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Northeastern Forest  
Experiment Station  
General Technical  
Report NE-123



## PROCEEDINGS

# Lymantriidae: A Comparison of Features of New and Old World Tussock Moths



## FOREWORD

Tussock moths constitute the major forest pest throughout the world. While similarities in ecology, behavior, natural enemies, and means of control may be similar for different Lymantrid species, no previous effort had been made to synthesize this information.

These proceedings resulted from a five-day conference held in June 1988 at the Park Plaza Hotel in New Haven, CT. It offered scientists from Canada, the Soviet Union, the People's Republic of China, Great Britain, Switzerland, Japan, the Federal Republic of Germany, Denmark, Poland, the Netherlands, and the United States the unique opportunity to present research findings. Just as important, it provided a forum by which to discuss research issues and needs, clarify terminology and enhance international collaboration. The importance and timeliness of the conference was recognized by the USDA Forest Pest Sciences Competitive Grants Program, which provided funds for defraying travel expenses for invited foreign participants. The International Union of Forest Research Organizations (IUFRO) was the mechanism for identifying participants and, together with the Northeastern Forest Experiment Station which published these proceedings, co-sponsored the Conference.

The conference agenda allowed each participant 45 minutes for a presentation, followed by a 15-minute question and answer period. The comments that followed each presentation were recorded and are presented as a separate section at the end of the proceedings.

Most of the papers were submitted as camera ready copy. Some of the foreign authors requested that their papers be edited for clarity and retyped, and this has been done.

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## COVER

Elliptical projection of the world showing Lymantrid distribution, by Paul A. Godwin, USDA Forest Service, retired.

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Northeastern Forest Experiment Station  
370 Reed Road, Broomall, PA 19008

March 1989

**PROCEEDINGS**

**Lymantriidae: A comparison of features of  
New and Old World tussock moths**

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June 26-July 1, 1988, New Haven, Connecticut

Technical Coordinators

William E. Walker and Katherine A. McManus  
Northeastern Forest Experiment Station  
Center for Biological Control of  
Northeastern Forest Insects & Diseases  
Hamden, CT 06514

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Sponsored jointly by the  
Northeastern Forest Experiment Station and  
The International Union of Forest Research Organizations

### Welcoming Address

International cooperation on scientific issues  
of common interest

*The Honorable Bruce A. Morrison, Representative in Congress,  
Third Congressional District, Connecticut*

### Contents

#### FAMILY CHARACTERISTICS

<i>P. W. Schaefer</i>	Diversity in form, function, behavior and ecology: An overview of the Lymantriidae (Lepidoptera) of the world	1
<i>P. J. Grijpma</i>	Overview of research on Lymantriids in Eastern and Western Europe	21
<i>X. Sun</i>	Lymantriid forest pests in China	51
<i>W. E. Wallner</i>	An overview of pest Lymantriids of North America	65
<i>I. Chaudhry</i> <i>W. Rahman</i>	Population studies on Lymantriid pests in Pakistan	81
<i>H. Bogenschütz</i> <i>K. Maier</i> <i>C. Trebitzky</i>	Gypsy moth outbreak and control in Southwest Germany, 1984-1986	89
<i>J. Novotny</i>	Natural disease of gypsy moth in various gradation phases	101
<i>R. T. Carde</i> <i>M. A. Willis</i> <i>R. E. Charlton</i>	Mate-finding behaviors and chemical communication in the Lymantriidae	113
<i>N. J. Mills</i>	Life tables of the Lymantriidae with particular reference to <i>Lymantria obfuscata</i> in Kashmir	143

#### POPULATION DYNAMICS

<i>T. S. Jensen</i>	Latency characteristics of tussock moths (Lepidoptera: Lymantriidae)	155
<i>J. Schönherr</i>	Outbreak characteristics of Lymantriids	171
<i>R. R. Mason</i>	Monitoring population change in the Lymantriidae	183

A. M. Liebhold J. S. Elkinton	Spatial aspects of gypsy moth population dynamics	203
K. W. Gottschalk	Impacts, silviculture and the gypsy moth	217
J. S. Elkinton J. R. Gould A. M. Liebhold H. R. Smith W. E. Wallner	Are gypsy moth populations in North America regulated at low density?	233

#### GENETICS AND BEHAVIOR

C. A. Clarke	The control of <i>Lymantria dispar</i> : Some genetic and behavioural considerations	251
R. G. Harrison T. M. ODeil	Mitochondrial DNA as a tracer of gypsy moth origins	265
V. C. Mastro T. M. ODeil C. P. Schwalbe	Genetic control of Lymantriidae: Prospects for gypsy moth management	275
Y. Higashiura	Oviposition site selection by Japanese Lymantriid moths	303

#### HOST AND SITE RELATIONSHIPS

Y. N. Baranchikov	Ecological basis of the evolution of host relationships in Eurasian gypsy moth populations	319
M. E. Montgomery	Relationship between foliar chemistry and susceptibility to <i>Lymantria dispar</i>	339
D. B. Lyons T. J. Lysyk	Development and phenology of eggs of gypsy moth, <i>Lymantria dispar</i> (Lepidoptera: Lymantriidae) in Ontario	351
D. E. Leonard J. G. Kunkel	Nutritional ecology: <i>Lymantria dispar</i> as a model system for study of serum storage proteins	367
R. F. Shepherd G. A. vanSickle D. H. L. Clarke	Spatial relationships of Douglas-fir tussock moth defoliation within habitat and climatic zones	381

#### BIOLOGICAL CONTROL AND POPULATION MANIPULATION

<i>B. Glowacka</i>	Pathogenic viruses and bacteria of the nun moth ( <i>Lymantria monacha</i> L.) during the outbreak 1978-1984 in Poland	401
<i>E. A. Cameron</i>	<i>Bacillus thuringiensis</i> in the management of gypsy moth populations	417
<i>P. M. Kelly</i> <i>P. F. Entwistle</i> <i>P. H. Sterling</i> <i>M. M. Speight</i> <i>R. F. Laport</i>	Virus control of the brown-tail moth, <i>Euproctis chrysorrhoea</i>	427
<i>K. D. Murray</i> <i>J. S. Elkinton</i> <i>S. A. Woods</i> <i>J. D. Podgwaite</i>	Epizootiology of gypsy moth nucleopolyhedrosis virus	439
<i>M. L. McManus</i> <i>J. V. Maddox</i> <i>M. R. Jeffords</i> <i>R. E. Webb</i>	Evaluation and selection of candidate European microsporidia for introduction into U.S. gypsy moth populations	455
<i>H. R. Smith</i>	Predation: Its influence on population dynamics and adaptive changes in morphology and behavior of the Lymantriidae	469
<i>R. M. Weseloh</i>	Predation of Lymantriids by arthropods	489
<i>R. Fuester</i> <i>G. Ramaseshiah</i>	A comparison of the parasite complexes attacking two closely related Lymantriids	501
<i>J. R. Gould</i> <i>R. G. vanDriesche</i> <i>J. S. Elkinton</i> <i>T. M. Odell</i>	A review of techniques for measuring the impact of parasitoids of Lymantriids	517
<i>K. A. Sheehan</i>	Models for the population dynamics of <i>Lymantria dispar</i>	533
<i>P. S. Grinberg</i> <i>W. E. Wallner</i>	New and Old World Lymantriidae: Discussion and research issues	549

T H E C O N T R O L O F L y m a n t r i a d i s p a r :  
S O M E G E N E T I C A N D B E H A V I O U R A L  
C O N S I D E R A T I O N S

Cyril A. Clarke. Department of Genetics, University of Liverpool,  
P. O. Box 147, Liverpool L69 3BX, England.

Ernst Mayr wrote to me recently and told me that some years ago a new outbreak of dispar totally devastated the vegetation around his country house in New Hampshire. The massiveness of the infestation had been unbelievable - he counted 140 egg masses on the trunk of a single cherry tree. It was the first outbreak in his area for some twenty years, and he pointed out that the first instar larvae can apparently be carried by strong winds for hundreds of miles. When they land, at considerable densities, in an area free of parasites and pathogens, they multiply explosively. His outbreak soon subsided, as a result of viruses, bacteria and Braconid wasps. However, from this information I draw the conclusion that the only way to suppress dispar is by concentrating on areas where it is known to be endemic, since geographical "anticipation" is unreliable.

Let us now consider some methods of attacking the insect.

a) Manipulation of the sex ratio.

It is well known that in the USA and Canada there are no distinctive dispar races, probably because of the founder principle, which meant that the original genetic stocks were very small in number (see Wood and Way, 1988). In Japan on the other hand racial differences are marked. Sterile intersexes may therefore occur when races are hybridised and Goldschmidt (1931, 1934) made a special study of these. As a result, attempts have been made to suppress dispar in the USA by introducing sterile males (Mastro et al., 1961).

E. B. Ford and I thought it was worth repeating some of Goldschmidt's crosses and re-evaluating the results using a modern technique. We came to an entirely different conclusion, particularly regarding Goldschmidt's most striking cross, that between the race in Hokkaido and the one in the Aichi region of Japan (Clarke and Ford, 1980, 1982, 1983 and 1984).

The new technique consisted of testing somatic cells for the presence or absence of a heteropycnotic body which Smith (1945) had shown to be present in females of the spruce budworm (Arctips tusiferana) whereas the males lacked it. The same was found to be true in most species of Lepidoptera (Traut and Mosbacher, 1968) (Fig.1).

The procedure is as follows: using sharp dissecting scissors, an abdominal proleg from the larva (Fig.2) is removed, and enough tissue can be scraped from the inside of the proleg to make one good preparation, the material being teased out and spread as thinly as possible. After amputation each larva is kept separately and cotton wool applied to the wound. The larvae recover, and there is no deformity in the perfect insect. In the adult, gut or Malpighian tubule cells are used, from freshly killed insects (see appendix for staining technique).

In the cross female Hokkaido X male Aichi we confirmed Goldschmidt's finding of a big excess of males, but Table 1 shows that, as judged by Smith testing, they were normal males and not transformed females. The moths selected were taken at random, as were the larvae, which we usually tested in the 4th or 5th instar, sometimes earlier. As a control the pure races were also tested and here the overall sex ratio was near unity, and the larval Smith testing tallied with the sex of the moth. In Table 1 on only one occasion (brood 16510) was an individual scored as positive and yet developed into a completely male-like insect; this was Smith negative as an adult. We have occasionally found similar discrepancies in testing pure races, the Smith test not being entirely free from error.

From our broods there seems no doubt that Goldschmidt was wrong and that the excess of males was the result of the Haldane (1922) effect (see appendix) the female embryos or tiny larvae dying because of genic imbalance.

Our F1 males and the two F1 females were fertile and further evidence comes from the F2 and back cross broods, where there was a much more normal sex ratio, supporting Haldane's 'rule'. The details of these F2 and back cross broods are given by Clarke and Ford (1963) and in the same paper the sex ratio in some reciprocal crosses showed an excess of males, arguing against Goldschmidt.

b) Juvenile growth hormone as a possible suppressor of *L. dispar*.

Since the discovery of the juvenile hormones there has been much interest in the synthesis of analogues for use as insect control agents, but I do not know whether they have enjoyed any commercial success as far as *dispar* is concerned. But I believe that none of the synthetic compounds has closely resembled in chemical structure the natural juvenile hormone. However, with the recent discovery of juvenile hormone III in the Malaysian plant *Cyperus iria* there may be more hope (Toong et al., 1968). A summary of their Nature paper follows:

Third stadium grasshopper nymphs fed on the Malaysian plant *Cyperus iria* ate normally and continued to grow and moult just like control insects during their two subsequent instars. Following the final moult, however, 90% of the adults were abnormal compared to control adults reared on wheat seedlings. Typical transposition effects were observed, including twisted wings and colour changes reminiscent of treatment with excess juvenile hormone. In addition, the ovaries of females reared on *C. iria* contained markedly under-developed eggs com-

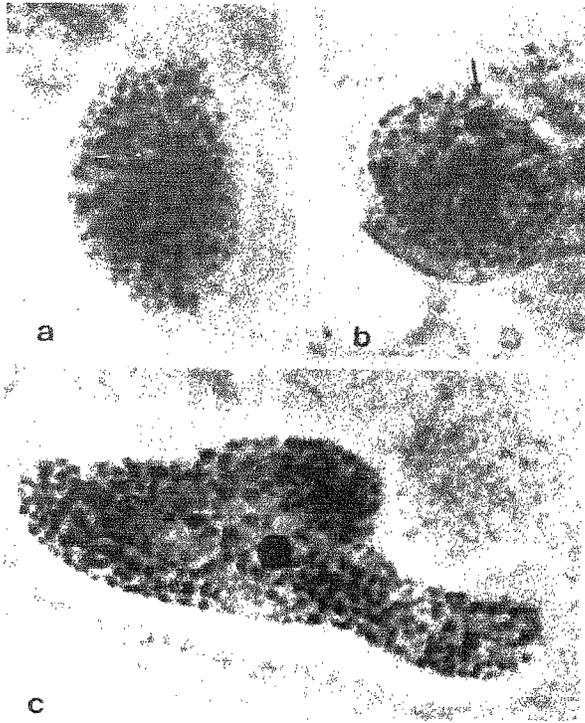


Figure 1. Cells from the proleg of *L. dispar* stained to show the absence a, (male) or presence b, c (female) of the heteropyknotic body.

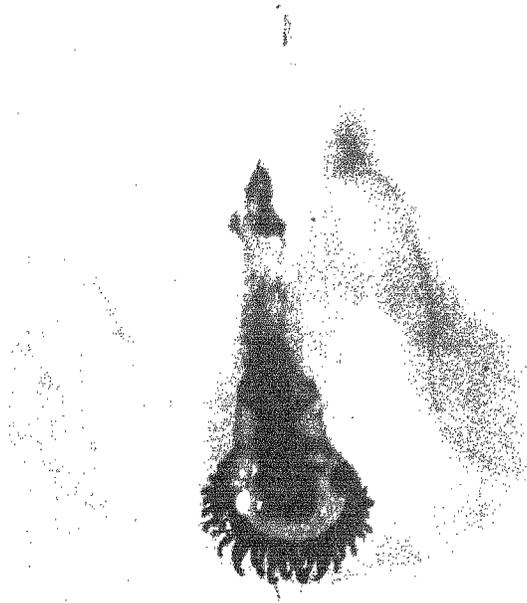


Figure 2. Proleg of *L. dispar* larva.

TABLE 1.

OUR BROODS OF THE CROSS FEMALE HOKKAIDO X MALE AICHI  
(GOLDSCHMIDT'S MOST CRITICAL CROSS)

Our brood no:	Offspring	No. of adults Smith-tested as moths	results of Smith-testing of adults	No. of adults Smith-tested prospectively as larvae	results of Smith-testing as larvae. (In dead larvae information about the gonada is given where known.)
16508	45 ♂♂	25 ♂♂	25 neg.	4 ♂♂	4 neg.
16509	33 ♂♂	21 ♂♂	21 neg.	2 ♂♂	2 neg.
16510	42 ♂♂	25 ♂♂	25 neg.	13 ♂♂	12 neg. 1 pos. as larva but neg. in both gut and Malpighian tubules as adult.  5 larvae died, all neg. (all had testes)
	2 ♀♀ †	1 ♀ †	1 pos.	1 ♀	1 pos.
16513	11 ♂♂	7 ♂♂	7 neg.	0	2 larvae died, both neg.
16514	31 ♂♂	15 ♂♂	15 neg.	2 ♂♂	2 neg. 2 died as larvae, both neg. 1 died as larva unscorable.  In addition four small 16514 larvae were killed. All had testes and were Smith-negative in the cells of the gut.
16519	18 ♂♂	13 ♂♂	13 neg.	0	2 larvae died; 2 killed and dissected, both had testes; all 4 neg.

† The two females appeared normal except that they were intermediate as regards colour between Aichi females (dark) and Hokkaido females (white). One female was mated to a sib and the other was back-crossed to a Hokkaido male. Both females were fertile and produced no intersexes.

pared with controls, and these females laid no egg cases.

