mass densities (25).

The increase in moth density that resulted from simulating mast failure by removing mice was similar in magnitude to that observed at the start of natural moth outbreaks, and the decrease in moth density on control grids was similar in magnitude to that previously observed after masting-induced increases in mouse density (1, 6).

Lyme disease in the northeastern and north central United States is transmitted to humans by black-legged ticks infected with *B. burgdorferi* (16, 26). Adult ticks feed and mate on white-tailed deer before dropping to the ground in autumn, laying eggs the following spring or early summer (17, 27). Larvae hatch in midsummer and are free from infection with *B. burgdorferi* because of extremely low rates of transovarial transmission (28). White-footed mice are primarily responsible for infecting ticks with *B. burgdorferi* during the larval blood meal (29, 30). Larvae then molt to nymphs that overwinter on the forest floor. In spring or early summer 1 year after egg hatch, infected nymphs seek vertebrate hosts, including humans, and may transmit *B. burgdorferi* to the host at this blood meal (16–17). The abundance of infected nymphs is the primary determinant of Lyme disease risk (16). Nymphs molt into adults that seek a deer host in the autumn. The location of deer in autumn determines the location of egg-laying adults and thus where host-seeking larvae should occur the following summer (1, 31–32).

In the autumn of mast years, deer spend more than 40% of their time in oak stands feeding on acorns but spend less than 5% of their time there in non-mast years (15). Larval tick density in oak forests reaches peak levels in the summer after mast production but is low during the summer after mast failure (1), corresponding to predictions based on habitat use by deer. Increased densities of mice in oak forests during the summer after masting coincide with peak densities of larval ticks (1). Because mice are the principal reservoirs for Lyme disease spirochetes, high densities of infected nymphal ticks and a high risk of exposure to Lyme disease should occur 2 years after heavy acorn production (32).

We took advantage of mast crop failure in the autumn of 1995, when acorn production was lower by a factor of 18 than in 1994, to add acorns to the three experimental grids but not to the three control grids (33), testing the chain of interactions linking acorns to mice, deer, and ticks. We added more than 811,000 acorns (>3500 kg) to experimental grids at densities of 60 m$^{-2}$ of oak canopy, approximating the 1994 acorn crop. We also simulated food caching by periodically supplementing mouse nest boxes on experimental grids with acorns, leaving boxes on control grids unsupplemented. Mouse density and reproductive status were monitored, and each month we measured the numbers of host-seeking ticks and ticks infesting mice (34). Although mice had been removed from the experimental grids in June–July, densities had returned to the levels measured on control grids by early October 1995, before acorn additions (Fig. 1; $P = 0.98$, unpaired $t$ test).

Acorn addition significantly increased mouse densities from March–August 1996

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Ecologists have hotly debated the relative importance of direct versus indirect species interactions as a cause of contingent ecological outcomes (38). Our studies clearly demonstrate that both gypsy moth dynamics and Lyme disease risk have contingent outcomes arising from a complex chain of strong pairwise interactions among taxonomically diverse species that are all interconnected within an ecosystem.

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9. ibid., p. 323.
20. Three pairs of open grids (165 by 165 m and 180 by 110 m for one grid) 100 to 250 m apart, with pairs separated by 1 to 3 km, were located in upland oak forests (57 to 70% oak relative basal area) at the Institute of Ecosystem Studies. Grids were not accompanied by moth population increases in forests that were acorn barren (13). Our studies indicate that attempting to simultaneously prevent moth outbreaks and minimize Lyme disease risk, by using silvicultural practices that alter acorn production, would be unlikely to succeed because decreasing the likelihood of moth outbreaks could increase the risk of Lyme disease and vice versa.

Fig. 3. Tick densities on control grids (open bars) and experimental grids (solid bars) in August 1996 after acorn additions to experimental grids in October–November 1995. The bars show grid means and within-grid SEs. Across-grid control and experimental means (=±SE) and statistical comparisons are also shown for each graph. (A) Number of host-seeking larval ticks per square meter. Control grids, 1.07 (=±0.21); experimental grids, 8.59 (=±2.93); P = 0.017, one-tailed paired t test. (B) Number of larval ticks per mouse. Control grids, 24.67 (=±6.7); experimental grids, 34.58 (=±13.64); P = 0.046, one-tailed paired t test.
animals during the removal period every 2 to 4 days on experimental grids, 26 June–30 July 1995; and (ii) monthly mark-recapture from nest boxes, Feb-

