# Biological Control Introductions as Grandiose Field Experiments: Adaptations of the Cinnabar Moth to New Surroundings

by
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While long term studies of insect populations are common (see Dempster 1975), these studies seem to have lead to little generality (to the species in other locations) or predictabality (to the succeeding years). An example is the winter moth study of Varley and Gradwell (Varley, Gradwell and Hassel 1973) which showed that in England the parasites *Cyzenis* and *Agrypon* had little effect on moth population density. However, the introduction of these parasites as biological control agents for the winter moth proved very successful. Therefore, in this case a long term population study did not predict which natural enemies would be successful biological control agents, i.e., the conclusions weren't general.

I feel that the lack of experiments in insect population studies has been responsible for the current state of affairs (see also Kitching 1976). One group of entomologists, however, have been setting the stage for experimental insect studies. Every time an insect introduction is made for biological control of weeds, we have the potential for an exciting experiment. Unfortunately this situation has been rarely exploited by the insect population ecologist.

The basic belief behind my investigations is this. If we can identify the characteristics of an insect species which are adapted to different environmental conditions, we will have pin pointed the biological aspects of the insects which are important generally to the population regulation mechanism. The specific mortality factors will vary from place to place and so enumerating them will not yield a general understanding of the population dynamics. However, by studying the same species in different localities, and by observing adaptation of introduced populations we can identify the biological aspects of the species which provide the potential for population regulation.

The Cinnabar moth *Tyria jacobaeae* is an ideal candidate for work following this approach. It has been studied extensively in its native range (England - Dempster 1971a, b, 1975 and the Netherlands - van der Meijden 1971, 1976 and this volume) and has been introduced to five areas in North America as a biological control agent for tansy ragwort, *Senecio jacobaea.* These areas are described by Myers and Campbell (1976). I will consider here some of the adaptations which the introduced populations have undergone and attempt to relate them to a previously proposed model of population regulations (Myers and Campbell 1976).

## GENETIC ADAPTATION — INTERPOPULATION MATING SUCCESS

To demonstrate adaptation in introduced populations of Cinnabar Moth, one must first determine if genetic change has occurred. As a first attempt at this, I determined if genetically isolated populations from England, Europe and North America could still interbreed (Table 1). Even though the sample sizes were very small, some pairs of all categories of crosses from European and North American populations produced viable eggs. Although further investigations might demonstrate reduced mating success among interpopulation pairs, I conclude that genetic changes among populations have not been sufficient to cause infertility.

Table 1. Proportion of pairs which mated successfully as indicated by laying eggs and as the proportion which produced eggs and the eggs hatched. Number in each group in parenthesis. Pairs of animals were placed in cardboard containers with a bouquet of tansy ragwort. Crosses were made between animals from Weeting Heath, England; Leiden, Netherlands and North America.

| TYPE OF CROSS | NO EGGS PRODUCED | EGGS PRODUCED | EGGS HATCHED |
|---|---|---|---|
| BETWEEN POPULATIONS | .61 (11) | .39 (7) | .57 (4) |
| WITHIN POPULATIONS | .54 (7) | .46 (6) | .67 (4) |

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## GENETIC ADAPTATION — ELECTROPHORETIC VARIATION

Protein variation has been commonly used as an indicator of genetic differentiation among individuals, since the development of electrophoretic techniques. I was interested to know if introduced populations differed from native populations in the amount of protein polymorphism or if differentiation had occurred among the introduced populations.

I approached the question by surveying 27 enzyme systems for electrophoretic variability in Cinnabar moths collected from 7 North American populations and one European population. Seventeen of these systems could be adequately distinguished but only one, phosphose hexose isomerase (PHI) was polymorphic. I therefore concluded that both native and introduced populations are highly monomorphic in terms of protein variation. This single polymorphic system could be used however to investigate genetic differentiation among the introduced populations. Three alleles occurred at the phosphose hexose isomerase locus coding for slow(*S*), medium(*M*), and fast(*F*) migrating bands. Allele frequencies are given in table 2. All populations have the allele for the intermediate band in the highest frequency. In the four more northern, North American populations the only other allele which occurs (with an exception of one individual in the Cultus Lake population which was heterozygous for *MS*) is the *F* allele. In the more southern and the Dutch population the *S* allele is second most common with the *F* allele most rare.