The detection of very high levels of JH III in the leaves of C. iria and the demonstration of clear morphological effects on insects raise some intriguing ecological implications for these plants in their native habitat. Furthermore, it is clear that C. iria and other species represent a valuable source of JH III for research purposes.

Whether juvenile hormone specific for dispar could be synthesised and used as a spray I do not know, but if this could be done and the expense were not excessive it might be useful in areas where dispar is endemic, and it would have no effect on other insect life. See also addendum, p.13 and 14.

c) "Phototherapy"

Less specific at present is the work of a research team at the University of Illinois which has developed a pesticide that kills insects in a few seconds when they are exposed to light. The pesticide is applied at night as a spray or as a bait for insects to eat. When the sun comes up a chain reaction is initiated which converts delta aminolevulinic acid into proto-porphyrin, which destroys cell membranes. The insects affected spin round, vomit and die within ten seconds. The team is now attempting to provide insecticides on the above lines which are specific for particular pests but otherwise harmless (New Scientist, 1988).

d) Clean air and the lack of genetic polymorphism in L. dispar.

It is first important to remember the proper definition of polymorphism. It is a type of variation in which individuals with clearly distinct qualities exist together in a freely interbreeding single population, for example, the different forms of female in the mimetic butterfly Papilio dardanus, or the human blood groups. The definition excludes several familiar types of variation, for example racial variation as occurs in L. dispar in Japan. This can be made clearer by considering the Caucasian, Mongolian and Negroid races in Man. These do not constitute a polymorphism since when interbreeding occurs the hybrid populations are intermediate and variable. Seasonal forms too, as in Arachnia levana, are excluded from the definition, since all members of a generation are alike. Finally, continuous variation, as in human height, is not an example of polymorphism. Here, as in racial differences, many genes are at work and the variation is brought about by the cumulative effects of segregation taking place at many loci, and not by 'switch' genes giving rise to distinct alternative forms. Genetic polymorphism is therefore not a characteristic of L. dispar, anyhow for visual characters, although some isozymes conform to the definition (Harrison et al., 1983; Bevegovoy and Gill, 1986). The lack of polymorphism in dispar might lead to its undoing. For instance, I read with interest that one way of subduing the pest was to improve the quality of the trees so that they could better withstand defoliation. A more concentrated attack on pollution might therefore help since lightening of the bark would make male dispar more conspicuous to predators. This is what we think has happened in England in the poly-

morphic Peppered Moth, Biston betularia, which we have surveyed at my home for the past 27 years (Clarke et al., 1985). The graph (Fig.3) shows how the proportion of the pale form of the insect has greatly increased and the likelihood is that as a result of cleaner air there has been a new critical level of background lightness at which birds fail to see f. typica. This form would then rapidly increase. In this case the polymorphism saves the moth, but the lack of it could be disastrous for dispar.

e) Molecular genetics (genetic engineering).

The essential feature of this new discipline is that DNA fragments from two different organisms can be combined to produce functioning DNA molecules. In this way one is bypassing the barriers normally imposed by sexual incompatibility. To do this, donor DNA - for example from a patient - or any organism - is incorporated with the recipient DNA of a virus which can infect bacteria. The hybrid DNA is therefore inserted into a bacterium so that replication can take place and many copies of the hybrid DNA can be produced. Theoretically therefore it is possible to exchange a bad gene for a good one or vice versa.

I have thought a good deal about applying this principle to eradicating L. dispar, but I could not see daylight, and neither could several experts whom I consulted. It seems that only an individual moth could be 'engineered' and how any damage could spread to the whole population of the moth unless it had compensating advantages (which would probably be undesirable) is hard to see. On the plant side, however, the genetic engineers have given the tobacco plant a pesticide gene (Hilder et al., 1987) but this is non-specific. An important new angle is set out in the addendum, p.13 and 14.

f) Hybrid dysgenesis.(Eggleston & Kearsey 1980; Bregliano et al.1980)

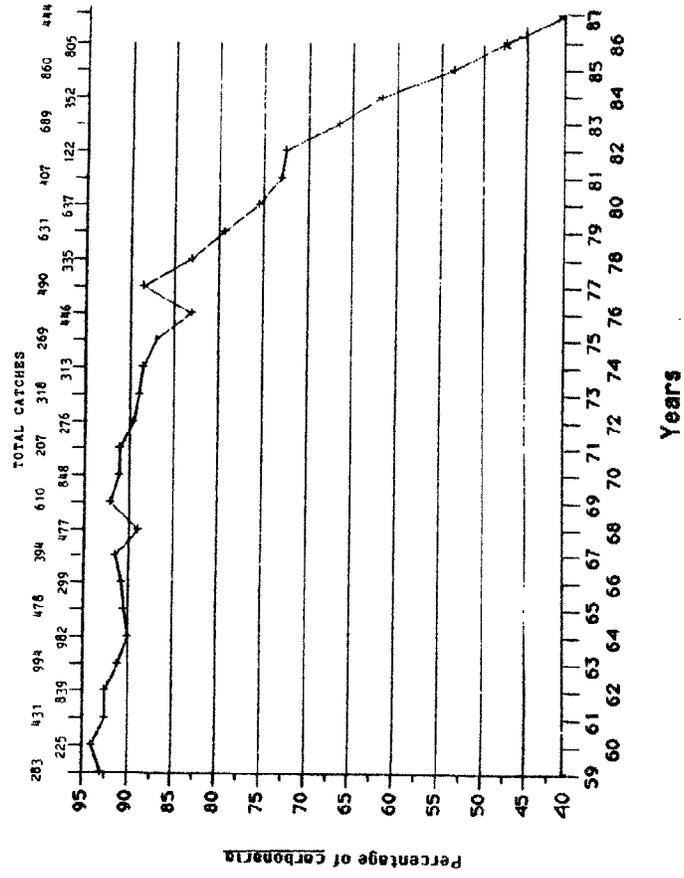
This might be thought of as a naturally occurring example of genetic engineering. It was much written about a few years ago and concerned Drosophila. When strains derived from wild males are crossed with long-established laboratory female flies, dysgenic (i.e. undesirable) traits occurred, e.g. enhanced mutation rates, chromosome aberration, distorted segregation and sterility. The mechanism appears to involve an interaction between the paternal genome and the maternal cytoplasm, and results in genes changing places - "transposable elements".

Hybrid dysgenesis sounds most promising in relation to dispar, but as far as I can make out it has never got further than the fruit fly, and even there less is heard of it than formerly. Nevertheless, it is worth remembering as there may be new developments.

g) Inbreeding.

This is often suggested without much thought having been given to it. Consanguineous matings certainly lead to the appearance of deleterious genes in double dose, but the argument can be turned so that if the population is big enough the deleterious traits are got rid of and

Fig. 3. The effect of the Clean Air Acts on *Biston betularia*.



the species prospers. The Japanese nation is the best example of this in modern times! Also, in dispar I cannot see how in practice one could encourage inbreeding.

h) An attack via pheromones.

We have some observations here on the genus Orgyia. In England bred Japanese O. thyellina females assembled O. antiqua males to a far greater number than did antiqua females (Clarke 1979) - previously we only knew that we had an occasional antiqua in our locality. Again, O. recens and antiqua fly together but neither assembles the other, and of particular interest is the fact that thyellina does not assemble O. recens (Greenberg et al., 1982). Lastly, on the Great Wall of China I could not assemble any species of Orgyia using thyellina female virgins that I had brought with me as pupae from UK. However I found, adjacent to the Wall, a full-grown Vapourer-like caterpillar which produced (in China) a wingless female which looked very like antiqua, but no males were attracted to it.

These results are puzzling, remembering that it is stated that all species in the genus Orgyia have similar pheromones, but whether measured by electroantennograms or by chemical means I am not certain.

In the case of dispar might it be possible to make some super-attractant, perhaps from a combination of the pheromones of dispar and monacha (see Leonard, 1981) which would parallel what happens with thyellina and antiqua?

Conclusions (see also addendum, p.13 and 14)

My view is that the best way to deal with dispar is to alter the moth's natural surroundings rather than employ ever more elaborate pesticides which may cause other biological damage and in any case engender antagonism. Although never a pest in England, in 1820 dispar was quite abundant in some of the Fens and also in the Norfolk Broads. However, Richard South, writing in 1892, states that somewhere about the fourth decade the species began to decrease in numbers and towards the end of the fifties it had practically ceased to exist as a wildling. What was the reason for its failure to survive in some of its former localities which still seemed suitable? Ford (1955) thinks there are two main reasons; one is that the area they could inhabit would be a minute fraction of that of the past, and small populations are in great danger if subject to fluctuation in numbers. The other was that the drainage of the Fens must have had a great effect on the areas that remained causing local climatic changes, so that Fenland is now probably drier in winter, hotter in summer, and less subject to mist than it was a century ago. Land reclamation has also caused an alteration in the bird population, concentrating the true Fenland bird species into very small areas and causing those normally found along hedgerows and in agricultural land to colonise the Fens, so adding to the hazards experienced by the insects which inhabit them. Such changes may well prove fatal to species which are rather accurately adjusted to this specialised environment.

## SUMMARY

The use of sterile males to upset the mating habits of L. dispar is discussed and evidence produced to show that the "Goldschmidt phenomenon" is untrue; in his most critical cross the excess males are fertile and the result of the Haldane effect, not intersexuality. This was discovered by sexing the moth in its early stages and staining for the heteropyknotic body which is present in female cells but not in those of the male. The larva is undamaged by this technique.

Other means of controlling L. dispar are discussed, among them the use of juvenile hormone, which would have possibilities if it could be made specific. Inbreeding is dismissed as impracticable, but the lack of a phenotypic polymorphism in the species might be exploited by reducing atmospheric pollution and thus rendering all male dispar more visible on tree trunks, as well as improving the nutrition of the trees. A comparison with the polymorphic Biston betularia in UK is made.

In general the author feels that natural means rather than the widespread use of pesticides should be used to control the moth, since sudden geographical spread is difficult or impossible to anticipate. If specific measures are used they should be employed in areas where the species is endemic and not necessarily a pest. See also addendum on pages 13 and 14 and for new hope in genetic engineering.

## APPENDIX

Definitions, and staining technique.

Intersexes: These are predisposed to by crossing races or species, the effect being that an insect develops for a time as one sex and then changes to the other, though the chromosomal sex remains that of the original zygote. If the change takes place early enough in development, e.g. at the formation of the gonads, the whole insect appears to be sexually converted, whereas if it occurs later only those structures formed during the end of development, e.g. the wings, will be affected. Intersexes are commonly regarded as being sterile.

Haldane's "rule": Haldane (1922) stated that 'when in the F1 of a cross between two animal species or races one sex is absent, rare or sterile, that sex is always the heterozygous sex'. The rule holds well in the Lepidoptera and here, when there is an upset in the sex ratio, it is nearly always in the direction of a marked deficiency of the heterogametic sex, i.e. females; nevertheless, F1 females do occasionally 'get through'.

Haldane's explanation of the deficiency of females in the F1 was that it is the result of genic imbalance either between the X and the Y chromosomes or between the autosomes and the sex chromosomes or a combination of both. Thus there will be an intermediate effect between the action of the complete set of genes for each race (or species) of a cross in the homogametic sex, so that the sex balance is not impaired. In the heterogametic sex, however, this holds good but only for the

autosomes. The single X in the female is aligned with the Y, but part of the Y is non-pairing and in this section the genes are non-allelic with those on the X, and selection may cause them to be different in the two races. The normal XY balance may therefore be upset and it is assumed that this has pathological effects in the female.

F1 hybrids may, like intersexes, be sterile, but they are often normally fertile or may have hybrid vigour. Thus they differ from intersexes.

The sex chromatin staining technique: The cells are not fixed before staining. Two drops of 2% orcein in 45% acetic acid are placed over the tissue and a coverslip added. After 10 to 15 minutes the coverslip is firmly pressed to make a 'squash' preparation. The 'Smith' body, when present, can be clearly seen under a X 40 objective, as well as under a X 90 (oil immersion) (see Cross and Gill, 1979).

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## ADDENDUM

Since writing this paper I have come across two other pieces of information which I think are worth mentioning.

A) Juvenile hormone and the gypsy moth (see p.3)

Professor R.P. Dales, Professor of Zoology at the Royal Holloway and Bedford New College, University of London, asked the entomologist in his Department, Dr. Peter Credland, for his views. He did so as follows:-

"Juvenile hormone (JH) is a term now used to include a number of native compounds found in insects. At least three forms are known to occur, sometimes in combination, in different species. There are also a number of factors with JH-like activity which have been isolated from plants, the first being the 'paper factor' discovered by Slama in Canadian fir balsam in 1961. There is a rapidly increasing number of synthetic analogues of which methoprene has been registered for several uses, notably the control of some dipterous pests.

The major problem with the use of JH analogues is that they are all fairly unstable and degrade rapidly under field conditions. Since their action is effective only at certain critical stages in the life histories of their targets, application times are most important. Therefore for species with several generations in each year and imperfect synchrony in life histories, they are expensive both in financial and in 'time' terms.

Chitin synthesis inhibitors are sometimes more stable and diflubenzuron has been registered for gypsy moth control purposes. These compounds are not hormones or their analogues but disrupt the deposition of cuticle following and during a moult. As such, they are known, with JH and its analogues, as Insect Growth Regulators (IGRs).

For what it may be worth, I do not see the current generation of JH analogues as serious contenders for the gypsy moth control programme. Compounds with greater stability are required but then, of course, effects on non-target insect species become a potential difficulty."

B) Genetic engineering (see p.7)

In the British Medical Journal of the 18th June 1988 there was a leader entitled "Release of genetically altered viruses into the environment", by D.H.L. Bishop, Professor of Virology in the Institute of Virology in Oxford. In it he refers to an earlier paper (Bishop 1986) describing how a baculovirus (baculus = rod), Autographia californica, well known for producing nuclear polyhedrosis in caterpillars, can be modified by genetic engineering to become

more efficient and specific, by adding, replacing or deleting a particular sequence of genetic information. For example, an insect hormone gene can be inserted into the genetic make-up of the virus so that early during a caterpillar infection the pest becomes physiologically deregulated, causing it to stop eating. Baculoviruses are good subjects for genetic engineering as they can be restricted in their host ranges to particular insect species and do not infect or affect other invertebrates, plants or vertebrates.

Dr P. Entwistle, who is a colleague of Professor Bishop and who is a participant in this symposium, can give more details and tell us whether or not this particular form of genetic engineering has been used in the control of L. dispar. It sounds a promising treatment for endemic areas.

BISHOP, D.H.L., 1986. "UK release of a genetically marked virus".  
Nature, (Lond.) 323, 496.

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## MITOCHONDRIAL DNA AS A TRACER OF GYPSY MOTH ORIGINS

Richard G. Harrison and Thomas M. Odell, Section of Ecology and Systematics,  
Corson Hall, Cornell University, Ithaca, NY 14853 and USDA Forest Service,  
Northeastern Forest Experiment Station, 51 Mill Pond Road, Hamden, CT 06514

### INTRODUCTION

The dynamics of gypsy moth populations have been a subject of considerable interest to entomologists, primarily because of the periodic outbreaks of this serious forest pest. A variety of empirical and theoretical approaches have been used in an attempt to elucidate the factors responsible for the dramatic changes in population density. In contrast, relatively little attention has been focused on patterns of genetic variation within gypsy moth populations and on the genetic consequences of population fluctuations. Furthermore, despite intense interest in the moth not only in North America, but also in Europe and Asia, there has been remarkably little effort devoted to documenting genetic variation among gypsy moth populations and to deciphering its recent evolutionary history. Here we discuss the use of mitochondrial DNA (mtDNA) as a genetic marker for gypsy moth - its utility as a tool for documenting patterns of genetic relatedness among populations worldwide and for studying local population outbreaks. We focus attention on two issues: (1) Can mtDNA comparisons provide information on the source of the North American introduction, relationships of European and Asian moths, and patterns of geographic variation within east Asia? (2) Can mtDNA be used as a genetic marker within North America to distinguish among persistent low density populations and to provide a tracer of the origins of outbreaks?

