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12. We performed molecular modeling using MACRO-MODEL 5.5 (Amber* force field); F. Mohamadi et al., J. Comput. Chem. 11, 440 (1990). The volumes for the guests 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:

\[ \begin{align*}
K_A^N & = \frac{[H^+G]^N}{[H^+][G]} \\
K_A & = \frac{[H^+G]^N}{[H^+][G]} \\
K & = \frac{[H^+G]^N}{[H^+][G]} \\
\Delta G & = \Delta G^0 + RT \ln K
\end{align*} \]

where \( K_A^{N} \) and \( K_A \) are the apparent association constants for the predominant and the subordinate complexes, respectively, and \( K \) is the apparent ionization constant between the two complexes.

In these equations \( H \) is the host; \( G \) is the chiral guest; \( [H^+G]^N \) and \( [H^+G]^P \) are the concentrations of the predominant and the subordinate complexes, respectively; \( \Delta G^0 \) is the free energy of formation; \( T \) is temperature; and \( R \) is the ideal gas constant; \( I_{pG} \) is the sum of all of the integrals corresponding to the guest (subscript T stands for total); \( I_{pG} \) is the integral for the signal of the guest outside the capsule; \( I_{pG} \) is the integral for the signal of the guest in complex \( A; I_{pG} \) is the integral for the signal of the guest in complex \( B. h \) is the initial amount of monomer (in millimoles); \( g \) is the amount (in millimoles) of guest added to the solution; \( a \) is the amount of guest (in millimoles) in complex \( B; \) and \( V \) is the total volume (in milliliters).

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

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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 autumnal 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, Lycormia 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 = 0.004).
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 3 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 significantly lower (Fig. 2B) and 43-fold higher (Fig. 2C) on experimental than on control grids. Comparison of control grids in 1993 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 stimulating moth outbreaks 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 during mast-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 following 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 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 mast failure 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 81,000 acorns (>350 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.
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.
21. 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 11–11 by 11–11 arrays (12 by 12 for one grid) of trap stations 15 m apart, with two Sherman-live traps per station at the centers of 15-by-15-m grid cells used for gypsy moth sampling. A wooden nest box, containing cotton batting nesting material replaced twice a year, was attached at 1.3 m to a large tree [0.6 to 0.9 m in diameter at breast height (dbh)] in each of 20 grid cells; boxes were stratified to maximize intercell distances. These nest boxes have no effect on mouse densities [J. O. Wolff and D. S. Dunn, J. Mammal. 67, 409 (1986)]; D. T. Krohn, J. F. Merritt, S. H. Vessey, J. O. Wolff, Can. J. Zool. 66, 2170 (1988) and were colonized by mice only. Mouse densities, measured as minimum number known alive (MNK) per hectare, were estimated from (i) monthly mark-recapture over 2-day periods from the 120 or 121 trap stations on all grids, April–November 1995 and 1996, and July 1994 for two control grids; (ii) trapping of new and recaptured
animals during the removal period every 2 to 4 days on experimental grids, 26 June–30 July 1995; and (iii) monthly mark-recapture from nest boxes, Feb-
ruary–April 1996. Traps were baited daily with oats, set for about 10 days in July andackbar to check the traps following two mornings. Traps were not operated during the day or in December–March to avoid risks of overheating or hypothermia of animals. During the breeding season, unbanded metal ear tags at first capture, Trap station or nest box, tag number, gender, body mass, and age and reproductive condition (for females, vaginal pat

tena, confirmation of pregnancy, and pubic hair development for males, whether testes were descended) were re-
corded at all captures, and animals were released at the point of capture. During the removal period, the traps were not recorded, but tagged and unbanded untagged animals were moved to a site 4.5 km away. One hundred forty, 230, and 230 mice were removed from the three experimental grids, re-
spectively. None of the tagged relocated animals were recaptured on grids. Care of animals was in accordance with institutional guidelines.

21. Unless otherwise noted, response variables were examined for grid pair effects, using an appropriate parametric or nonparametric comparison, followed by treatment comparisons using paired tests if P < 0.05 for grid pair effects and unpaired tests if P > 0.05 for grid pair effects. Where one-tailed treat-
ment comparisons were used because there were a priori hypotheses, this is reported; otherwise, comparisons were two-tailed. A single time point was used for comparisons before and after mouse

22. Two hundred forty to 242 trees, greater than 7 cm DBH and greater than 2 m in height, without nests of birds, bees, or other insects, were used as moth sampling on trees. Half the trees were band-
ed with slitted, folded, burlap skirts (30 cm) tied at 1.3 m. One-third of all tree pairs were oak-oak, one-third were oak-non-oak, and one-third were non-oak-oak. Banded and unbanded tree pairs were alternated across each grid. Late-stage larvae use burlap bands as daytime refuges (17), and their development was monitored until fourth and fifth instars were prevalent. The number of living larvae of all instars on or under burlap bands on all banded trees was counted during dry days before 4 p.m. When expressed as a mean number of larvae per tree, with a tree-to-tree

23. Pupation, eclosion, mating, and egg-laying occur in the daytime resting locations of late-stage larvae, and the number of flightless females determines egg mass density. Female pupae took a mean (±SE) of 12.7 (±0.4) days (n = 42) to develop to adults, before immediately mating and laying egg masses. The time taken for female pupae to eclose was determined by monitoring individuals every other day from 7 August on grid cells with banded oak-oak tree pairs (n = 34 monitored survivors). Because no monitored pupae survived on control grids, additional female (sixth-instar) lar-

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 15×15-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. New egg masses were distinguished from standing dead eggs by freshly hatched eggs by gentle prodding. Mean (±SE) new egg mass densities per grid cell were 0.028 (±0.028) on control grids and 0.305 (±0.121) on experiment-
alan grids (t = 2.7, df = 19, P < 0.05). Three most egg masses are laid at heights less than 2 m in low-density moth populations (P. Skelker, Environ. Entomol. 14, 106 (1985)).

25. Only two contrasting grid marks of vertebrate predation in 1994, limiting the statistical power of pairwise compar-
isons between 1994 and 1995. Mean (±SE) mouse densities, MNKA ha−1, were 2.59 (±0.36) in July 1994 and 0.64 (±0.14) in July 1995 (one-
tailed paired t-test on interrelated data, P = 0.004). Mice attacked a mean (±SE) of 1.06 (±0.44) freeze-dried per day in 1994 and 0.6 (±0.14) in 1995 (t = 1.3, df = 13, P = 0.104). Mean (±SE) egg mass densities per tree on or under burlap bands were 0.1 (±0.02) in 1994 and 0.004 (±0.004) in 1995 (one-tailed paired t-test P = 0.002).

26. The abundance of infected host-seeking nymphal lyme-
icks is the primary determinant of Lyme disease risk (15). Because acorn addition caused higher densities of host-seeking larval ticks, higher densities of lice on spruce-incected mice, and higher densi-
ties of mice, Lyme disease risk is expected to be substantially increased 2 years after mast. This inference was supported by our observation that the average density of infected June 1997 was 70% 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 greater cause of some variation is the possible density-dependent emigration by mice from our experimental grids during the 1996 period of larval infestation, neither of these differences was statistically significant (July 1997: P = 0.21 and 0.15 for June and July, respectively).


sues in the Consequences and Contro-

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