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The Disease Complex of the Gypsy Moth

I. Major Components

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A study was undertaken to elucidate the impact of the various components of disease on natural populations of the gypsy moth, *Porthetria dispar*. Diseased larvae from both sparse and dense populations were examined and categorized on the basis of etiologic and nonetiologic mortality factors. Results indicated a significantly higher incidence of parasitoid involvement—but virtual nonexistence of polyhedral viroses—in the relatively stable sparse populations. Nuclear polyhedrosis probably represented the primary mortality factor in the dense populations. Many insects examined from both population types revealed no infectious agent or overt cause of disease, a fact that may indicate a major regulatory role of noninfectious disease in natural populations. Variation in the disease complex within the populations that have been studied indicates that minor causes of disease in one may well predominate in others. Thus, to fully understand this complex, it must be studied across a number of years within a series of populations from different geographical areas.

INTRODUCTION

Disease in general represents an important complex of factors in the population dynamics of the gypsy moth, *Porthetria dispar* (Campbell, 1963, 1967). Our overall objective is to develop a generally applicable mathematical model for disease incidence among natural gypsy moth populations to help us manipulate the environment in a way that optimizes the impact of this complex. The insect is host to many pathogenic microorganisms whose roles in the dynamics of the host are unknown. Furthermore, autopsy of apparently diseased larvae has frequently failed to reveal either microorganisms or evidence of parasitoid insects.

For these reasons we have decided to approach our overall objective through the following four steps: (1) Divide the disease complex into categories and determine which of these contain important elements in establishing overall disease incidence. (2) Identify these important elements. (3) Determine the range in the mortality caused by each of these particular elements. (4) Relate the variation found through the preceding step to variation in underlying environmental conditions. Since steps (1) and (2) should logically precede further work, this is a report of our progress on these steps.

METHODS AND PROCEDURES

Collection of Study Material

A total of 1,576 diseased insects were collected from a series of dense populations in the town of Old Lyme, Connecticut, during the summer of 1967 and from a series of sparse populations located near Eastford in northeastern Connecticut and an adjacent part of Massachusetts during the summers of 1967 and 1968. (Since the sparse populations were all near the town of Eastford, the material drawn from them will be called the "Eastford data".)

Three sites were studied in Old Lyme during 1967. All sites were supporting more

than 4,000 gypsy moth egg masses per acre in the spring of that year, and all were supporting stands composed primarily of oaks. Site number 2 exhibited relatively wet soil moisture conditions, compared with sites 1 and 3.

Fifteen trees in each of the Old Lyme sites were selected for sampling. Each tree, including as much of the crown as could be reached from the top of a 32-foot extension ladder, was visited twice each day from June 14 through 28 and once each day from June 29 until virtually all larvae had either died or pupated. Every diseased insect found was refrigerated immediately and forwarded to the laboratory for autopsy. (We considered all dead, nonpredated gypsy moth larvae as diseased; so parasitoids were considered as disease causing organisms.)

The Old Lyme sites were established exclusively to obtain material from dense gypsy moth populations for this study. Eastford sites (averaging fewer than 25 egg masses per acre) were established to study many aspects of the dynamics of sparse gypsy moth populations. Thus, field procedures followed in Eastford were necessarily some what different from those in Old Lyme. In particular, each tree marked for study in Eastford was examined only twice each week, so the diseased gypsy moths that were collected from Eastford may have been dead for as many as 3 or 4 days before they were collected and refrigerated. Also, foliage was not routinely examined in Eastford—as had been done in Old Lyme. This was because larger gypsy moth larvae in these sparse populations were almost never found on foliage during daylight hours. Rather, they spent this time in sheltered places, which were on both the tree itself and in the litter around the tree base. Consequently, only these sheltered places were examined routinely in Eastford.