Table 2. Frequencies of 3 alleles at the PHI locus of North American and a Dutch population of the Cinnabar moth. * Includes new populations established form natural dispersal in 1974.

| Area | Frequency S | Frequency M | Frequency F | Total Sample Size |
|---|---|---|---|---|
| Linn, Co. Oregon | .00 | .90 | .10 | 103 |
| Cultus Lk. B.C.* | .00 | .82 | .18 | 155 |
| Nanaimo, B.C. | .00 | .85 | .15 | 47 |
| Nova Scotia | .00 | .78 | .22 | 119 |
| Dutch Dunes | .06 | .92 | .01 | 43 |
| Coos, Co. Oregon | .29 | .71 | .00 | 12 |
| Ft. Bragg, Calf. | .23 | .74 | .03 | 179 |
| Comptche, Calf. | .21 | .77 | .02 | 92 |

These genetic differences among populations can be interpreted in two ways. First, selection could have led to the elimination of the *S* allele in the more northern populations. Secondly, the differences could have been due to founder effects. The history of the Cinnabar moth introduction is given in Table 3. I think that this argues against a historical interpretaton of these differences. The populations in Linn Co., Oregon and Ft. Bragg, California originated from the same area near Paris, France. The Canadian introductions originated from a different area all together. However the Linn, Oregon population is more similar to the Canadian populations than to the California population. This argues in favor of selection causing the differentiation at the PHI locus. Furthermore, some individuals from the Ft. Bragg population were introduced to Nanaimo, B.C. The Cultus Lake population was later initiated from the Nanaimo population. The single individual with an *S*. allele found at Cultus Lake is an indication that this allele was at least introduced to the area. The lack of spread of the *S* allele in the British Colum-

Table 3. The history of the introduction of the Cinnabar moth to North America.

| ORIGIN | PARIS, FRANCE | | SWITZERLAND & SWEDEN |
|---|---|---|---|
| STAGE COLLECTED | LARVAE | ADULTS | ADULTS |
| STAGE SHIPPED | PUPAE | LARVAE | ADULTS |
| FIRST RECEIVED | LABORATORY—NEW JERSEY (1959) | FIELD—CALIFORNIA (1959) | LABORATORY—ONTARIO (1961) |
| STAGE SHIPPED | ADULTS | LARVAE | LARVAE |
| SITE RELEASED | LINN CO., OREGON (1960) | COOS CO., OREGON (1965-67) | NOVA SCOTIA (1963)<br>BRITISH COLUMBIA (1964) |

bia populations also argues that selection against the allele is responsible for its near elimination in more northern populations.

The test of the selection hypothesis will be to introduce Californian moths into presently uninhabited areas of B.C. and to observe changes in allele frequencies over time. In another presently vacant site both Californian and local animals should be released so that the influence of locally adapted animals on the frequency of the introduced allele can be studied. Until these tests are done we can only speculate that there has been genetic change in the introduced populations caused by a response to different selection pressures.

Another way of looking for selection acting on the PHI locus is to look for temporal changes in allele frequencies. In two of the three populations with multiple samples, little variation occurred from year to year (Table 4). However, in the third, allele frequencies differed significantly between 1975 and 1976. This was not associated with any obvious demographic change in the population and further studies will be necessary to determine if this change is permanent or temporary.

Table 4. Frequencies of the M allele of PHI over time. * Significantly different from 1976 ($X^2=8.7$ $P<.01$). Sample size in parentheses.

| Year | Cultus Lk., B.C. | Linn Co., Ore. | Nova Scotia |
|---|---|---|---|
| 1974 | .86 (107) | — | .79 (50) |
| 1976 | — | .85 (66)* | .78 (56) |
| 1975 | .80 (31) | .99 (37) | .73 (76) |

Samples taken from May to July 1974, in the Cultus Lake population show a marked constancy of allele frequency (Table 5). Therefore animals emerging late in the summer are not different from the early emergers. An interesting difference

Table 5. Frequencies of the *M* allele of the PHI locus in Cultus Lake population during the summer of 1974. Sample size in parentheses.

| Date | May 26 | June 4 | July 11 | July 18 | July 22 |
|---|---|---|---|---|---|
| Frequency | .86 (15) | .86 (18) | .85 (48) | .88 (12) | .86 (15) |