Goldschmidt (1934) suggested that the origins of the genus *Lymantria* were in southeast Asia and that this was also the original home of the gypsy moth. *Lymantria dispar* is now found across central Asia, throughout Europe and in North Africa (Giese and Schneider 1979). Its presence in the United States is clearly a result of an accidental introduction in 1869 (Forbush and Fernald 1896). Goldschmidt (1934) documented patterns of geographic variation, using a variety of morphological and life history characters. He argued that the gypsy moth is extremely variable in Japan and eastern Asia, but relatively homogeneous throughout much of the rest of its range. Such a pattern of variation is consistent with east Asian origins and with a relatively recent spread of the moth across Eurasia. However, characters examined by Goldschmidt and others (Pintureau 1980) do not vary concordantly, and patterns of morphological and developmental variation may reflect local adaptation (rather than common ancestry). Moreover, Goldschmidt's interpretation of his data on

intersexuality (which served as an important component in his classification of "races" of gypsy moths) has recently been challenged (Clarke and Ford 1980, 1982). Clearly, more reliable genetic markers are needed to document patterns of genetic relationships and to resolve the evolutionary history of the gypsy moth.

Allozyme data (Harrison et al. 1983) revealed significant differences in frequencies between a Japanese population and populations across Europe. European populations have lower levels of genetic variation and are relatively homogeneous across a broad geographic area. The allozyme data also suggest that European populations may be derived relatively recently from an east Asian ancestor. North American populations exhibit virtually no allozyme variation (observed heterozygosities ranged from 0.000 to 0.008), presumably a consequence of the severe population bottleneck that accompanied the introduction.

According to records of gypsy moth population outbreaks, which span several decades in New England, certain discrete (2-10 ha in area) forest sites are historically "first to be defoliated" (Bess et al. 1947; Houston and Valentine 1977). These sites have a reputation for always having detectable gypsy moth populations. It has been hypothesized that gypsy moths disperse from these susceptible forests, which serve as reservoirs from which area-wide outbreaks emanate when abiotic or biotic conditions change in adjacent stands. If these susceptible/focal sites are the sources for periodic outbreaks and if genetic markers can be found that distinguish among populations from these sites, such markers could be used to trace the origin and spread of outbreaks.

Methods for assessing DNA sequence similarity or difference have recently become important tools in population and evolutionary biology. Sequence variation provides reliable markers for distinguishing populations and species, and sequence comparisons can be used for estimating genetic relationships and inferring evolutionary history. Data on DNA variation in natural populations have come principally from comparisons of restriction endonuclease fragment patterns and site maps. Restriction endonucleases are enzymes that cleave double-stranded DNA at specific recognition sequences. Digestion of a length of DNA with a restriction enzyme yields a series of fragments, the number and sizes of which will vary depending on where within the DNA the recognition sequence occurs. Differences in fragment patterns following digestion of homologous sequences from two or more individuals must reflect DNA sequence divergence. Therefore, fragment pattern differences can conveniently serve as markers of genetically distinct lineages. Moreover, comparisons of restriction fragment patterns or restriction site maps can be used to derive measures of genetic distance among individuals or populations and can provide data sets appropriate for phylogenetic analysis.

We have used comparisons of restriction fragment patterns and site maps to examine sequence variation of gypsy moth mitochondrial DNA. mtDNA has a number of properties that make it particularly attractive as a genetic marker in population and evolutionary biology (Avise 1986; Avise et al. 1987; Moritz et al. 1987). First, it is relatively easy to isolate and purify. Second, there is no ambiguity about the homology of the sequences being compared. Third, mtDNA is a small DNA molecule (15-30 kilobases), a size convenient for restriction site mapping. Fourth, mtDNA is maternally inherited and therefore provides direct information about matrilineal genealogies. This property makes mtDNA especially useful in tracing patterns of colonization. Fifth, there is no recombination between mtDNA molecules, so that a series of restriction site differences form a completely linked set. This provides exceptionally high resolution for discriminating common ancestry (e.g. in identifying the source of a colonization event or an introduction).

## MATERIALS AND METHODS

Gypsy moths were collected from a series of sites in eastern North America (Table 1). Four of these sites (ONT, MA, CT, PA) were selected to provide individuals from geographically diverse populations. The other three North American sites (VT1, VT2, RI) are localities that have been identified as possible "focal sites" by Houston and Valentine (1977). Moths from Japan and France were collected by personnel at the USDA Agricultural Research Service's Asian and European Parasite Laboratories, respectively, and shipped as pupae to the USDA Forest Service Laboratory in Hamden, CT. Adults were frozen as they emerged and have been stored at -80° since that time. These samples correspond to the France-2 and Japan populations for which we have published data on allozyme variation (Harrison et al. 1983). Gypsy moths from China were brought back by one of us (TMO) following visits to China in 1983 and 1987. Again, adults from each of these collections were frozen as they emerged.

Table 1. Geographic origins of samples used for mtDNA analyses.

Region/Population	n <sup>1</sup>
NORTH AMERICA (NA)	
Ontario (ONT)	2
Vermont 1 (VT1)	6
Vermont 2 (VT2)	3
Massachusetts (MA)	2
Rhode Island (RI)	7
Connecticut (CT)	2
Pennsylvania (PA)	2
FRANCE (FR)	
Provence	6
CHINA (CH)	
Heilongjiang Province (CH1)	4
Beijing (CH2)	2
JAPAN (JN)	
Hokkaido	6

<sup>1</sup> n is the number of individuals for which mtDNA data are available.

For a majority of the collections, total DNA was prepared from individual frozen moths following standard procedures (e.g. see Harrison et al. 1987). For the

three "focal sites", isofemale lines (lines derived from single females) were reared and pure mtDNA prepared from eggs taken from female progeny. Since mtDNA is maternally inherited (Awise 1986; Wilson et al. 1985), all progeny of a single female will have identical mtDNA genotypes. In addition, we prepared pure mtDNA from eggs of females from a standard laboratory strain (NJSS - Forest Service, see ODell et al. 1985). Details of procedures for preparing pure mtDNA from gypsy moth eggs will be described elsewhere.

In order to characterize variation in mtDNA restriction fragment patterns, total DNA from each individual was digested with an array of restriction enzymes. The resulting fragments were separated on 0.7% agarose gels, transferred to nitrocellulose filters or nylon membranes and hybridized with a  $^{32}\text{P}$ -labeled pure mtDNA probe (for details of this whole genome Southern blot technique see Maniatis et al. 1982). Fragment patterns were visualized by autoradiography, and fragment sizes estimated by comparison with a series of known standards.

Having characterized restriction fragment patterns, we proceeded to construct restriction site maps for each of the observed mtDNA genotypes. Maps were constructed using information obtained from double-digests (Maniatis et al. 1982). The restriction site maps (which will be presented elsewhere) enabled us to estimate the proportion of restriction sites shared between different mtDNA genotypes and to estimate the percent nucleotide difference between these genotypes.

The techniques described above are suitable for analysis of restriction fragment patterns produced by cutting the mtDNA molecule into relatively few large fragments. In order to obtain higher resolution, it is necessary to use restriction enzymes that cut the mtDNA molecule into many (small) fragments. By so doing, we are able to examine a much larger number of fragments (sites) and therefore assay a much larger proportion of the mtDNA genome. Pure mtDNA from the isofemale lines derived from moths collected at VT1, VT2 and RI was digested with enzymes that cut gypsy moth mtDNA many times, i.e. enzymes that have recognition sequences that occur many times within the mtDNA molecule. Following digestion, the resulting fragments were end-labeled, using  $^{32}\text{P}$ -labeled nucleotides (dNTPs) and the Klenow fragment of *E. coli* DNA polymerase. Labeled fragments were separated on 5% or 7% acrylamide gels and visualized by autoradiography. Note that this approach requires preparation of pure mtDNA from each individual or isofemale line and will not work on total DNA preparations. Pure mtDNA is difficult to obtain from frozen material, and therefore, we did not attempt to use this approach on material obtained from abroad.

## RESULTS

Using the whole genome Southern blot technique, we characterized mtDNA fragment patterns for moths from North America (ONT, MA, CT, PA), France (FR), China (CH1 and CH2) and Japan (JN). Our survey included fifteen different restriction enzymes, each of which produced 1-5 fragments. For most enzymes, the survey included 26 moths (at least two individuals from each site - see Table 1), but due to partial digestion and/or weak hybridization, data are missing for some individuals for six of the enzymes. However, we have characterized fragment patterns for the entire set of enzymes for individuals from each of the four major collecting areas (NA, FR, CH, JN). Furthermore, we have mapped all restriction sites for the fifteen enzymes - a total of 39-44 sites for each composite mtDNA genotype.

Except for a single restriction site polymorphism in the CH1 population, we found no variation in mtDNA composite genotype within any of the populations or even within any of the four major areas. The observed polymorphism involved the absence of a single *Msp*I restriction site in one moth.

Although separated by 1000 km, the two Chinese populations had identical mtDNA genotypes. They shared 39 restriction sites, with no unique sites found in either population. The mtDNA genotypes of North American moths were identical to those of moths collected in south France; these moths had 44 restriction sites in common.

Despite the remarkable homogeneity within populations and regions, mtDNA genotypes of moths from different regions exhibit substantial amounts of divergence. In all of our samples (ignoring the single polymorphic site in the CH1 populations), we observed only three composite mtDNA genotypes - one characteristic of both North American moths and the single French population, a second characteristic of the Chinese populations, and a third found in Hokkaido, Japan. The NA/FR genotype differs from the CH genotype by nine restriction site gains or losses and from the JN genotype by eleven site changes (Table 2). The two Asian genotypes differ in the presence/absence of fourteen restriction sites, i.e. they are more different from each other than either is from the NA/FR genotype. The number of restriction sites shared between mtDNA genotypes (and the total number mapped for each genotype) are summarized in Table 2.

Table 2. Number of mtDNA restriction sites mapped for each population or region (on the diagonal) and number of restriction sites shared between pairs of populations

	NA	FR	CH1	CH2	JN
North America	44	44	37	37	36
France		44	37	37	36
China 1			39	39	32
China 2				39	32
Japan					39

We used the method of Nei and Tajima (1983) to estimate genetic distances among the three genotypes - in terms of the percent nucleotide difference (Table 3). Nucleotide sequence divergence between gypsy moth mtDNA genotypes ranges from 2.1-3.6%. If gypsy moth mtDNA sequences are evolving at about the same rate as those in vertebrates, the differences among the three genotypes reflect divergence times of 1-2 million years (the rate of mtDNA sequence divergence between lineages is generally estimated to be 2-4% per million years in vertebrates- see Brown 1985, Wilson et al. 1985).

Table 3. Estimates of the percent nucleotide sequence divergence between gypsy moth mitochondrial genotypes.

	NA	FR	CH	JN
North America	0.0	0.0	2.1	2.6
France		0.0	2.1	2.6
China			0.0	3.6
Japan				0.0

If useful genetic markers are to be found within North American gypsy moth populations, we clearly must resort to higher resolution techniques. That is, we must assay a larger proportion of the mtDNA genome. We have recently initiated a survey using restriction enzymes that cut gypsy moth mtDNA into many (40-60) fragments. Gel banding patterns of such complexity are difficult to interpret, but our initial goal is simply to discover whether any mtDNA polymorphism exists within North America and whether individual morphs are unique to specific localities. The preliminary data involve comparisons of moths from three "focal sites" within New England - two sites in Vermont (VT1 and VT2) and one in Rhode Island (RI). There are clearly polymorphisms, i.e. differences in gel banding patterns. At this point, however, we have not discovered any restriction fragment patterns that are characteristic of only one site.

#### DISCUSSION

Mitochondrial DNA composite genotypes, defined by the presence and absence of restriction endonuclease recognition sites, provide high resolution markers which clearly define maternal lineages. The utility of mtDNA as a marker in both inter- and intra-specific comparisons has been clearly demonstrated for a variety of animal species (Avisé et al 1987; Moritz et al 1987). Our preliminary data on mtDNA genotypes in worldwide gypsy moth populations indicate that this molecule will be of considerable value as a tracer of gypsy moth origins.

The existing mtDNA diversity within North American gypsy moth populations is a function of the original diversity of genotypes that escaped in Medford, Massachusetts in 1869. Because mtDNA is both maternally inherited and effectively haploid, the effective population size for mtDNA is only one-fourth that for nuclear gene markers. Hence mtDNA genotype diversity is particularly sensitive to population bottlenecks and founder events. If more than one mtDNA genotype were present immediately after the introduction, the chance extinction of certain maternal lineages may have led to a subsequent decrease in diversity. This would be opposed by an increase in variation due to the accumulation of mutations in *L. dispar* populations in the 120 years since the introduction. The extremely low level of mtDNA diversity observed within North American gypsy moth populations suggests that the original founder event involved very few moths.

However, the absence of mtDNA restriction site differences between moths collected in North America and those collected in France (Provence) suggests that French populations may also exhibit very low levels of variation (in which case the amount of variation within North American populations also reflects the absence of

variation in the source population). It is thought that French moths were the source of the North American introduction (Forbush and Fernald 1896; Burgess 1944), but the exact site from which they came is not known. If significant mtDNA variability is present within French populations, then we have fortuitously sampled a population that has a mtDNA genotype identical to that introduced into North America. It seems far more likely that French populations of *L. dispar* represent a single mtDNA genotype (with respect to the restriction sites that we have mapped). If true, this would suggest that French (and perhaps all European) gypsy moths are relatively recent arrivals or that recent population bottlenecks have reduced mtDNA diversity. Data from other characters (e.g. morphology and development (Goldschmidt 1934), allozymes (Harrison et al. 1983)) also suggest homogeneity of gypsy moth populations across Europe. Indeed, Goldschmidt (1934) went so far as to say that across much of Eurasia the gypsy moth "behaved practically as a unit". We find significant differences in mtDNA genotype between French and Chinese populations, but no differences between two Chinese populations that are separated by at least 1000 km.

Although Goldschmidt (1934) argues for an east Asian origin of the gypsy moth, there is little direct evidence for deciphering the evolutionary history of this important insect pest. Our data suggest that mtDNA may be a good marker for tracing the Asian origins of European (and North American) gypsy moth populations. The marked differences among the French, Chinese and Japanese mtDNA genotypes reflect a relatively ancient divergence. It is probable that several (many) other distinct genotypes will be found upon further sampling of Asian moths. It is possible that a genotype very similar to that found in Europe and North America may be present in east Asia. Given that mtDNA is a set of completely linked markers, it provides excellent discrimination between common ancestry and convergence and will allow us to trace phylogeographic patterns with considerable confidence. With only three distinct genotypes, we cannot yet draw any conclusions about the population phylogeny of the gypsy moth. Based on mtDNA comparisons, French moths are more similar to Chinese moths than they are to those collected in Hokkaido, but the differences in genetic distance are not significant. The observed divergence between French and Japanese gypsy moths is consistent with earlier observations based on allozymes (Harrison et al. 1983).

Obviously, additional sampling of east Asian populations should be a high priority. Recently, we have discovered that satisfactory total DNA preparations can be made from ethanol preserved specimens. This may allow us to circumvent the obvious problems associated with importing live Asian material into North America.

One advantage of the approach we are taking is that it allows us to gain a very high level of resolution in distinguishing lineages. Our ongoing studies of mtDNA variation in North American gypsy moth populations have not yet provided any markers that distinguish among the sites we have sampled. However, we can clearly identify mtDNA restriction fragment pattern variants within populations. At this point we are not sure whether these reflect variation in presence/absence of restriction sites or size variation of the gypsy moth mtDNA (i.e. small insertions or deletions). In either case, these mtDNA variants may prove useful either in continuing attempts to document the genetic structure of North American populations or in release experiments in which it is important to have a presumably neutral genetic marker.

## SUMMARY

We have initiated a study of mitochondrial DNA restriction site variation within and among gypsy moth populations from North America, Europe and Asia. MtDNA is an excellent marker for determining the history of colonization and introduction. One objective is to use mtDNA comparisons to decipher the recent evolutionary history of the gypsy moth. For the small set of populations sampled to date, we find no restriction site differences between North American and French moths (and no variation within North America for the 44 mapped sites). However, there are many site differences between these moths and those from China and Japan and even greater differences between the two Asian localities. These results suggest a relatively ancient divergence among existing gypsy moth lineages. The approach used here would seem to hold great promise for determining the source of European populations. Additional sampling of east Asian populations is a high priority. Our second objective is to develop high resolution techniques that will provide us with mtDNA markers within North America for tracing the origin of local outbreaks and perhaps for use in sterile male releases.