Examination of Study Material

Diseased insects were divided into three broad groups: (1) those killed by primary pathogens or parasites, (2) those whose cause of death was undetermined, but whose autopsies revealed possible contributing factors, and (3) those whose autopsies revealed no apparent cause of death. We further divided these groups into 16 categories, including parasitized; pathogenic bacteria; viruses; undetermined; cause unknown; and combinations of the first four.

As parasitized we included insects that contained larvae of parasitic tachinids or hymenoptera or that showed evidence of parasitization in the form of an "emergence scar."

We considered primary pathogens to be bacteria that were demonstrated in later feeding trials to be pathogenic and polyhedral virus inclusion bodies that were revealed in numbers greater or equal to 10 per field in a stained larval smear. (The designation of 10 polyhedra has no profound significance. It was simply chosen as a reference point for separating insects that probably contained enough polyhedra for the virus alone to have caused death from those in which the low number of polyhedra did not allow a definite diagnosis.)

Autopsies often revealed microorganisms or other conditions that were not shown to be primary causes of mortality, but which could have been contributing factors. So we categorized cause of death "undetermined" if (1) fewer than 10 polyhedra were seen per field in a stained larval smear; (2) bacteria were present in a larval smear, but were not shown to be pathogenic through feeding trials; (3) fungal spores or hyphae were seen in larval smears; and (4) the insect displayed any wound other than a parasite emergence scar.

Those diseased larvae not killed by primary pathogens or parasites, and showing no signs of contributory factors, were assigned to the cause unknown category.

Examination of diseased insects. Each dead larva was examined for preliminary assignment into one of our categories. Final cate-

gorization was made after feeding trials to test for pathogenicity of bacterial isolates.

Mature larvae, puparia, or cocoons of parasitoid insects were sometimes found outside their host. These were identified. If no parasitoid was found, the host integument was scrutinized for a parasitoid emergence scar. Wounds and any evidence of fungal disease were also noted at this time.

Each larva was placed in a sterile screw-cap tube and surface treated (5 min with agitation) in 0.2% aqueous Hyamine 10-X (Rohm and Haas)[1] containing 0.01% Triton X-100 (Rohm and Haas). Each larva was then rinsed five times in sterile distilled water to remove the sterilant, dissected aseptically, and examined for parasitoid larvae. Larval remains were then triturated in 1 ml of sterile distilled water. Larvae not suitable for surface sterilization or internal examination were simply triturated in 1 ml of sterile water. In either case, a loopful (5 mm) of the macerated remains was smeared over a 2-cm^2 area and was fixed and stained with dilute Giemsa (1:1,000) for 2 min.

Ten random fields per smear were examined under the light microscope at 1,000× under oil immersion. Insects whose smears revealed less than 10 polyhedral inclusion bodies per field were placed in the undetermined category. Those showing 10 or more per field were relegated to a primary cause category. Insects harboring fungal spores or hyphae were also placed in the undetermined category.

If sufficient numbers of bacteria were detected in the stained smear from a larva, a loopful of the macerated remains of that larva was streaked on trypticase soy agar (TSA) (BBL) and incubated aerobically at 32°C for 48 hr. At the conclusion of the incubation period, a representative of the colony type(s) appearing in the greatest number was picked and placed on TSA slants and was incubated acrobically at 32°C for 48 hr and stored at 4°C.

Our criteria for isolating bacteria were based upon the relative number of bacteria present per field, again subjective; but our experience has been that aerobic bacterial pathogens of the gypsy moth are present in great numbers immediately after death. If bacteria were not observed in great numbers, we assumed that they were not independent agents of mortality. However, any larvae harboring bacteria, no matter in what number, were placed in a category containing bacteria as one of its components.

Those nonparasitized larvae containing no bacteria, virus, or fungi, and displaying no wound or obvious deformity, were placed in the cause unknown category.

Feeding trials. In designing the feeding trials, we assumed that: (1) bacterial pathogens enter the host orally; (2) bacteria pathogenic for fourth, fifth, and sixth instar larvae would most likely be pathogenic for second instar larvae also; (3) the chance of infection was directly proportional to dosage.