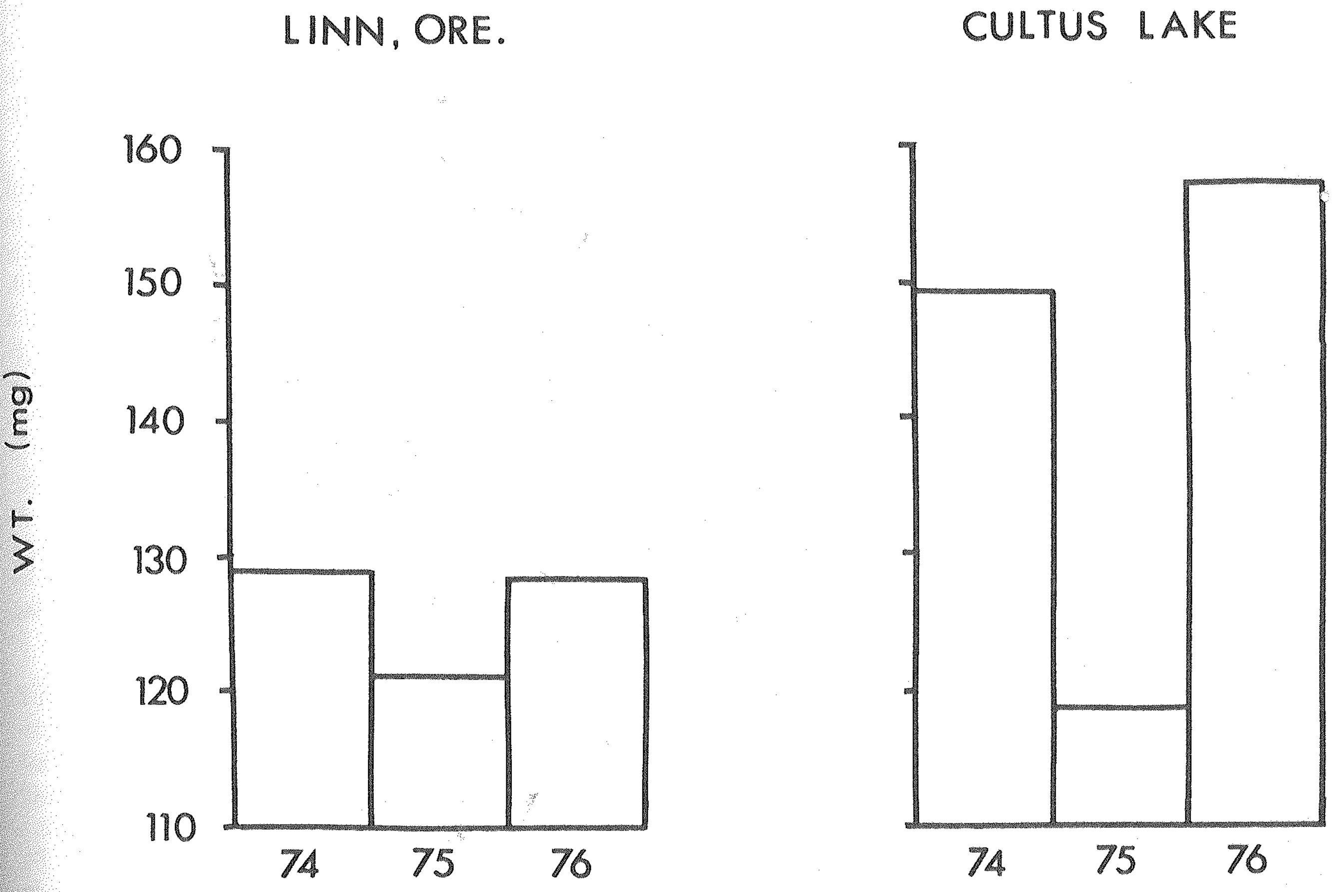

Figure 1. Variation in pupal weights from year to year in two Cinnabar moth populations. From this the Linn Co. population is classified as "stable" and the Cultus Lake population "fluctuating." The Linn Co. site is also known as the Jordan site (Stimac and Isaacson, this volume) and the Silbernagel site (Myers and Campbell 1976).

which did occur in the population in 1974, however, was between animals which had dispersed to adjacent uninhabited fields and residents. Cinnabar moths reached high densities for the first time that year since the introduction in 1971. In previous years there had been little movement from the population to surrounding fields and few larvae were found in two fields which had high tansy ragwort populations. These fields were separated from the original site by only 20 to 50 m of woods. In the summer of high density we caught 17 adults in one day's sample which appeared to have dispersed to the adjacent fields. The allele frequency of the group was significantly different from that of the residents (Table 6). The allele frequencies of the two new populations established by these dispersers has remained low even though by now they are quite well established (Table 6).