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GENETIC CONTROL OF LYMANTRIIDAE:  
PROSPECTS FOR GYPSY MOTH  
MANAGEMENT

V. C. Mastro  
Otis Methods Development Center  
USDA, APHIS, PPQ  
Otis ANGB, MA 02542

T. M. Odell  
Forest Service  
Northeastern Forest Experiment Station  
Hamden, CT 06514

C. P. Schwalbe  
Otis Methods Development Center  
USDA, APHIS, PPQ  
Otis ANGB, MA 02542

SUMMARY

Progress in the current program to develop the sterile insect technique for control of gypsy moth, *Lymantria dispar* L., is reviewed. The discussion includes a synopsis of radiation biology studies, competitiveness evaluation of sterile adults, and field testing of three application techniques. A field test involving the release of fully sterile male gypsy moths was successful in eradicating an isolated population, but labor demands were high and execution was logistically difficult. Releases of partially sterile males in another isolated population was equally successful in that their F<sub>1</sub> progeny were observed the year after release and eradication was achieved. But again, this approach was logistically and economically costly. The current method of releasing F<sub>1</sub> eggs (progeny of irradiated males and normal females) is described. These egg masses can be produced and stockpiled in the laboratory in large numbers. Release of F<sub>1</sub> egg masses prior to native egg eclosion establishes a population of sterile insects that ultimately mate with the native population, imparting a suppressive effect. Treatment of a sparse, isolated infestation using this technique resulted in population eradication and other results indicate promise for use in control of low density, isolated populations. Problems associated with estimating native population densities, determining the impact of treatment, and evaluation of competitiveness of immature and adult stages are discussed.

## INTRODUCTION

Investigations into the use of the sterile insect technique (SIT) for gypsy moth, *Lymantria dispar*, control were initiated over thirty years ago (Godwin et al., 1964). Godwin's work and studies by other investigators using gamma radiation and chemosterilants to induce sterility were reviewed by Mastro et al., (1981). Although this early work laid the basis for establishing the radiation biology of the gypsy moth and resulted in some early field trials, there were practical barriers to the successful demonstration of SIT as a control technique. Some technological problems that prevented progress in this approach included lack of: 1) adequate rearing techniques, 2) evaluation criteria and techniques for measuring insect competitiveness and 3) techniques for monitoring densities of wild populations and the subsequent impact of a sterile insect release.

However, even if the technical problems confronting development of the SIT were overcome, the inconsistent management policies and objectives that have persisted almost since the gypsy moth's introduction into North America (Dunlap, 1980; McManus & McIntyre 1981; Ravlin et al., 1987) may have proven a greater barrier. In the 1960's and 1970's, no overall management strategy prevailed within the area generally infested by the gypsy moth. Objectives of management programs ranged from prevention of defoliation, abatement of nuisance, protection of esthetics, protection of high value timber and watershed protection to no action when defoliation threatened. Control programs tended to be defensive and were reactions to outbreak or near outbreak insect population densities. Use of the sterile insect technique would have been of little practical value in this type of reactionary situation. Because of the inherent density-dependency of the technique, it would not be practical or economical to rear sterile insects in large enough numbers to overflow these types of defoliating populations. However, techniques such as SIT can theoretically be used in area-wide IPM programs where the emphasis is on population maintenance at densities well below defoliating levels.

Despite quarantine measures, introductions of the gypsy moth occur yearly outside of the generally infested area, largely through the movement of outdoor household articles. The United States Department of Agriculture, Animal and Plant Health Inspection Service, in cooperation with individual states, maintains a survey and detection program for identifying these introduced populations (Ravlin et al., 1987; Schwalbe, 1981). The policy for introduced populations has been clear: to eradicate populations as quickly as possible. In the past, these eradication programs mainly utilized applications of chemical insecticides. These introduced populations are isolated from the generally infested area and are usually detected when they are sparse and of limited size (usually less than 200 ha). The sterile insect technique can theoretically be used economically in these circumstances. Enhancing the SIT's value in these situations is

the public's increasing resistance to broadcast insecticide applications in residential areas where, because of the mode of transport, these infestations are usually detected.

The area generally infested by the gypsy moth is also increasing through natural spread. Along this expanding edge, no uniform management strategy prevails. Currently, there is an ongoing dialogue about the feasibility of containment or a slowing of the rate of natural spread of gypsy moth. A stated objective of the Appalachian Integrated Pest Management (AIPM) Project, a large demonstration program along a portion of the leading edge, is to "minimize the spread and adverse effects of the gypsy moth within the project area". This objective implies treatment of newly established and numerically sparse populations along the expanding edge of the currently infested area. Theoretically, this is an ideal place for the application of SIT, when applied in combination with other management tools. If populations are carefully monitored in an IPM program, intervention measures could be initiated when populations are sufficiently sparse for sterile release to be biologically and economically feasible.

How the SIT will ultimately fit into the varied management strategies for gypsy moth is the subject of much discussion. If the strategy involves suppression or eradication of sparse populations, then the potential for use of SIT is promising. The challenges are greater to develop the technology for management of large infested areas in the Eastern States. Reevaluation of the SIT technique for gypsy moth control was undertaken in 1977 because of developments in mass rearing techniques (Bell et al., 1981), a broader understanding of the insect's biology and behavior, and the recognized need for alternatives to chemical control.

#### EVALUATION OF CLASSIC STERILE INSECT TECHNIQUE

Our initial approach evaluated the use of the sterile insect technique in the "classical" way: large numbers of fully sterile adult insects are released to "overflow" a wild population. In this approach, as the overflowing ratio of sterile:fertile (wild) insects increases, the probability of successful mating between wild adults decreases, which results in population suppression. The first major success using this technique was a small eradication trial for the screwworm fly, Cochliomyia hominivorax (Coquerel), over thirty years ago (Baumhover et al., 1955). Since that time, the technique has been successfully applied to a number of pest species. Perhaps the most widely known are extensive programs for the control of the Mediterranean fruit fly, Ceratitidis capitata (Wiedemann) (Patton, 1982; Rode, 1970; Steiner et al., 1962).

Initially, we investigated the radiation biology of the gypsy moth and the competitiveness of laboratory reared males. Irradiation studies demonstrated that near total sterility was

induced when males were exposed to 15 krads of gamma radiation (Cobalt 60) as pupae or adults, and somatic damage was minimized when pupae were treated 8 days or more after pupation. Evaluation of laboratory reared male moths (irradiated and untreated) indicated that they were competitive with their wild counterparts. Laboratory and field tests evaluated male longevity, flight behavior, pheromone response, mating periodicity and frequency and other competitive indices. Table 1 summarizes competitiveness traits examined (see Lance et al., 1988; Mastro, 1980; Waldevogel et al., 1982).

Table 1. Behavioral traits examined for competitiveness comparisons of adult male gypsy moths.

Field comparisons using mark-release-recapture techniques

Pupal eclosion periodicity  
 Adult dispersal periodicity  
 Response to pheromone sources and periodicity of response  
 Response to pheromone sources as a function of male age  
 Horizontal and vertical distribution in the forest canopy

Field cage comparisons

Frequency of mating of males of various ages  
 Periodicity of mating  
 Length of mating

Laboratory comparisons

Response to pheromone (flight tunnel)  
 Periodicity of activity (actograph)  
 Propensity to fly (actograph)

Pilot Test of Sterile Insect Technique

In 1980, an isolated population in Berrien Co., Michigan, was selected for a field evaluation of the impact of release of sterile males. Males were irradiated (15 k) as pupae 8-11 days after the pupal ecdysis. To facilitate release, pupal release containers were positioned systematically throughout the infested area on a 50m grid in consideration of the relatively short dispersal distances of male gypsy moths (Elkinton & Carde, 1980; Schwalbe, 1981; Schwalbe & Paszek, 1978). Male loss from a population is rapid (Mastro & Obell, unpublished) and one or two-day old males comprise the largest proportion of a male population which is actively mating. To maintain high overflooding rates throughout the wild flight period, sterile male pupae were placed at the

release sites daily. Released sterile males were internally marked with calco oil red dye. Sterile:fertile overflooding ratios were carefully monitored by examining the abdomen of males recovered at pheromone-baited traps for presence of dye. In addition, females were placed daily throughout the release area to monitor mating success. Egg masses produced by monitor females were evaluated for hatch to determine the type of male parent (sterile male matings resulted in no hatch). After three years of sterile male release, the native population was eradicated. Based on analysis of the observed sterile:fertile overflooding ratios, the native population was reduced in a systematic and predictable manner (Table 2). Released sterile males appeared to be highly competitive with the target population.

Table 2. Results of treating an isolated gypsy moth population in Berrien County, Michigan, with sterile<sup>1/</sup> male gypsy moths.

Year	No. of sterile males released/day	No. of wild males trapped	Observed sterile:fertile ratios	
			Trapping <sup>2/</sup> method	Female mating ratios
1979	0	90	-	-
1980	10,000	274	14.4:1	5.9:1
1981	10,000	50	128.6:1	29.8:1
1982	6,000	1	5842:1	287:0
1983-86	0	0	-	-

<sup>1/</sup> Moths irradiated as 8-12 day-old pupae with 15 krad.

<sup>2/</sup> Mean male trapping ratios were computed from daily ratios of sterile and fertile males captured in traps during peak native flight - 1979 (7/31-8/11), 1980 (8/1-8/10) and 1981 est. (8/1-8/10).

This pilot study also identified difficulties in the operational use of the technique. Male pupae are large (approximately 0.5g) and somewhat fragile, necessitating special packing and shipping provisions to minimize damage and prevent eclosion during transit. The release cages, which were designed to eliminate predators but allow exit of eclosed moths, were costly and difficult to maintain. Broadcasting pupae was considered as a release strategy but rejected because of projected losses caused by predators. However, as already mentioned, the major impediment was the need to release males frequently throughout the flight period to maintain desired overflooding ratios. The expense of

maintaining personnel at the release site throughout the adult flight period added appreciably to the cost of applying the technique. On the relatively small scale of this test (approximately 2.59 km<sup>2</sup>), the technical and logistic problems were overcome. However, applying the technique on a larger scale was judged to be impractical.

#### EVALUATION OF INDUCED INHERITED STERILITY

Our initial irradiation studies suggested that sterility was induced in the F<sub>1</sub> generation when the radiation dose was approximately halved. Indeed, earlier investigators had induced F<sub>1</sub> sterility in the gypsy moth using low doses of radiation [reviewed by Mastro (1981)]. The mechanism of inducing sterility in the F<sub>1</sub> generation has been investigated by Bauer (1967) and North & Holt (1968). It is now generally believed that F<sub>1</sub> sterility can be induced in any lepidopteran species, and has been demonstrated for a number of species and was reviewed by North (1975) and La Chance (1985). Our interest in F<sub>1</sub> sterility stemmed from the theoretical advantages of using it as opposed to releasing totally sterile males (Knipling, 1969, 1970; Brower, 1980; Nielsen & Brister, 1980; Carpenter et al., 1983).

#### Radiation Biology

To explore the feasibility of using the induced-inherited sterility technique (F<sub>1</sub> technique) for gypsy moth, we initiated a study of the radiation biology in 1980. Male gypsy moths were (P<sub>1</sub>) irradiated at different pupal ages (6 groups) with seven levels of radiation, i.e. 42 dose/age treatments. Irradiated laboratory-reared males were mated with untreated laboratory-reared females (50 pairs per treatment) and the resulting F<sub>1</sub> progeny were evaluated in the egg, larval, pupal and adult stages. Egg masses were evaluated for number of eggs, degree of embryonation and percent hatch. Length of development and survival of other stages were also observed and any abnormalities were noted. Resulting F<sub>1</sub> adults were increased within their own treatment group and outcrossed to normal insects. In the F<sub>2</sub> generation, insects were evaluated in the egg, larval and pupal stages in the same manner as their parents and again outcrossed to untreated insects and increased within their treatment groups. All treatments were evaluated through the F<sub>3</sub> egg stage and some selected treatments were evaluated through the F<sub>4</sub> egg stage. All insects used in this study were from a colony maintained by the Otis Methods Development Center for twenty generations (NJS-G20) [see O'Bell et al., (1984)] and were reared under standard laboratory conditions as outlined by Bell (1981). Because of the quantity of data generated, only a synopsis of this study will be presented.

#### Effects on F<sub>1</sub> Generation

The results of our irradiation studies closely parallel those of studies with other Lepidoptera species (Brower, 1979, 1980; Carpenter et al., 1983, 1987). Debilitating effects of irradiation on  $P_1$  males are most pronounced in youngest pupal age classes at the higher radiation doses. When males were exposed to only 4 krads 0-1 days after pupation, only 44% of the females produced an egg mass vs. 98% in a control group. This effect was not observed in the next older pupal age class (2-3 day old) until the dose was increased to 8 krads, and not pronounced until the dose was increased to 10 krads (i.e., respectively 78% and 36% of the pairs produced egg masses). Also, when paired with males irradiated in younger age classes, those females that did oviposit produced fewer eggs. Other effects noted were incomplete eclosion and wing deformities of irradiated  $P_1$  males. Matings with males irradiated in older age classes resulted in  $F_1$  egg masses with high levels of embryonation. Pairs with males irradiated with 15 krads greater than 1 day old averaged 84.4% embryonation vs. 96.6% for control matings.

Hatch from  $F_1$  egg masses produced by the various male irradiation treatments is summarized in Figure 1. The degree of sterility increased (i.e. the proportion of eggs which hatched decreased) as the radiation dose increased from 2 to 15 krads. Also, as the pupal age at irradiation increased, generally the degree of sterility decreased. However, males irradiated in the oldest age class (10-11 days old) were more sterile than the next younger age class (8-9 days old).

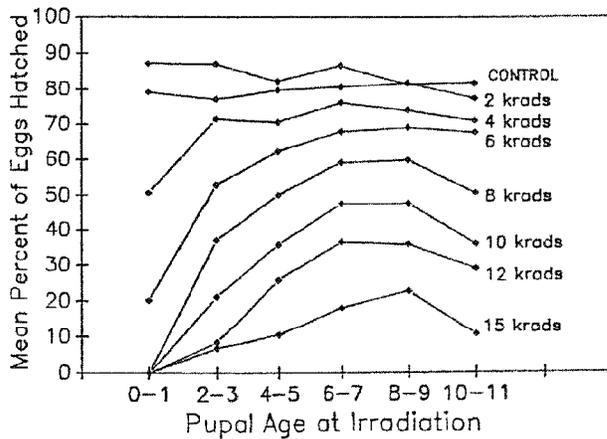


Figure 1. Mean percent hatch from  $F_1$  egg masses which resulted from mating males (treated at various ages with different doses of radiation) with untreated females.

Effects on F<sub>1</sub> Generation

Survival of F<sub>1</sub> immatures from all treatment groups was generally high (60-98%). The proportion surviving, however, was dose dependent; F<sub>1</sub> larval survival decreased as the radiation dose of the P<sub>1</sub> male increased (Figure 2). At lower radiation doses between age classes, survival rates were nearly constant. For example, survival of progeny of males irradiated with 10 krad ranged from 82% to 91%, respectively, for the 2-3 and 8-9 day-old age classes. Larval developmental time from eclosion to pupation increased as dose increased. Mean larval developmental times (all age classes) for F<sub>1</sub> male larvae for the 2 and 15 krad treatment groups were, respectively, 30.5 days and 35 days. Developmental times of female larvae were similarly dose dependent (i.e. respectively 32 and 37 days in the 2 and 15 krad treatment groups). LaChance (1985) states that delayed development of F<sub>1</sub> progeny may be a common phenomenon. Also, as in studies with other Lepidoptera, there was a male-biased sex ratio shift in the F<sub>1</sub> generation which resulted in an approximately 2:1 ratio at the 10 krad level of P<sub>1</sub> treatment.