Test insects were reared from egg masses that were collected from areas of sparse populations where disease incidence was low. Eggs were stored at 4°C before being dehaired and hatched. Test and control larvae for a given set of trials were hatched from the same egg mass. One lot of 10 larvae constituted each trial, while one lot of 10 was used as a control for each 10 trials.

Larvae were hatched from surface treated eggs (0.5% Clorox for 10 min) and reared to second instar on artificial diet (Odell and Robinson, 1966) in sterile plastic petri dishes (13 cm) at room temperature (24 ± 2°C). As soon as they had molted to the second instar, they were segregated into groups of 10 and starved for 24 hr before being fed tender lettuce leaves dipped in a 24-hr trypticase soy broth (TSB) (BBL) culture containing between 10^6 and 10^9 viable cells of the test microorganism per milliliter. Control insects were fed on lettuce leaves dipped in sterile TSB. All larvae were

[1] Mention of a particular product should not be taken as endorsement by the Forest Service or the U. S. Department of Agriculture.

TABLE 1

COMPONENTS OF DISEASE AMONG GYPSY MOTHS; OLD LYME *vs.* EASTFORD

Mortality category	Old Lyme		Eastford	
	No.	% of total	No.	% of total
Parasitoid	169	17.6	155	25.2
Virus[a]	102	10.6	3	0.5
Bacteria	12	1.3	5	0.8
Undetermined	226	23.5	190	30.8
Cause unknown	166	17.3	124	20.1
Par-vir	16	1.7	3	0.5
Par-bac	5	0.5	7	1.1
Par-und	53	5.5	116	18.8
Vir-bac	7	0.7	0	0.0
Vir-und	151	15.7	2	0.3
Bac-und	18	1.9	7	1.1
Par-vir-bac	10	1.0	0	0.0
Par-vir-und	14	1.5	0	0.0
Par-bac-und	3	0.3	4	0.7
Vir-bac-und	7	0.7	0	0.0
Par-vir-bac-und	1	0.1	0	0.0
Total	960	100	616	100

[a] At least 10 polyhedra per field.

allowed to feed undisturbed for 48 hr on the lettuce dips before being transferred to artificial diet, on which they were reared for the remainder of the trial (8 days).

Any larvae that died in either the test or control groups were surface sterilized and triturated in 1 ml of sterile water. A smear was made from the resulting suspension, stained, and examined microscopically. A loopful of the suspension was cultured on TSA for the presence of the test microorganism. Pathogenicity was assessed on the basis of the number of larvae killed per trial, reisolation of the test bacterium, and numbers present in the larval smear.

RESULTS

Percentages of diseased insects within each of the 16 mortality categories are shown in Table 1. Over 80% of the larvae examined from Old Lyme were assigned to the parasitoid, virus, undertermined, cause unknown, and virus undetermined categories; the last representing mainly virus-killed wounded animals. On the other hand, over 90% of the larvae examined from Eastford fell into four categories: parasitoid, undetermined, cause unknown, and a parasitoid-undetermined category, which reflected the involvement of microorganisms other than primary pathogens.

Parasitoid involvement was substantially higher in Eastford than in Old Lyme, since 46.3% of the Eastford larvae contained parasitoids versus 28.2% in Old Lyme. On the other hand, virus incidence was high in Old Lyme and virtually nonexistent in Eastford. Of the insects from Old Lyme 32.1% contained a virus burden classified as lethal, while only 1.3% of the insects from Eastford area contained a lethal virus burden. Pathogenic bacteria were found in 6.6% of the Old Lyme insects and in 3.7% of those from Eastford.