male pupae (USDA Animal and Plant Health Ins-

service, MA) were freeze-dried and affixed with beeswax, which retains an imprint of the spinose bark-louse predaceous predators. Tarsals, in groups of five on burlap panels 20 by 15 cm. Twenty or 21 panels per grid were stapled under the bark in each of the oak-paw tree traps on 10 m grids. Pupae were monitored daily to day 9 (except day 6) and then every other day to day 18 or until all pupae had been attacked. Judging by tooth marks and damage characteristics, the main predators of oak-paw were verte-

brates, invertebrates, both, or an unknown agent. (10). The total number of successfully eclosing pu-
pae was determined from counts on all banded trees and was expressed as the mean number per tree, with a tree-to-tree within-grid variance estimate.

24. New egg masses on or under burlap bands were counted on all banded trees and expressed as the mean number per tree, with a tree-to-tree within-grid variance estimate. Twelve randomly selected 15- by 15-m grid cells per grid were also censused for egg masses on or under bands, at heights less than 2 m on banded and unbanded trees, on small saplings, dead trees, woody debris, litter, and rocks. Fem new egg masses were distinguished from empty egg masses or unhatched eggs by gentle pressing. Mean (±SE) new egg masses per grid cell were 0.028 (±0.028) on control grids and 0.036 (±0.016) on experimen-
tal grids (P = 0.038, one-tailed Mann-Whitney U test). Most egg masses are laid at heights less than 2 m in low-density moth populations (Skalter, Environ. Entomol. 17, 1496 (1988)).

25. Only two control grids were fully operational in 1994, limiting the statistical power of pairwise comp-

arisons between 1994 and 1995. Mean (±SE) mouse densities, MNKA ha−1, were 2.59 (±0.26) in July 1994 and 38.81 (±24.74) in July 1995 (one-
tailed paired t test on ln-transformed data, P = 0.045). Mice attacked a mean (±SE) of 1.06 (±0.46) free-living pupae in 1994 and 0.14 (±1) in 1995 (one-tailed paired t test on ln-transformed data, P = 0.003). Mean (±SE) egg mass densities per tree on or under burlap bands were 0.11 (±0.02) in 1994 and 0.004 (±0.004) in 1995 (one-tailed paired t test, P = 0.386).


33. Acorn production (mostly red oak) on control grids in 1995 versus 1994 averaged 7.6 (range 7.2 to 8) versus 3.6 (range 2.9 to 4.6) free-living pupae per square meter of canopy (estimated from 0.5-m2 seed traps, of which five per tree were under canopy oaks), from an average of 88 versus 142 masting oaks per grid at the beginning of the sampling period. The acorn density added was the average of 1994 val-

dues and that found by Sork et al. (3) for red oak (25 m2 of canopy). To calculate the amount of acorns to add per grid, we used the mean DBH of masting oaks in 1994 to calculate average crown radius (in meters) [1.71 + 7.92 × DBH (C. D. Canham, A. C. Finzi, S. W. Pacala, D. H. Burbank, Can. J. For. Res. 22, 734 (1992)] and the number of oaks masting in 1994. Based on these calcula-
tions, we added an average of 270,525 (1172 kg) acorns (primarily red oak, locally collected and do-
ned as noted) to each of the three experimental grids.

Acorns were added in three events between late Oc-

tober and late November 1995 and were evenly scatter-
ted in a 4.45-m average crown radius circle around the center of each of the three experimental grids. The 10 nest boxes on each experimental grid received acorn supplements of 10 per box every month from mid-

September to December 1995; then 5 per box every 2 weeks to early June 1996.


36. The abundance of infected host-seeking nymphal ticks is the primary determinant of Lyme disease risk (15). Because acorn addition caused higher densi-
ties of host-seeking larvae, higher densities of ticks on spirochete-infected mice, and higher densi-
ties of mice, Lyme disease risk is expected to be substantially increased 2 years after mastication. This inference was supported by our observation that the average density of nymphs in June 1997 was 73.9% higher and in July was 31.3% higher on experimental (acorn-supplemented) grids than on control grids. However, because of high variability among sites, a drought causing small sample sizes of nymphs, and possible density-dependent emigration by mice from our experimental grids, the period of larval infestation, neither of these differences was statistically significant (paired t tests, P = 0.21 and 0.15 for June and July, respectively).


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