Characteristics of F<sub>2</sub> egg masses produced by incrossing and outcrossing F<sub>1</sub> adults from selected treatments are summarized in Table 3. Generally, F<sub>1</sub> insects whose male parents received higher radiation doses were more sterile than those whose parents were irradiated with lower doses.

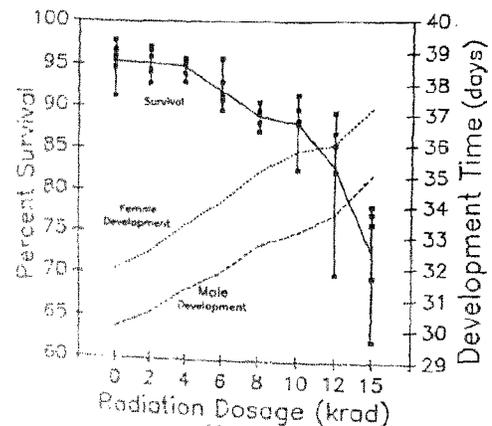


Figure 2. Survival and developmental times of F<sub>1</sub> larvae that hatched from egg masses produced by untreated females mated to irradiated males. Data presented are mean development times for all age classes at each dosage. Mean percent survival for individual age classes are represented by ■.

Table 3. Viability and size of F<sub>2</sub> egg masses resulting from incrossing (within treatment groups) and outcrossing F<sub>1</sub> adults (progeny of an irradiated male and an untreated female).

Dose	Age	Treatment of P <sub>1</sub> Mating type ♂ x ♀	No. successful mating pairs (n=25)	Mean no. of total eggs produced	Mean percent of eggs embryonated	Mean percent of total eggs which hatched
6	8-9	F <sub>1</sub> x C	24	809.5	43.9	6.5
		C x F <sub>1</sub>	21	747.5	74.7	13.0
		F <sub>1</sub> x F <sub>1</sub>	20	572.2	44.1	1.3
6	10-11	F <sub>1</sub> x C	21	673.0	46.1	13.0
		C x F <sub>1</sub>	26	823.3	77.2	13.6
		F <sub>1</sub> x F <sub>1</sub>	24	659.4	31.8	1.2
8	8-9	F <sub>1</sub> x C	21	612.4	22.1	1.2
		C x F <sub>1</sub>	12	495.2	13.6	0.0
		F <sub>1</sub> x F <sub>1</sub>	12	495.2	13.6	0.0
8	10-11	F <sub>1</sub> x C	19	624.4	23.5	1.9
		C x F <sub>1</sub>	22	920.4	79.3	4.5
		F <sub>1</sub> x F <sub>1</sub>	18	505.4	13.7	0.1
10	8-9	F <sub>1</sub> x C	17	607.0	39.8	7.4
		C x F <sub>1</sub>	25	661.4	74.1	0.5
		F <sub>1</sub> x F <sub>1</sub>	20	548.6	9.7	0.1
10	10-11	F <sub>1</sub> x C	19	460.1	16.8	0.1
		C x F <sub>1</sub>	22	622.8	66.8	0.9
		F <sub>1</sub> x F <sub>1</sub>	16	584.3	10.9	0.1
Control		C x C	145 (n=150)	947.3	93.7	76.5

Egg masses produced by crosses of  $F_1$  males and untreated females characteristically contained less than half the proportion of embryonated eggs that normal crosses produced. Abnormal sperm and poor cupyrene sperm transfer are possible explanations (Ashrafi & Roppel, 1973; North, 1975; Brower, 1979; LaChance et al., 1979; LaChance, 1985). At radiation doses of 6 krad or greater, the degree of sterility induced in the  $F_1$  parents, as measured by percent hatch of  $F_2$  egg masses, was relatively high for all age groups and mating types. Generally,  $F_1$  sterility increased as the  $F_1$  male parent's radiation dose increased. At lower doses,  $F_1$  females outcrossed to untreated males produced more highly embryonated  $F_2$  egg masses and more progeny than the reciprocal cross. At higher radiation levels, and as total sterility is approached, differences between the fertility of  $F_1$  males and females disappears (Fig. 3).

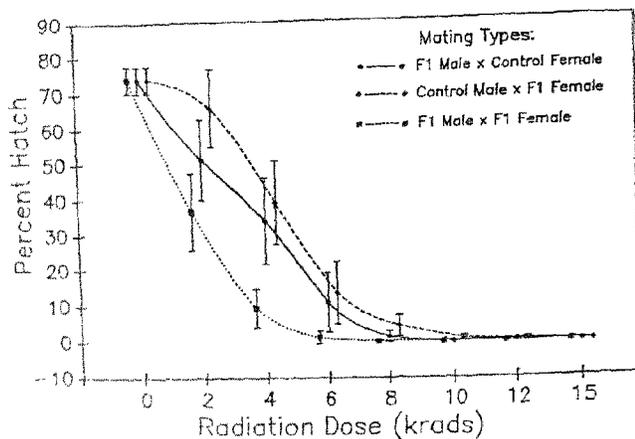


Figure 3. Mean percent hatch and 95% confidence intervals for  $F_2$  egg masses, progeny of  $F_1$ s whose  $P_1$  male parent was irradiated as 10-11 day-old pupae.

#### Effect on the $F_2$ Generation

Survival and developmental characteristics of  $F_2$  progeny are summarized in Table 4. Survival of  $F_2$  progeny resulting from outcrossing either  $F_1$  males or females from the 6 krad treatment group was high (i.e. respectively 59.7 and 60.7%) vs 76.6% for control insects. Length of larval development for both  $F_2$  males and females from these same treatment groups also averaged only 1 and 2 days longer than control insects. Data from  $F_2$  groups originating from radiation treatments above 6 krad are limited because  $F_1$  sterility is high and few  $F_2$  neonates were available for

rearing. The limited amount of observations, however, indicate a general trend of longer larval development times with increasing dose.

Effects of radiation on fertility are apparently carried into at least the F<sub>2</sub> adult stage. F<sub>3</sub> egg masses produced by outcrossing F<sub>2</sub> adults were less embryonated than control matings and had lower proportions of eggs which hatched. Egg masses produced by incrossing F<sub>2</sub> adults generally contain the smallest proportions of eggs which were embryonated and which hatched. When progeny of males irradiated with a dose as low as 2 krad were inbred for two generations, reduced viability was noted (data not shown).

#### Effects on the F<sub>3</sub> Generation

Survival and development of F<sub>3</sub> progeny, regardless of the parentage, approximates the control group better than the F<sub>2</sub> generation. Survival of first instars to the pupal stage was similar to control insects for nearly all treatment groups. Male and female larval development times, however, were longer than those of control insects. This difference, however, was not as pronounced as in the F<sub>2</sub> generation. Although the radiation effects are not as pronounced in the F<sub>3</sub> larval stage, they still are apparent. Incrossing and outcrossing F<sub>3</sub> adults resulted in egg hatch rates only slightly lower than control matings.

#### Competitiveness of F<sub>1</sub>s

Results of radiation biology studies defined potential fitness deficiencies of F<sub>1</sub> progeny, e.g., longer larval developmental times, decreased survival and possible poor sperm transfer of F<sub>1</sub> males. How these and other characteristics would be effected by natural conditions was our next area of investigation.

In preliminary studies we found that when F<sub>1</sub> larvae were reared on excised oak foliage in an insectary or on caged oak foliage, survival to the adult stage was significantly lower than the survival rate of wild insects reared in the same manner (Lance et al., 1983; 1984). Untreated insects from the laboratory colony (NJS), from which F<sub>1</sub>s were derived, developed faster than wild insects. Mean F<sub>1</sub> male larval developmental rate was approximately 1 to 2 days longer than wild immatures. Also, when unfed first instar F<sub>1</sub>s were placed on branches of a host, they tended to establish in a manner similar to wild first instars. However, a larger proportion of first instars from the untreated laboratory strain dispersed (Lance et al., 1982). We found that when late instar larvae of three strains (NJS, wild and F<sub>1</sub>) were released on trees, patterns of activity were similar. However, F<sub>1</sub> larvae tended to leave the tree more often than the untreated laboratory reared NJS or a strain reared from field collected eggs (Mastro & Schwalbe, 1986).

Adult F<sub>1</sub> male progeny paternally treated with three levels of



radiation (6, 8 and 10 krads as 8-11 day-old pupae) were compared in field trials. Pupal eclosion, adult dispersal and response to pheromone sources located at various distances from the eclosion site were monitored. In the parameters measured, there were not significant differences between  $F_1$ s and wild or untreated laboratory reared insects. In field cage studies designed to evaluate mating propensity,  $F_1$  males mated an initial time with lab-reared females as frequently as did wild males, however, fewer  $F_1$  males mated a second time on the same day, e.g.,  $F_1$ s mated 1.8 times vs. 3.4 and 2.5 respectively for wild and untreated laboratory reared males (Odell & Mastro, unpublished). The impact of this competitiveness shortcoming is still to be determined in field tests.  $F_1$  females generally attracted males and mated in patterns similar to wild females (Mastro et al., 1987). Competitiveness testing related to the areas just described is ongoing in order to define more precisely, potential impact on the overall competitiveness of sterile  $F_1$  progeny.

When considering a particular management objective (e.g. eradication vs. suppression), the characteristics of  $F_1$  progeny of the various dose/age treatments must be considered carefully. Treating  $P_1$  males with 10 krads in either the 8-9 or 10-11 day age groups provides  $F_1$  progeny which are very sterile (see Table 3). However, the proportion of  $F_1$  eggs which hatch is small (approximately 34-40%, Figure 1), and mean larval developmental times are approximately three days longer than that of control insects and survival is reduced. Only a small number of  $F_2$  larvae result from outcrossing  $F_1$  adults from this treatment group (Table 3) perhaps making it the best irradiation treatment for eradication programs. The fertility of the  $F_1$  female and the survival of any of her  $F_2$  progeny should be closely scrutinized when making a selection (Tables 3 & 4). Selection of a lower treatment dose (6 krad) provides  $F_1$  larvae with a faster development time and a greater number of  $F_1$  adults (e.g. higher proportions of  $F_1$  eggs hatch and survive); these adults, however, are more fertile than  $F_1$  progeny of males receiving higher radiation treatments and the outcrossed  $F_1$  female is more fertile than the reciprocal cross. Although 6 krads may not be considered as a suitable treatment dose when the objective is eradication, it may be a better choice for population suppression purposes. Simulation modeling is essential to accurately predict the consequences of various treatments and release options.

#### Pilot study of $F_1$ technique

The information compiled from radiation and competitiveness studies and the theoretical advantages of  $F_1$  sterility prompted us to initiate a field trial. In 1982 an isolated, low-density population in Horry Co., South Carolina, was selected. Irradiated (10 krads) males treated as pupae (8-12 days old) were released throughout the flight period. Release procedures were similar to the fully sterile male release described previously. The site was intensively monitored in 1982, and in the following year.

Interaction with the native population was demonstrated, and the population was declared eradicated in 1985 after two years of negative pheromone-baited trap survey (traps deployed on a 268m grid). Although the outcome of the trial was positive, the technique was nearly as difficult and costly as releasing fully sterile insects. The theoretical advantage is that a greater suppressive effect results from releasing partially sterilized males because the F<sub>1</sub> progeny of released males which mate with wild females are available in the next generation for continued suppression. In 1983 we began to consider the release of F<sub>1</sub> egg masses (progeny of males treated with a substerilizing dose and mated with untreated females). Since F<sub>1</sub> eggs can be easily produced and held for months in diapause, large numbers could be stockpiled either in the laboratory or at the release site under field conditions. Just prior to native egg hatch, F<sub>1</sub> egg masses could then be distributed throughout the target population. Release rates, theoretically, can be adjusted to result in the desired overflooding F<sub>1</sub>:wild adult ratio, provided that the native density is known, F<sub>1</sub> and native eggs hatch in synchrony, and comparative survival and synchrony information on immature stages is accounted for.

Reasonably accurate estimates of the native egg mass density are essential to establish the desired sterile:fertile overflooding ratios. However, in sparse insect populations where application of this control technique should be considered, current techniques for estimating egg mass densities are not sensitive (Wilson & Fontaine, 1978). Studies with grids of pheromone traps, have demonstrated a relationship between the proportion of males captured and actual male density (Schwalbe & Paszek, 1978; Elkinton & Carde, 1980). Currently, we use a technique based on pheromone trap information for estimating densities in isolated populations. The applicability of using trap information for monitoring densities within areas that are continually infested is under investigation (Ravlin et al., 1987).

#### F<sub>1</sub> Egg Mass Release Pilot Study

To date, nine isolated gypsy moth infestations have been treated using releases of paternally irradiated F<sub>1</sub> eggs whose male parent was irradiated. In addition, approximately 400 ha. of infestation have been treated with F<sub>1</sub> eggs at sites within the endemic area (plot size ranged from 7.5 to 225 ha.) Generally, the release and monitoring techniques described below are similar to those used at all release sites.

To describe the details and effectiveness of the F<sub>1</sub> egg release technique, the treatment of one isolated population in Bellingham, Washington, is summarized below. The infestation was detected in a residential area in 1983 through the use of pheromone-baited traps. More intensive trapping in 1984 succeeded in delimiting the boundaries of the infestation and capturing more male moths (n=62). Also egg masses and larval and pupal exuviae

were found. Based on the previously described relationship between trap density and proportion of males captured, we estimated that approximately 20 percent of the males were captured in 1984 resulting in a population estimate of 400 wild males. To arrive at an estimate of the overwintering egg mass density, we assumed that there was a 1:1 adult sex ratio during the 1984 flight period and that all females mated and produced viable egg masses that survived and hatched in the spring of 1985. The resulting estimate of 400 native egg masses may have been too high. Campbell et al., (1976a) and others have shown that skewed sex ratios occur in sparse populations. We assumed that the residential nature of the area and the abundance of man-made objects would foster female larval and pupal survival (Campbell et al., 1976b). At worst we believed our native egg mass estimate would be too high and we would err only in overflooding at a higher than projected rate.

The desirability of establishing a very high overflooding ratio had to be balanced against other factors. Because we would be releasing a feeding stage in a residential area with limited numbers of ornamental and fruit trees, we did not want to release numbers of gypsy moths which would produce objectionable defoliation. We circumvented this problem by releasing numbers which would provide the desired overflooding ratios and produce the desired outcome, eradication, over a two-year period. Also, in 1985, the release area was thoroughly inventoried for numbers and size of host trees in order to estimate the number of insects which could be released without causing objectionable damage. Thirty-four thousand  $F_1$  egg masses were released in the initial year. We calculated that this was equivalent to 13,600 "wild" egg masses (wild egg mass equivalents = W.E.M.E.) in terms of the numbers of adult males produced. The factors that were used to compute the W.E.M.E. are the number of eggs per mass, reduced  $F_1$  egg hatch, a skewed 2:1 male:female sex ratio, reduced  $F_1$  larval survival and asynchronous development. Egg masses were distributed in 1985 according to location and size of host trees. Based on all of the previously mentioned assumptions, the 1985 release density should, theoretically, have produced a 34:1  $F_1$  sterile male:wild male overflooding ratio. Based on the preliminary results of the initial release, an additional 12,769 egg masses (5,108 W.E.M.E.) were released in the spring of 1986, which should have produced an overflooding ratio high enough to effect eradication. The distribution of wild, immature insects located through sampling in 1985 was also used as an additional weighting factor in the 1986 release, i.e. more  $F_1$  egg masses were placed at these sites in 1986.

The impact of the release was monitored using a variety of techniques. To compare the proportions and synchrony of  $F_1$  and wild egg mass hatch, samples of both were held within the release area in a screened enclosure designed to prevent escape, but which maintained ambient temperature conditions. Larval hatch from individual egg masses was generally monitored daily. Theoretically, successful establishment and survival of  $F_1$  neonates would be reduced if hatch of  $F_1$ s was out of synchrony with host

development (Hough & Pimentel, 1978; Raupp et al., 1988).