A substantial number of all larvae fell into the undetermined category, which is subdivided in Table 2. Differences can be seen between the two areas, particularly a higher incidence of microorganisms in the

TABLE 2

STATUS OF GYPSY MOTHS THAT WERE PLACED IN THE UNDETERMINED CATEGORY; OLD LYME *vs.* EASTFORD

Status	Old Lyme		Eastford	
	No.	% of total	No.	% of total
Virus[a]	35	15.5	16	8.4
Microorganism[b]	51	22.6	86	45.3
Wound	65	28.8	32	16.8
Vir-mic	22	9.7	10	5.3
Vir-wou	19	8.4	5	2.6
Mic-wou	22	9.7	37	19.5
Vir-mic-wou	12	5.3	4	2.1
Total	226	100	190	100

[a] Fewer than 10 polyhedra per field.

[b] Includes both aerobic bacteria that did not prove to be pathogenic and fungal mycelia or spores.

TABLE 3

PERCENTAGE OF TOTAL INSECTS FROM OLD LYME THAT CONTAINED ONE OR MORE OF THE FOLLOWING TYPES OF PRIMARY DISEASE AGENTS: PARASITOID, VIRUS, PATHOGENIC BACTERIA; COMPARISON BETWEEN SITES

Primary disease agent	Site No. 1		Site No. 2		Site No. 3	
	No.	% of total	No.	% of total	No.	% of total
Parasitoid	106	27.3	73	22.7	92	37.1
Virus[a]	122	31.4	131	40.8	55	22.2
Bacteria	31	8.0	22	6.9	10	4.0
Total	259	66.7	226	70.4	157	63.3
Total examined[b]	388	—	321	—	248	—

[a] At least 10 polyhedra per field.

[b] Includes both undetermined and cause unknown categories.

Eastford insects and a higher incidence of wounds and virus in Old Lyme.

Differences were noted between sites within Old Lyme (Table 3). More insects from site 2 were killed by virus than in either sites 1 or 3, whereas parasitism was highest in site 3. On the other hand, only minor differences were found among the primary agents of disease between insects collected in 1967 and those collected in 1968 in Eastford (Table 4). The major components of disease differed greatly from one instar to the next within both Old Lyme and Eastford (Table 5). In Old Lyme, the percentage of animals killed by virus or bacteria tended to increase with instar while the percentage parasitized or dying from cause unknown decreased. The number of insects whose cause of death was undetermined remained relatively stable from one instar to the next. In Eastford, parasitism increased from 39% in the third instar to 59% in the fifth instar. Virus, bacterial, and undetermined incidence remained relatively stable; and cause unknown, as in Old Lyme, decreased.

TABLE 4

PERCENTAGE OF THE TOTAL INSECTS FROM EASTFORD THAT CONTAINED ONE OR MORE OF THE FOLLOWING TYPES OF DISEASE AGENTS: PARASITOID, VIRUS, PATHOGENIC BACTERIA; 1967 *vs.* 1968

Primary disease agent	1967		1968	
	No.	% of total	No.	% of total
Parasitoid	102	42.1	183	48.9
Virus[a]	7	2.9	1	0.3
Bacteria	7	2.9	16	4.3
Total	116	47.9	200	53.5
Total examined[b]	242	—	374	—

[a] At least 10 polyhedra per field.

[b] Includes both undetermined and cause unknown categories.

DISCUSSION

Southern Connecticut towns such as Old Lyme have been supporting dense gypsy moth populations for some years now, and the pest remains the predominant hardwood defoliator in these areas. Contrarily, the Eastford area has maintained sparse, innocuous gypsy moth populations for at least 50 years.

Comparisons between Old Lyme and Eastford data indicate some striking differences between the major components of the disease complex in the two areas. The higher incidence of viral and bacterial diseases in the

TABLE 5

PERCENTAGE OF THE OLD LYME AND EASTFORD LARVAE, SEPARATED BY INSTARS, THAT CONTAINED ONE OR MORE OF EACH OF THE FOLLOWING TYPES OF DISEASE AGENTS: PARASITOID, VIRUS, PATHOGENIC BACTERIA; ALSO PERCENTAGE IN THE UNDETERMINED AND CAUSE UNKNOWN CATEGORIES