Daily random samples of larvae and pupae were collected throughout the release area to provide material for monitoring developmental synchrony, survival, and overflooding ratios. Collected larvae were placed individually in 44.4 ml cups provisioned with artificial diet (Bell et al., 1981) and held in the laboratory until type (i.e., F<sub>1</sub> sterile or wild) could be determined. Male larval type was determined using two techniques: chromosome analysis and mating-egg mass evaluation.

A portion of the male larvae collected as first through fourth-instars was typed by the examination of sperm cells. Type was determined by scoring early metaphase cells for number of normal pairs of chromosomes (normally n=31) and presence or absence of translocations. Males were typed as wild if two cells were found with the normal numbers and appearance of chromosome pairs. To type a male as an F<sub>1</sub> required observing translocations in two cells.

Type of the second group of males (approximately half of all larvae collected as first through fourth-instars and all fifth-instar and male pupae) was determined by using a mating-egg mass evaluation technique. Field collected insects were reared in the laboratory until adult emergence. On the following day, one-day-old adult males were mated to similarly aged virgin laboratory-reared females. Resulting egg masses were held 30 days (25° C, 50%-60% RH) for embryonation and type determinations were based on the proportion of eggs which were embryonated. The proportion of embryonated eggs in each mass was compared using Chi-square techniques with mean values for mating type 2 and type 4 (Table 5) (Mastro et al., 1984).

Similar to the second group of males, all field collected female larvae and pupae were similarly held until adult eclosion and mated as one-day-old females to normal laboratory males. Resulting egg masses were held for the normal 30-day embryonation period and an additional 150 days (4-5° C) to satisfy diapause requirements. A determination of the female type was then based on percent hatch of the eggs. Characteristics of each egg mass were compared statistically to mean values for percent hatch for mating types 3 and 4 (Table 5). Holding egg masses produced by females of unknown type is necessary because F<sub>1</sub> females, when mated to normal males, produce highly embryonated egg masses often indistinguishable from egg masses produced by normal mating pairs.

Table 5. Characteristics of egg masses produced by incrossing and outcrossing  $F_1$  adult gypsy moths progeny of males irradiated (10 krad) as 6-11 day-old pupae mated with normal females.

Type	Mating type (male x female)	n <sup>1/</sup>	Mean proportion of eggs		Mean proportion of total eggs which hatched	
			embryonated	SE		SE
1	$F_1 \times F_1$	50	0.0910	0.0155	0.0012	0.0006
2	$F_1 \times$ Normal	52	0.2630	0.0380	0.0277	0.0104
3	Normal $\times F_1$	60	0.6711	0.0353	0.0098	0.0020
4	Normal $\times$ Normal	145	0.9374	0.0118	0.7653	0.0154

<sup>1/</sup> Number of mating pairs producing an egg mass.

Sterile  $F_1$ :fertile wild male mating ratios were monitored by placing one-day-old, virgin  $F_1$  females throughout the release area. In other studies, we found that normal laboratory-reared and  $F_1$  females attract and mate with wild males with the same periodicity and frequency as wild females (Mastro et al., 1987). Egg masses oviposited by these females were evaluated for male parental type based on the proportion of eggs which were embryonated (Table 5, mating types 1 & 3).

After the adult flight season, egg masses from the release area were collected and evaluated for mating type. Evaluations were based on the proportions of embryonated eggs and the hatch of total eggs (Table 5). These field collected egg masses could potentially be the progeny of all four possible mating types.

A 23.3 sq. km. area centered over the release area was trapped throughout the adult flight period with USDA high capacity milk-carton pheromone baited traps during both years of treatment. Traps were placed on a grid at a rate of 13.9 traps/sq. km. Trapping information was used to determine if the treated area was adequately isolated from other infested areas, and to provide estimates of the total adult male density during both treatment years.

#### Results and discussion of treatment with $F_1$ eggs

Hatch from samples of wild-type and  $F_1$  egg masses during the spring of 1985 was nearly synchronous. Wild egg masses were not available for monitoring in the spring of 1986 but samples of  $F_1$  egg masses appeared to hatch in synchrony with bud break of host trees. Wild egg masses in 1985 produced a mean of 279 larvae per mass while  $F_1$  egg masses produced a mean of 162 larvae.

Results of evaluation of 1985 male larval samples provided estimates of overflooding ratios close to those expected. Of the 466 male larvae that were typed using chromosome analysis, 458 were

F<sub>1</sub>s and 8 wilds, resulting in a 57:1 F<sub>1</sub>:wild ratio (Table 6). F<sub>1</sub> larval establishment and survival studies on host foliage have shown that most F<sub>1</sub> mortality occurs in the first stadium. Therefore, a better estimate of the eventual adult overflooding ratio would be based only on determinations of later stadia larvae. When only those insects collected after the first observation at the release site of a fourth stadium larva are considered, the F<sub>1</sub>:wild ratio was approximately 32:1 (254 F<sub>1</sub>s and 7 wilds).

In all, 693 field-collected males were reared to the adult stage and crossed with normal laboratory reared females for type determination. Evaluation of resulting egg masses disclosed that male parents of 677 of these samples were sterile F<sub>1</sub>s and 11 were fertile wilds. In 5 cases, the proportion of eggs embryonated was not different than mean proportions of embryonated eggs for either mating type (Table 5). If only the later collections of late stadia larvae and pupae are considered, as in males typed using chromosome analysis, the estimate of the overflooding ratio becomes approximately 80 F<sub>1</sub>:1 wild (n=483). Wild male larvae were found widely scattered throughout the release area, indicating that the native population distribution was well represented by trap captures the previous year.

Results from sampling and evaluating female larvae and pupae in the release site are consistent with results of male evaluations. Of the 372 field collected females reared to the adult stage and paired with normal laboratory-reared males, 309 produced egg masses which could be evaluated; 241 females produced egg masses characteristic of a fertile male x F<sub>1</sub> female mating, while 9 females produced egg masses characteristic of a fertile x fertile mating. Of the remainder, 49 egg masses contained all unembryonated eggs (i.e., possibly did not mate or had an F<sub>1</sub> male parent) and 12 females produced egg masses with characteristics which could not be distinguished from mean values of the two possible mating types. Computing a female overflooding ratio from these data results in an approximately 27 F<sub>1</sub>:1 wild ratio. Theoretically, the F<sub>1</sub>:wild female ratio in the field should be approximately half the male ratio because F<sub>1</sub> egg masses produce adults in an approximately 2:1 male to female ratio. The observed overall female F<sub>1</sub>:wild ratio in 1985 of 27:1 was approximately half of the observed male ratios arrived at by chromosome analysis (57:1) and mating-egg mass evaluation (62:1). These female data have not been separated to exclude early stadia larvae.

In 1985 monitor F<sub>1</sub> females produced 93 egg masses which could be characterized as being the result of a mating with an F<sub>1</sub> male (n=88) or a wild type male (n=5). The calculated overflooding F<sub>1</sub>:wild ratio is 17.6:1. An additional 734 egg masses could not be characterized because they contained all unembryonated eggs. Unembryonated egg masses can be the result of an F<sub>1</sub> male mating or no mating. Undoubtedly, some of these 734 egg masses were the result of an F<sub>1</sub> male mating, but it is impossible to separate these from egg masses produced by unmated females. In unpublished studies we have determined that mated females begin to oviposit

soon after mating (generally within an hour), while unmated females generally do not begin to oviposit until they are 3 days old or older. Unfortunately, we did not note when the monitor females had begun to oviposit in 1985. In 1986, we recorded if oviposition had commenced when the female was retrieved and if not, how soon afterward, in an attempt to separate females producing unembryonated egg masses into mated and unmated categories.

After the 1985 adult flight period, 63 egg masses were collected from within the release area. These egg masses could potentially be progeny of all four mating types. Of the 63 egg masses collected, 1 was determined to be from a (male x female) wild x wild mating, 7 from wild x  $F_1$  matings, 19 from  $F_1$  x  $F_1$  matings and 17 from  $F_1$  x unknown female matings. The computed mating ratios follow: sterile to fertile male 5.1:1 (n=43) and sterile:fertile female 26:1 (n=27). This female ratio agrees with the ratio from female larval and pupal collections. However, the male ratio is much lower than the predicted ratio or the ratios determined by larval sampling or monitor females. The difference between these male ratios is unexplained. Overall, the ratio of sterile egg masses (where at least one parent was an  $F_1$ ) to fertile egg masses (both parents fertile wilds) was 62:1.

Pheromone traps in 1985 captured a total of 869 males and 745 of these were captured within the release area. The remaining males were captured within ca. 0.5 km of the release site.

It was only possible to collect a small number of immature male insects (n=39) during the 1986 larval and pupal sampling period. Of these, 30 males could be evaluated. Five of these were dissected for chromosome analysis and all were typed as  $F_1$ s. The remaining males were reared to the adult stage and mated with normal females. Twenty-four produced egg masses characteristic of an  $F_1$  male parent and 1 produced an egg mass characteristic of a wild male parent. Based on all males, the  $F_1$ :wild ratio was calculated as 29:1.

Also, in 1986 twenty-six field collected immature females were reared to the adult stage. Mating of these females with normal laboratory males resulted in 14 egg masses on which a determination could be made. Thirteen of these egg masses were evaluated to be from  $F_1$  females and 1 was determined to be from a wild female.

Of the monitor  $F_1$  females placed in 1986, 116 of these produced egg masses determined to be the result of an  $F_1$  male mating. No monitor females produced an egg mass which could be characterized as the result of mating with a fertile male.

Trapping in 1986 resulted in 209 males captured within the  $F_1$  release area. This was approximately 28% of the males captured in the same area in 1985 (n=745). This roughly corresponds to the reduced numbers of  $F_1$ s released in 1986 (35% of the 1985 release). In all of the area trapped in 1986, 244 males were captured.

Based on 1986 results, no further release was made in 1987. Trapping in 1987 was carried out at the same trap density as in previous years, but the high-capacity USDA milk carton trap was replaced by the more efficient USDA delta trap. No males were trapped in 1987 or 1988.

A summary of monitoring this release site is presented on Table 6. Generally, the sterile F<sub>1</sub>:fertile wild male ratios observed were near the expected 34:1 overflooding ratio in 1985. The observed ratios from male chromosome analysis (57:1) and male mating egg mass evaluation (62:1) are not different at the 5% level of significance (Chi-square analysis). Also, ratios calculated from reduced data sets (i.e., using only later stadia insects for chromosome analysis (32:1) and mating-egg mass evaluation (80:1)) are not significantly different from ratios derived from their parental data sets or each other. Both of the overall male ratios computed from larval sampling are significantly different (5% level) from the ratio computed using monitor female egg mass evaluation (17.6:1) and post season egg mass evaluation (5.1:1). Ratios derived from these two evaluation techniques are also significantly different (5% level) from each other.

Table 6. Expected and observed results of releasing F<sub>1</sub> progeny of irradiated males (10 krad) in an isolated gypsy moth population in Bellingham, Washington.

Method of Estimation	Year			
	1984	1985	1986	1987
Expected spring wild egg mass density	-	400	8	0
Numbers of F <sub>1</sub> s released (W.E.M.E.)	0	13,600	5,108	0
Expected F <sub>1</sub> :Wild male ratio (Range)		34:1	640:1 (189:1 to 2,254:1)	
Observed F <sub>1</sub> :Wild <u>male</u> ratios				
1) Chromosome analysis				
A) All instars		57.3:1	5:0	
B) Later instars		31.8:1		
2) Mating egg mass evaluation				
A) All samples		61.5:1	24:1	-
B) Later instar & pupae		79.5:1		
3) Monitor female mating ratio				
		17.6:1	116:0	-
4) Post season egg mass evaluation				
A) Male ratio		5.1:1	no data	-
E) Overall ratio		(62:1)		
Observed F <sub>1</sub> :Wild <u>female</u> ratios				
1) Mating egg mass				
		27:1	13:1	-
2) Evaluation of post season egg masses				
female ratio		26:1	no data	-
No. of males trapped	82	869	244	0
Estimated male population	400	9,549	2,681	0

At the beginning of the 1986 field season, an estimate of the residual wild population was needed to determine the number of F<sub>1</sub> egg masses to be released to achieve eradication. From pheromone trap catches, we estimated that the 869 males trapped represented 9.1% of the total male population (i.e., 9,549). Only three estimates of male overflooding ratios in 1985 were available at the time of the 1986 egg release (i.e., male chromosome and mating-egg mass evaluations and monitor female-egg mass evaluation). Based on these ratios (ranging from 17.6:1 to 79.5:1), we calculated that the wild male population could have been between 119 and 513 males (i.e., 1.25 to 5.38 percent of 9,549 males). Again, we made the assumption of a 1:1 sex ratio in the wild population and that F<sub>1</sub> males were completely competitive. The number of successful wild mating pairs in 1985 was calculated to be from 2 to 27, respectively, for F<sub>1</sub>:wild mating ratios of 17.6:1 and 79.5:1. We believed that the estimated mating ratio of 17.6:1 provided by monitor female data was too low for previously mentioned reasons and the true mating ratio was nearer 34:1 or higher. When the appropriate calculations are made using a 34:1 F<sub>1</sub>:wild male ratio, wild egg density in the spring of 1986 was estimated at 8 wild egg masses. We released 5,108 F<sub>1</sub> W.E.M.E. which, if the wild egg mass population in the spring of 1986 consisted of 8 egg masses, would provide a male overflooding ratio on the order of 640 F<sub>1</sub>:wild male.

In sparse gypsy moth populations immatures are difficult to locate and we were only successful in locating a small number in 1986. Based on all males collected as immatures and typed (both techniques) the male ratio was 29 F<sub>1</sub>:1 wild. Monitor female data provided a much higher estimate of the sterile male overflooding ratio (116:0). It was mentioned earlier that in 1986 egg mass release was weighted at known sites of wild insects. Sampling was concentrated around these same sites because immatures could only be located at these sites. Monitor females, however, were distributed as in 1985 (i.e., generally throughout the area). A ratio based on assessment of immature males, because of our skewed sampling, could be biased. Monitor female data may provide a better estimate of the ratio throughout the release area.

We conclude that the F<sub>1</sub> egg mass release in Bellingham, WA, was successful in eradicating the native population. Two years of negative trap survey data is generally considered necessary for confirming the outcome of an eradication attempt. The evidence of successful interaction with, and suppression of the native population is apparent.

In other studies in isolated sites and within the generally infested area, we have not always achieved predicted overflooding ratios and results do not appear as clear cut. These results suggest that we need to develop better techniques for estimating native insect density and distribution. Several investigators are currently exploring the utility of pheromone-baited traps for monitoring populations in the generally infested area, as well as techniques for estimating densities of other life stages (Liebhold & Elkinton, 1980ab). We are also far from a clear understanding

of how, compared to wilds,  $F_1$  immatures survive and develop in the field. Several ongoing studies are exploring the comparative behavior, relative egg viability, immature development and survival of  $F_1$ s. Also, the possible impacts of parasites and predators on  $F_1$  egg releases are unknown. In some field release sites, we have noted rapid disappearance of late instar larvae and pupae, and although the overall population density was lowered, we do not know how this precisely affected the sterile:wild ratio. Propensity of  $F_1$  males, as described earlier, to multiple mate on the same day has been shown to be lower than wild males. The impact of this competitive deficiency on the actual mating ratio under field conditions is still to be determined.

Studies have been initiated to explore the impact of female multiple mating on viability of resulting egg masses. It was generally believed that female gypsy moths are monandrous, however, in unpublished laboratory studies, we found 15% of the females mated twice. If mating with an  $F_1$  male does not elicit the normal monocoitic response, or if there is sperm precedence in multiple-mated females, then the  $F_1$  male competitiveness could be adversely affected. A better understanding of these areas of exploration should enable us to apply the technique more effectively and interpret our field results more clearly.

Results from the Bellingham site and other sites where  $F_1$  eggs have been released have also posed several problems which must be addressed: 1) how to predict when native hatch will occur and how to time  $F_1$  egg mass release so that hatch is synchronous, 2) what is the impact of dispersal (neonate and adult) on the overflooding ratios, and 3) how does egg distribution within a site affect survival.

Evaluation techniques used in this study and other similar studies are labor intensive and require that the insects be held for a period of time before a determination of type ( $F_1$  or wild) can be made. A method to rapidly discriminate between  $F_1$  and wild insects would allow a more intensive evaluation of releases, lower the cost of evaluation and provide a valuable tool for the ongoing research described previously. Some techniques under investigation which would provide for a rapid discrimination between wild and  $F_1$  insects, have a genetic basis (i.e. larval setal patterns, morphometrics of adult male wing venation) and others, such as paternity assays may depend on identification of damage induced by radiation.

We believe that the  $F_1$  technique is at present a viable technique that can be operationally useful for treating isolated gypsy moth infestations. Furthermore, we believe that the technique may be potentially useful for management of gypsy moth populations within the generally infested area. This possibility becomes more attractive when considering  $F_1$ s that are progeny of males receiving lower (6 or 8 krad) doses of radiation. Finally, all the investigations associated with the technique have led to a better understanding of the biology and behavior of the insect.

With a concentrated effort to resolve outstanding monitoring and competitiveness questions and additional efforts to improve laboratory colonization, the sterile egg technique can be operationally used within a short period of time.

An as yet unexplored potentially beneficial area for control of the gypsy moth is the use of other genetic control techniques reviewed by Whitten & Foster (1975). Downes (1959) suggested that, based on the work of Goldschmidt (1931, 1933) males from different geographic races of gypsy moth could be used in a control program. Both Downes and later Leonard (1974) recognized that the Japanese genome would enter the North American population, and potentially impart some factors which would make it more successful or destructive. However, no experimental work was initiated to explore the possibility of using this type of sterility.

An exciting possibility is the use of backcross sterility, which was originally described by Laster (1972), if it could be found in the gypsy moth. Although the genetic basis for backcross sterility is poorly understood, male sterility is associated with abnormal eupyrene sperm formation which is not transferred to females (Goodpasture et al., 1980; La Chance, 1984). This system offers a number of advantages over the  $F_1$  sterile insect technique. Foremost, is that theoretically, once the sterility factor is introduced into a population it can persist indefinitely. Although this technique has not been explored for the gypsy moth, a number of closely related old world species, including Lymantria obfuscata, L. mathura, and L. monacha, offer potential candidates for investigation. Although it is impossible to predict if backcross sterility will be found with the gypsy moth, at least one of these species, L. obfuscata, will hybridize (Lenek, 1974). The advantages and potential benefits appear to be so great that the effort and expense of examining backcross sterility in gypsy moth appears well justified.

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OVIPOSITION SITE SELECTION  
BY JAPANESE LYMANTRIID MOTHS

Yasutomo Higashiura, Hokkaido Forest Experiment Station,  
Ecology Lab., Shintoku, Tokachi, Hokkaido 081, Japan

INTRODUCTION

Oviposition site selection is one of the most important habitat selection in insects. As for the oviposition of the gypsy moth, Lymantria dispar L., overwintering egg masses have been shown to be protected against avian predators (Higashiura, 1980; Schaefer 1981) and low winter temperatures (Leonard, 1972) by the insulative effect of snow. Thus, habitat suitability for oviposition is higher beneath the snow than above (Coulson & Witter, 1984). However, more egg masses are deposited above the eventual snow line than beneath (Leonard, 1972; Higashiura, 1980). To solve this puzzling distribution, I present a conceptual model of oviposition site selection by using the fitness set approach of Levins (1968). The prediction by the model is tested in comparison of oviposition sites by Japanese lymantriid moths.

Predation on lepidopteran eggs is thought to be small, and so relatively little is known about it (Torgersen & Mason, 1987). The egg masses of the gypsy moth is, however, known to be subject to high avian predation (Higashiura, 1980; Brawn & Cameron, 1982). During 1974-83, I estimated the bird predation on the egg mass to reveal the habitat suitability in oviposition of the gypsy moth in a snowy region, Hokkaido northern Japan.

Finally, I present a control method of gypsy moths utilizing the strategy of the oviposition site selection by the moths.

THE MOTH

Pintureau (1980) treated Japanese gypsy moths as two species, Lymantria dispar hokkaidoensis Goldschmidt for Hokkaido populations, and L. japonica Motschulsky for Honshu populations. Here, however, I treat the two species as the same, L. dispar L., in examination of their behavior in oviposition, because both females can fly during oviposition site selection as in other Asian populations (Goldschmidt, 1934).

Adults of the gypsy moth emerge between July and September. A female moth deposits only one egg mass. Larvae hatch during the following April and May. The eggs withstand the winter low temperatures and are rarely attacked by egg parasites in Japan (Schaefer, 1981).

## METHODS

During 1974-83 I studied bird predation on gypsy moth egg masses in a birch, *Betula platyphylla* Sukatchev, forest at Bibai, Hokkaido, northern Japan (Fig. 9). Three study plots (Plots 1-3) neighboring each other were established in autumn 1974. The plots were 0.51 ha, 0.52 ha, and 0.52 ha. Plot 4 (0.63 ha) was added in autumn 1975. Plot 1 was reduced to 0.18 ha in May 1980 and disappeared in May 1982. The density of canopy layer trees was 1096/ha. The birch accounted for 57% of them. During the study period, almost all egg masses (1077) were deposited on bark of the birch as in the previous studies (Schaefer, 1978; Higashiura, 1987), only two egg masses being laid on *Sorbus commixta* Hedle.

The predator left characteristic scratches on the bark surface wherever egg masses had been removed (Higashiura, 1980). The scratches made it possible to distinguish egg masses eaten by birds from those physically damaged by the compression of accumulating snow.

Predation rate was visually estimated for a single egg mass to seven grades, i.e. 0, 10, 30, 50, 70, 90, and 100%, in early May just before larval hatching. The mean predation rate in each plot, or in each height class, was obtained by averaging these visually estimated predation rates of all egg masses. Because the mean predation rate hardly differed among egg-mass sizes (Higashiura, 1980), egg-mass size

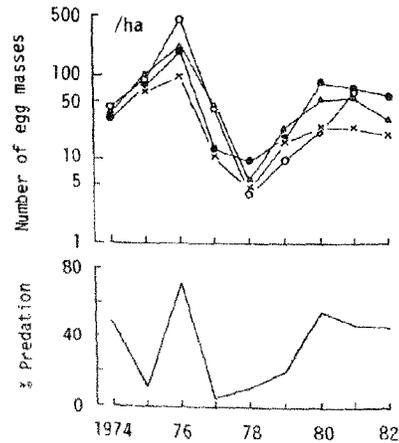


Figure 1. Population trends in egg masses in autumn (upper) and changes in predation rate on egg masses by birds (lower) at Bibai. Predation rate during the winter was plotted in the year of oviposition. ○: Plot 1, ●: Plot 2, △: Plot 3, ×: Plot 4.

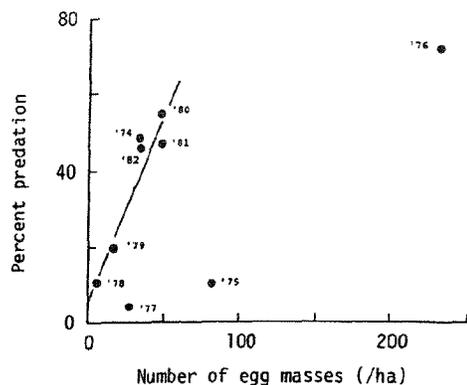


Figure 2. Predation rate in relation to initial egg mass density over the entire study area at Bibai. Suffix numbers denote the year of oviposition. The relationship was significantly density-dependent in the latter five winters, winters 1978-1983 ( $y=5.80+0.98x$ ;  $r=0.965$ ;  $P<0.01$ ). The straight line shows this regression.

is not taken into account here. Sample sizes from winter 1974-83 were 56, 179, 503, 59, 13, 36, 88, 89, and 56 egg masses, respectively. During winter 1980-81, predation rates were estimated at intervals of one to four days to reveal the progression of bird predation. In each year, during September, the aboveground height of every egg mass in the study plots was recorded except in 1974. Additional studies of predation were carried out at Asahikawa, Hokkaido in winter 1982-83 using the same methods.

The aboveground heights of egg masses were measured in seven stands in five regions of Japan by January (Fig. 9). The heights of the tree and of the lowest branch were recorded for each tree having eggs. At Bibai and Asahikawa the two heights were measured for each of ten study trees.

## RESULTS

Population trends in egg masses, or in ovipositing females were similar in the four plots, though there were considerable differences in the density among plots (Fig. 1). Mean density over all plots varied from 6 /ha in 1978 to 231 /ha in 1976.

Predation rate by birds in the plot also varied considerably, between 0% and 84%, the latter occurring in Plot 4 in winter 1976-77.

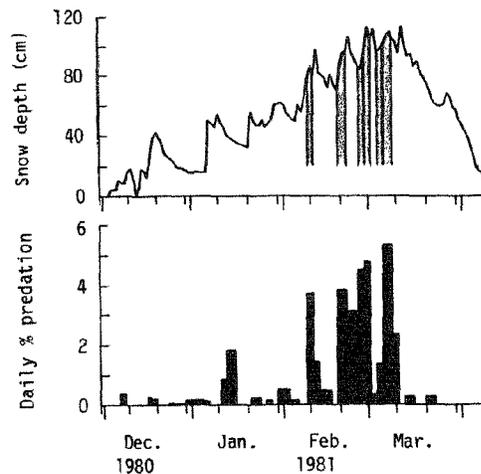


Figure 3. Snow depth (upper) and daily predation rate on egg masses above the snow (lower) in winter 1980-81 at Bibai. Stippled bars in the upper correspond to periods of increase in the daily predation rate between 9 February and 9 March 1981.

The most common predator was the nuthatch, *Sitta europaea baicalensis* Taczanowski, recognized from direct observations and from the characteristic scratches left from their beaks and claws. Other predatory species were *Parus palustris hensoni* Stejneger, *P. major minor* Temminck & Schlegel, and *Garrulus glandarius pallidifrons* Kuroda. The mean predation rate during the nine generations was 38.8% in the geometric mean. The percentage of egg masses physically damaged by the compression of accumulating snow was negligibly low; 8.8% in winter 1982-83 was the highest during the study period.

Figure 2 shows the relationship between egg mass density averaged over all plots (/ha) and predation rate by birds. There was no significant relationship during the nine years of the study ( $r=0.589$ ;  $P>0.05$ ). However, a highly significant relationship was observed through the latter five winters, winter 1978-79 to winter 1982-83 ( $r=0.965$ ;  $P<0.01$ ). During the other four winters 1974-78, there was no significant relationship ( $r=0.706$ ;  $P>0.2$ ).

The daily percentages of egg masses eaten by birds are shown in Figure 3 for winter 1980-81. Since attacking egg masses beneath the snow was impossible for any birds, I calculated the percentage only for egg masses above the snow. Moreover, I assume that birds maintain a constant predation rate during the interval.

Predation rate was 54.7% in the winter, the second highest during

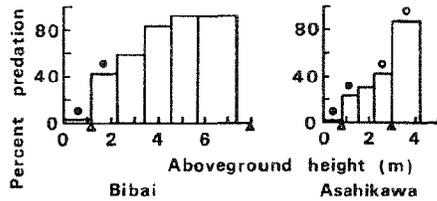


Figure 4. Vertical distributions of mean predation rate at Bibai in winter 1980-81 (left), and in a *Larix* forest at Asahikawa in winter 1982-83 (right). Open and closed triangles show heights of maximum snow depth in the winter and of the lowest branch, respectively. Open and closed circles indicate the significant difference between the adjacent two height classes at the 1% and 5% levels, respectively (the Tukey-Kramer method using the arcsine transformation).

the nine winters. In this winter of high predation, birds attacked egg masses mostly in February and March, when the snow depth was highest of the year, and never attacked any egg mass after March when the snow began to melt. Figure 3 also suggests that snow accumulation intensified predation in February and March.

Since predation was concentrated in snowy season, the height at which the egg mass was laid on a tree affected mortality from bird predation at Bibai and Asahikawa (Fig. 4). Predation rate beneath the snow was significantly lower than just above the snow (the Tukey-Kramer method,  $P < 0.05$ ; Sokal & Rohlf, 1981). Although percent predation gradually increased with height above the snow, the most intensive predation was concentrated above the lowest branch at Asahikawa even in the year of 23% predation (Fig. 4). At Bibai, females rarely oviposited above the branch.

Table 1. Maximum snow depth and mean height of egg masses at Bibai, Hokkaido.

Year of oviposition	1975	1976	1977	1978	1979	1980	1981	1982
Maximum snow depth (cm)	116	156	99	129	90	114	92	99
Mean height of egg masses (cm: 95% C.L.)	187 +16	249 +15	265 +56	233 +90	248 +68	298 +41	271 +37	229 +35

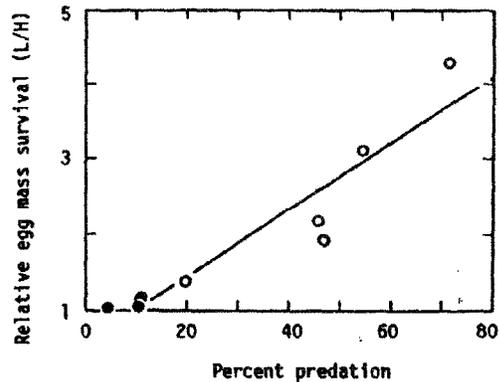


Figure 5. Correlation between relative egg mass survival and percent predation measured from overall egg masses at Bibai in winters 1975-83. Correlation was highly significant ( $y=0.569+0.044x$ ;  $r=0.939$ ;  $P<0.001$ ). Relative egg mass survival (L/H) is expressed by dividing the proportion of egg masses surviving beneath the snow (L) by that above the snow (H) (snow height used was the average maximum through the eight winters, or 112 cm). Open and closed circles indicate that the difference of the proportion of egg masses surviving between the two height classes (L and H) was statistically significant ( $P<0.01$ ) and insignificant ( $P>0.1$ ), respectively (t-test using the arcsine transformation).

The insulative effect of snow can be demonstrated more obviously by the survival of the egg masses beneath the snow (L) relative to those above the snow (H). Throughout the eight winters at Bibai, relative survival (L/H) increased with average percent predation on egg masses (Fig. 5). Oviposition beneath the snow had the advantage of producing high egg mass survival in five of eight winters. However, survival approached equality in the year of low predation.

Since snowfalls at Bibai did not vary so much (Table 1: C.V. in maximum snow depth was 20%), inferences from mean snowfall values accurately indicated above or beneath the snow.

The population density in larval and egg stages is correlated with oviposition heights in Europe (Luciano & Prota, 1981). At Bibai, however, mean height of egg masses were not different among years during 1976-82 (Table 1:  $P>0.25$ ), irrespective of egg density. Significant differences were observed only between the height in 1975 and others ( $P<0.05$ ), except between 1975 and 1978 and between 1975 and 1979 ( $P>0.1$ ).

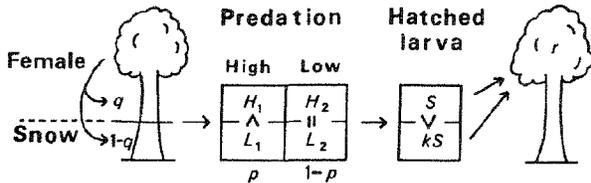


Figure 6. Two factors, winter predation and cost of larval dispersion, affect oviposition site selection by the gypsy moth as assumed by the model.  $q$  denotes the proportion, or probability of females ovipositing above the eventual snow line. Egg masses above the snow have much lower survival than those beneath the snow in the year of high predation. In the year of low predation, however, the proportion of surviving egg masses above the snow is almost the same as beneath the snow. Probability of high predation is  $p$ . Larvae hatched on the lower site experience lower survival ( $kS$ :  $0 < k < 1$ ). The rate of increase ( $r$ ) after larval dispersion is not affected by hatching sites.

#### Model of oviposition

The data presented above showed that oviposition beneath the snow was advantageous in snowy regions. In the year of low predation, however, egg mass survivals were almost the same above and beneath the snow. The question that arises is, why do not all females oviposit beneath the snow in snowy regions? In this section I present a conceptual model, based on the fitness set approach of Levins (1968), which suggests that a mixed strategy of ovipositing both above and beneath the snow is optimal in unpredictably changing environments.