Disease agent or category	Instar II % of total	Instar III % of total	Instar IV % of total	Instar V % of total	Instar VI % of total
			Old Lyme		
Parasitoid	20.0	55.1	32.0	16.0	26.3
Virus[a]	2.5	6.8	27.4	46.5	48.5
Bacteria	2.5	2.5	5.1	9.6	8.1
Undetermined	30.0	17.8	24.4	23.4	23.2
Cause unknown	45.0	20.3	14.9	17.3	9.1
Total examined	40	118	369	312	99
			Eastford		
Parasitoid	—	38.9	44.0	59.1	—
Virus[a]	—	0.0	0.7	2.9	—
Bacteria	—	0.9	4.4	2.3	—
Undetermined	—	25.0	33.5	30.4	—
Cause unknown	—	36.1	19.3	13.5	—
Total examined	—	108	275	171	—

[a] At least 10 polyhedra per field.

Old Lyme populations, relative to that noted in Eastford, might easily be rationalized on the basis of the higher host densities in the former area. On the other hand, the high percentage of parasitoid involvement in the Eastford populations might imply that this area supported a greater relative abundance of alternate hosts, which most of these parasitoid species require to exist in any given area from one year to the next.

Differences in the disease complex among sites in Old Lyme may have been a reflection of the environment as well as density. The greatest incidence of viral disease occurred in site 2, a low, wet site that supported the highest density of *P. dispar*.

We could not make comparisons between the Eastford sites because only a small number of larvae were drawn from any given site. However, comparing the 2 years in which diseased insects were collected in Eastford, we found only minor differences in the primary components of disease, as might be expected from this stable population system.

The percentage of the larvae dying from cause unknown decreased with instar in both Old Lyme and Eastford. This may imply that young larvae are predisposed to certain physiological disorders; are less susceptible to disease agents (this seems unlikely from the results of feeding young larvae known pathogens); or are less likely to come in contact with certain disease agents at this early age. Pathologists have long observed insects dying of unknown causes, and our cause unknown category accounted for nearly 20% of all diseased animals examined. It seems unlikely that disease agents (noninclusion viruses, etc.) were overlooked in the majority of these cases, yet this remains a possibility. At any rate—because this category included a relatively large portion of the total insects ex-

amined and thus, in all likelihood, was representative of a good percentage of the natural population—it seems appropriate, indeed necessary, that in further studies we delineate this enigmatic classification.

Our undetermined category represented more insects than any other; both in Old Lyme and in Eastford. In all probability many of the insects relegated to this classification in fact died of cause unknown, but our rationale for establishing this category was to segregate the occasional animal that might have succumbed to the combined action of two or more agents that acting alone would show no effect. Insects harboring microorganisms, such as fungi, that were not tested in feeding trials—but which may have proved to be pathogenic—were placed in this category. These were relatively few in number. Most insects placed in this category harbored bacteria that were not pathogenic. This may be explained by the extended time lag between the death of the insect and collection that prevailed in Eastford and, to a limited extent, in the later collections from Old Lyme. This time lag would of course allow the development of saprophytic bacterial populations within the dead host.

The variation among sites in the Old Lyme populations leads us to believe that other dense populations might be equally as variable; and what appear to be minor causes of disease in Old Lyme, pathogenic bacteria for example, may well be predominant in other populations. Thus, to fully understand the disease complex of this insect, we must study it within a number of dense populations in a number of geographical areas; a rather obvious conclusion in retrospect, but one that obviously has not been fostered.

We fully recognize the limitations imposed by our exploratory techniques and the need for more refined methodology in subsequent studies. However, our primary goal was to obtain a larger sampling of insects in order to relate our results to the natural populations under consideration, rather than to exhaustively diagnose a few dead insects. There are many disease agents we did not observe, indeed did not seek out, most obvious of which are anaerobic bacteria, noninclusion viruses, protozoans, and rickettsiae. However, we now have a foundation on which to base further studies involving the impact of disease in natural populations of this insect; a foundation that we have sorely needed.

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