Ovipositing in higher locations is thought to be advantageous for hatched larvae. Newly hatched larvae of the gypsy moth move up trees. As they move, they spin a thread of silk without feeding on any host leaves (Mason & McManus, 1981). Therefore, larvae from egg masses deposited lower must crawl longer distances and spin a longer thread to disperse or reach a food source. However, during years of high predation these larvae gain the benefit of high survival during the egg stage beneath the snow. In winters of low predation, the same proportion of egg masses survived beneath the snow as above the snow in three of the eight winters (Fig. 5). Natural selection favours ovipositing above the snow in this case, though ovipositing above the branch results in high predation on the egg masses even in the year of low predation in the snowy region (Fig. 4). Females should oviposit beneath the snow only in years of high predation, or in years when survival differs above vs. beneath the snow. However, since oviposition precedes predation, the predation stress makes the environment unpredictable and changing for the adult females. Our aim is to find the proportion, or probability, of oviposition above the snow,  $q$ , that

maximizes the long-term expectation of fitness, i.e., the optimal oviposition strategy.

I assume two types of temporally varying environments, environment 1 (in which predation is high) and environment 2 (low predation), and two sites for oviposition (above and beneath the snow) (Fig. 6).

Table 2. Definitions of symbols in the model: H and L denote the proportion of egg mass survivals above and beneath the snow, respectively.

Symbols	Definitions
$H_1$	H in the year of high predation (environment 1)
$L_1$	L in the year of high predation (environment 1)
$H_2$	H in the year of low predation (environment 2)
$L_2$	L in the year of low predation (environment 2)
S	Survival rate of larvae hatched from the higher site
$kS$	Survival rate of larvae hatched from the lower site ( $0 < k < 1$ )
r	Rate of increase after dispersion

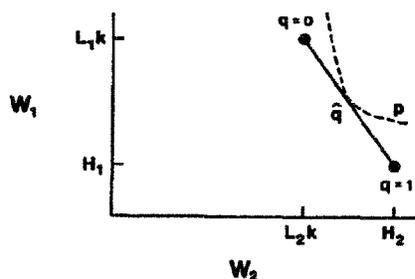


Figure 7. A fitness set analysis of the strategy of oviposition site selection by the gypsy moth. The point at  $q=1$  represents females ovipositing above the eventual snow line. They have very different survival rates in the two environments. While the point at  $q=0$  represents females ovipositing beneath the snow line, survival rates are almost the same in these forms.

Definitions of symbols in the model are given in Table 2. Then the adaptive function in environment 1 ( $W_1$ ) is

$$W_1 = [qH_1S + (1-q)L_1kS]r,$$

and the adaptive function in environment 2 ( $W_2$ ) is

$$W_2 = [qH_2S + (1-q)L_2kS]r.$$

Since the moth is univoltine and of discrete generations, the alternative environments are "coarse-grained" (Levins, 1968), so that the average fitness ( $\bar{W}$ ) can be estimated as the geometric mean of the fitness in each environment according to its probability ( $p$  for environment 1, and  $1-p$  for environment 2) (Levins, 1968).

$$\log \bar{W} = p \log W_1 + (1-p) \log W_2$$

Setting  $d \log \bar{W} / dq = 0$ , the optimum  $\hat{q}$  is calculated as

$$\hat{q} = k \frac{L_1(H_2 - L_2k) - p(H_2L_1 - H_1L_2)}{(L_1k - H_1)(H_2 - L_2k)} \quad (1)$$

This fitness set analysis is shown in Figure 7. Since the value  $\hat{q}$  must lie between 0 and 1, we must adjust it as follows.

$$\text{If } p \geq \frac{L_1(H_2 - L_2k)}{H_2L_1 - H_1L_2}, \text{ then } \hat{q} = 0.$$

Thus, if there are many years of high predation, natural selection favours always ovipositing beneath the snow.

$$\text{If } p < \frac{H_1(H_2 - L_2k)}{k(H_2L_1 - H_1L_2)}, \text{ then } \hat{q} = 1.$$

Thus, if there are many years of low predation, selection favours always ovipositing above the snow.

If  $p$  lies between those values mentioned above, then the optimum  $\hat{q}$  equals the  $\hat{q}$  calculated in eqn (1). Thus the mixed strategy is optimal. This strategy corresponds to the oviposition site selection at Bibai.

#### Tests of the model

Costs for larvae hatched from lower egg masses,  $k$  in eqn (1), have been included in the model, because the larva must spin a longer thread of silk without feeding. The height of the lowest branch is

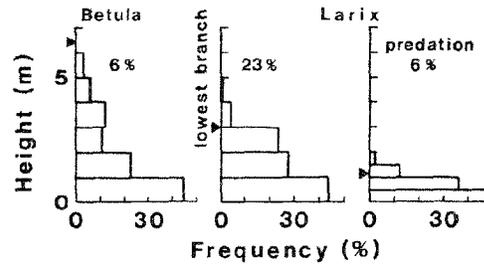


Figure 8. Oviposition sites by the gypsy moth in relation to the height of the lowest branch at Asahikawa, Hokkaido in autumn 1982. Triangles show the mean height of the lowest branch. Predation rates of egg masses in winter 1982-83 are also shown. Left: *Betula* plantation. The mean tree height was 16.0 m. Sample size was 97 egg masses. Middle and Right: Two forests were in the same *Larix* plantation. The mean tree height was 9.8 m. Branches below 3 m were pruned in the middle forest. Sample sizes in the middle and right were 177 and 86 egg masses, respectively.

important in this case. Since hatched larvae can easily reach a food source on trees branching out beneath the snow line, oviposition beneath the snow line should be suitable for both egg mass and larval survivals.

The gypsy moth at Asahikawa (snowy region) deposited her eggs beneath the branch, irrespective of the heights of branches (Fig. 8). Egg masses were distributed to higher sites on trees without lower branches than those on trees having lower branches. Predation rate was lower in the later forest because of snow protection. The maximum snow line was 83 cm in the winter. Predation rate was also low in the *Betula* forest. Some birds, even the nuthatch, slipped off the smooth surface of birch bark during predation, their claws leaving slip marks on the surface. Once they slipped down, they left the tree for another.

Highest predation occurred during the snowy season, February and March; snowfalls accelerated predation (Fig. 3). Consequently, differences in both predation pressure and the insulative effect of snow against predation should be expected in southern populations where snowfall is lighter. Oviposition site selection in northern and southern Japanese populations should reflect these differences.

Oviposition sites were considerably different among geographical regions (Fig. 9). Egg mass distribution is represented by relative aboveground heights (letting the height of the lowest branch equal one, and the top of the tree equal two) in Figure 9. Using the relative height, we can compare the distributions above and beneath the lowest branch and also above and beneath the snow, irrespective the

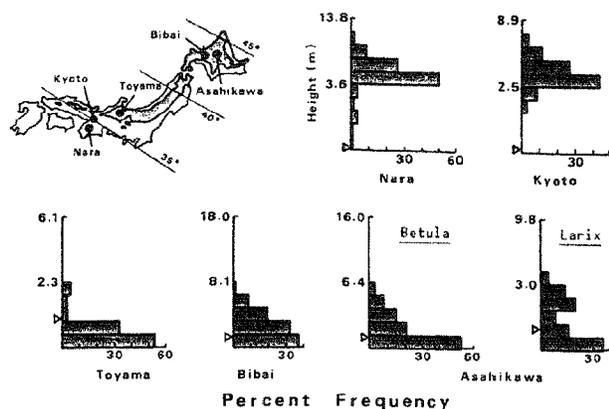


Figure 9. Geographical variation of the oviposition site in Japanese gypsy moth. The abscissa shows the frequency distribution of egg masses. The ordinate represents the aboveground height relative to the heights of the lowest branch and of the top of tree. Both average absolute heights are shown, each interval being divided into five classes. Stippled area in the map shows the region having snow depths over 1 m. The average maximum snow depths (1976-83) are shown by triangles. Snow is rare at Nara and Kyoto, where snow temporarily covers the ground for 5 and 11 days each year, respectively. In contrast, the other sites are characterized by heavy snow: snow lies for 64 days (Toyama), 132 days (Bibai), and 146 days (Asahikawa) through the year. Sample sizes were 75 egg masses on 20 trees (mainly *Zeikova serrata* and *Sarix* spp.) at Nara, 67 on 21 trees (*Prunus* spp. and *Quercus glauca*) at Kyoto, 54 on 33 trees (*Magnolia obovata* and *Ilex macropoda*) at Toyama, 503 on 288 trees (*Betula platyphylla*) at Bibai, 97 on 28 trees (*B. platyphylla*) at Asahikawa, and 177 on 81 trees (*Larix leptolepis*) at Asahikawa.

absolute heights of trees and branches. I compared frequency distributions of egg masses between the two forests in Figure 9 using the G-test (Sokal & Rohlf, 1981). Of the fifteen pairs, the distributions were significantly different in thirteen pairs ( $P < 0.05$ ), and were not significantly different in only two pairs (Kyoto and Nara,  $G = 1.105$ , d.f.=3,  $0.75 < P < 0.90$ ; Toyama and the *Betula* forest at Asahikawa,  $G = 5.366$ , d.f.=2,  $0.05 < P < 0.10$ ).

In the regions with light snow, Nara and Kyoto, almost all egg masses were deposited in tree canopies, frequently on undersides of branches. Low predation should be expected in these regions, or p is

small. In contrast, females oviposited mostly beneath the snow at Toyama, where snow depths reached 96 cm on an average and were most variable during each year. The largest p should be expected. Egg masses were deposited both above and beneath the snow at Bibai and Asahikawa, where predation rate considerably fluctuated between years (Figs. 1 & 5).

Oviposition sites in Japanese Lymantriid moths are mainly divided into two sites (Table 3). Species overwintering in larvae oviposit on undersides of host leaves, except *Leucoma candidum*. In contrast, species overwintering in eggs oviposit on tree trunks, except *E. pseudo-conspersa*. The most interesting selection for oviposition site is observed in *Orgyia thyellina*. Female moths, which are bivoltine, switch oviposition sites from undersides of host leaves for nondiapauses eggs to tree trunks for diapauses eggs (Kimura & Masaki, 1977).

#### DISCUSSION

In the present study, the mean predation rate on the egg mass was 38.8% during the nine years, although this fluctuated considerably. The highest predation (84%) was observed in Plot 4 in winter 1976-77. These high frequencies suggest that bird predation on the egg mass is as important a mortality factor as parasites are in the egg and larval stages. Although predation clearly showed a density-dependent trend in the later five winters, the trend was ambiguous during the nine study period. Thus females cannot predict predation pressure through their density.

Eggs of the moth are covered by a dense coating of hairs, sloughed from the female abdomen. The hair covering is believed to provide some protection against bird predation (Leonard, 1981). The behavior of birds attacking the egg mass suggests that it is unpalatable. Birds ate the egg mass not wholly at once, but bit by bit (Higashiura, 1980). Egg masses were eaten mainly during winters with heavy snowfalls (Fig. 3), which is when adult nuthatches experience the highest mortality from starvation (Nilsson, 1982). Nuthatches may be feeding on the egg mass simply to avoid starvation during the deep snow cover that prevents them from feeding on their favorable foods.

During the intensive predation period, egg masses deposited under the snow line survived from the predation. Thus, in snowy regions, habitat suitability for oviposition was higher beneath the snow than above. Egg mass survival beneath the snow was 1.9-4.3 times higher than above the snow in years of high predation, four out of eight years. However, survival was almost the same on both sites in the three years of low predation. Thus bird predation created unpredictably changing environments for ovipositing female gypsy moths. In contrast, height suitability was suggested to be different in regions with little snowfall: not only were egg masses rarely attacked by birds, but egg masses suffered predation at all heights. Egg mass distributions at Nara and Kyoto are thought to reflect these differences of predation pressure. There are three strategies for oviposition site selection shown in Figure 9: oviposition in tree canopies (Nara & Kyoto), oviposition beneath the snow (Toyama), and oviposition

Table 3. Oviposition sites in Japanese lymantriid moths.

Species	Host	Stages in overwintering	Oviposition site		References
			Diapause eggs	Non- diapause eggs	
<u>Cifuna locuples</u> Walker	D	Larva		L	Mutuura et al., 1965
<u>Calliteara abietis</u> (D. & S.)*	E	Larva		L	Matsushita, 1943
<u>Euproctis similis</u> (Fuessly)	D	Larva		L	Mutuura et al., 1965
<u>E. subflava</u> (Bremer)	D	Larva		L	Matsushita, 1943
<u>E. pseudoconspersa</u> (Strand)	E	Egg	B+L	B+L	Mutuura et al., 1965
<u>Orgyia recens</u> (Hübner)	D	Larva		L	Higashiura, unpublished
<u>O. thyeellina</u> Butler	D	Egg	T	L	Kimura & Masaki, 1977
<u>Leucoma candidum</u> Staudinger	D	Larva		T	Sirota et al., 1976
<u>L. salicis</u> (L.)	D	Larva		T	Burgess & Crossman, 1927
<u>Lymantria mathura</u> Moore	D	Egg	T		Matsushita, 1943
<u>L. fumida</u> Butler	E	Egg	T		Matsushita, 1943
<u>L. monacha</u> (L.)	D+E	Egg	T		Matsushita, 1943
<u>Ivela auripes</u> (Butler)	D	Egg	T		Matsushita, 1943

Species: (D. & S.)\*=(Denis & Schiffenmüller)  
 Oviposition site: L=Leaf, B=Branch, T=Trunk  
 Host: E=Evergreen, D=Deciduous

both above and beneath the snow (Hokkaido). Although the model is an oversimplification, it predicts these three strategies.

Japanese Lymantriid moths mostly deposited nondiapause eggs on host leaves, but diapause eggs on tree trunks. *Orgyia thyellina* use the two site each for the egg. Tauber, Tauber & Masaki (1986) have regarded such the use of oviposition sites for diapause eggs as a strategy for depositing eggs on permanent sites. However, avoidance of winter predation on eggs accounts more precisely for the behavior, because the oviposition site is on considerably lower parts of tree trunks (never on twigs). Other members depositing diapause eggs also use the lower parts, except *Euproctis pseudoconspersa* and *Lymantria fumida*. Reversely, depositing nondiapause eggs on leaves is probably to avoid the cost of dispersion, because the egg stage of nondiapause eggs is too brief to be attacked. For univoltine species, such as the gypsy moth, females should select oviposition sites at once to balance both costs of predation and dispersion in long-term expectation.

In the argument mentioned above, the height of the lowest branch is most important for the gypsy moth. In forests having branches near the ground, oviposition near the ground should satisfy both egg and larval survivals. Outbreaks of the gypsy moth frequently occurs in young larch, *Larix leptolepis* (Sieb. et Zucc.), plantations, just after canopy closure. Shinohara & Higashimura (1982) reported that the outbreaks in larch plantations at Iurano, near Asahikawa (Hokkaido), occurred only in 6-18 years old plantations. Egg masses were deposited in higher sites due to pruning of the lower branches (fig. 8). The environment in the forest is changed to be like in the older by pruning, and reduces both egg and larval survivals.

Pests in man-made forests are selected through adaptations to the environment created in such forests. Control methods utilizing their life history strategies are one of the most powerful methods against these pests.

I am grateful to the late I. Hirata who suggested the study of this problem and helped me on the field work at Nara. I am indebted to my colleagues at Bibai and Shintaku, especially to S. Yamaguchi. I am also grateful to J.L. Bronstein for her helpful comments to improve early drafts and correct the English.

#### SUMMARY

Mean predation rate on gypsy moth egg masses by birds was estimated at 38.8% during nine years at Bibai, Hokkaido, Japan. The predator, mainly the nuthatch, attacked egg masses during a snowy season, February and March. Thus oviposition beneath the eventual snow line had the advantage of producing high egg mass survival only in the year of high predation. Since predation rate varied considerably, the predation created changing environments for females. The model of optimal oviposition strategy was presented by using the Levins model. The prediction of the model was consistent with the geographical variation of oviposition sites in Japanese gypsy moth and in Japanese Lymantriid moths.

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