Table 6. Frequencies of the *M* allele of the PHI locus in the residents and dispersing animals of the Cultus Lake population in 1974 and of the resident and new populations established by the 1974 dispersers as observed in 1976. In both cases the differences are significant ($P < .05$). Sample size in parentheses.

| | 1974 | 1976 |
|---|---|---|
| Residents | .86 (107) | .80 (31) |
| Dispersers | .79 (17) | .72 (22) |

To summarize, genetic adaptation of introduced populations of Cinnabar moth appears to have occurred at the PHI locus. Patterns of allele frequencies cannot be explained by the introduction history of the populations and probably indicate the action of selection. Unexplained temporal changes have occurred in the Linn, Oregon population. Dispersing animals do not seem to be a random sample of the resident population and populations established by natural dispersal have maintained higher frequencies of the F allele than occur in the original population.

## POPULATION FLUCTUATION AND PUPAL SIZE VARIATION

A native population of Cinnabar moth at Weeting Heath, England has been observed to fluctuate dramatically in density (Dempster 1975, discussion in Myers and Campbell 1976). The population fluctuations occur because periodically the moth populations increase to levels which cause severe food plant defoliation and larval starvation leads to population crashes. An indicator of the population fluctuations are associated variations in pupal size (Dempster 1971). Following summers of defoliation and starvation pupae are small (see also van der Meijden 1976). When larval populations are low, they are well fed and metamorphose into large pupae.

Variation in pupal size can be used therefore as an indicator of population fluctuation. This measurement can be made with a minimum of effort if pupae are collected and weighed or measured once a year. I have done this now for field populations in Linn Co., Oregon and at Cultus Lake, B.C. Variation in pupal size in these two populations is shown in Figure 1. Pupal weights varied the most from year to year and pupal lengths showed similar trends.

Pupal widths, as used in Dempster's study were not as variable in the populations I observed. Figure 1 shows two things. First the average size of pupae in the Cultus Lake population is larger than that of the Oregon population. Secondly, the size of the pupae has varied considerably more in the Cultus Lake population. The association between pupal size reduction and defoliation and population decline was confirmed by field observations.

This observation indicates an exciting extension of the original experimental design. If, as seems to be the case, these two populations differ in the degree of density fluctuation which they exhibit, then we can make comparisons of adaptations in a "stable" and a "fluctuating" population. The Oregon population has remained relatively stable since its establishment (Isaacson 1973; Stimac and Isaacson, this volume; and personal observation). On the other hand the Cultus Lake population expanded rapidly after its introduction in 1971 and in the summer of 1974 population densities were high and food plant defoliation was almost complete. This led to the reduced size of the pupae collected in the spring of 1975.

We may now investigate the characteristics which differ between the animals of these two populations and the habitats in the two areas. What factors led to the stability of some Cinnabar moth populations (Linn, Co. Oregon; Coos, Co. Oregon - see Myers and Campbell 1976 and possibly Ft. Bragg California - see comment by Huffaker in discussion of Dempster 1971a), while other populations fluctuate wildly (Weeting, Heath, Dempster 1975; Dutch dunes, van der Meijden 1971).

## SIZE AT PUPATION

Cinnabar moth larvae deprived of food can pupate at a reduced size. Van der Meijden (1971) quantified the relationship between the weight of fifth instar larvae when they were removed from food and the proportion which pupated successfully. I tested animals from the same population studied by van der Meijden 6 years later and found a correspondence almost unheard of in an ecological study (Figure 2). Van der Meijden (1976) also repeated the measurement with the same result. Therefore, the threshold weight for pupation seems to be a consistent characteristic for populations established for long periods.

I obtained similar information on pupation weight thresholds for the Cultus Lake population by collecting fifth instar larvae from the field and placing them in containers without food at 22°C. The proportion of larvae, divided into 15 mg. weight categories, which pupated was determined. This was done in two years; in 1974 when the population reached peak densities and in 1976 when numbers were again beginning to increase after the decline associated with defoliation and starvation in the summer of 1974. Larvae in 1976 were able to pupate at lighter weights than those of the peak population of 1974. Therefore, exposure of the population to food limitation seems to have reduced the pupation weight threshold. Only future studies will tell if this characteristic will be maintained in the Cultus Lake population, which would possibly indicate a genetic basis, or if it will fluctuate in association with density fluctuations indicating an environmental basis.

Pupation weight thresholds of stable and fluctuating populations are summarized in Table 7 and

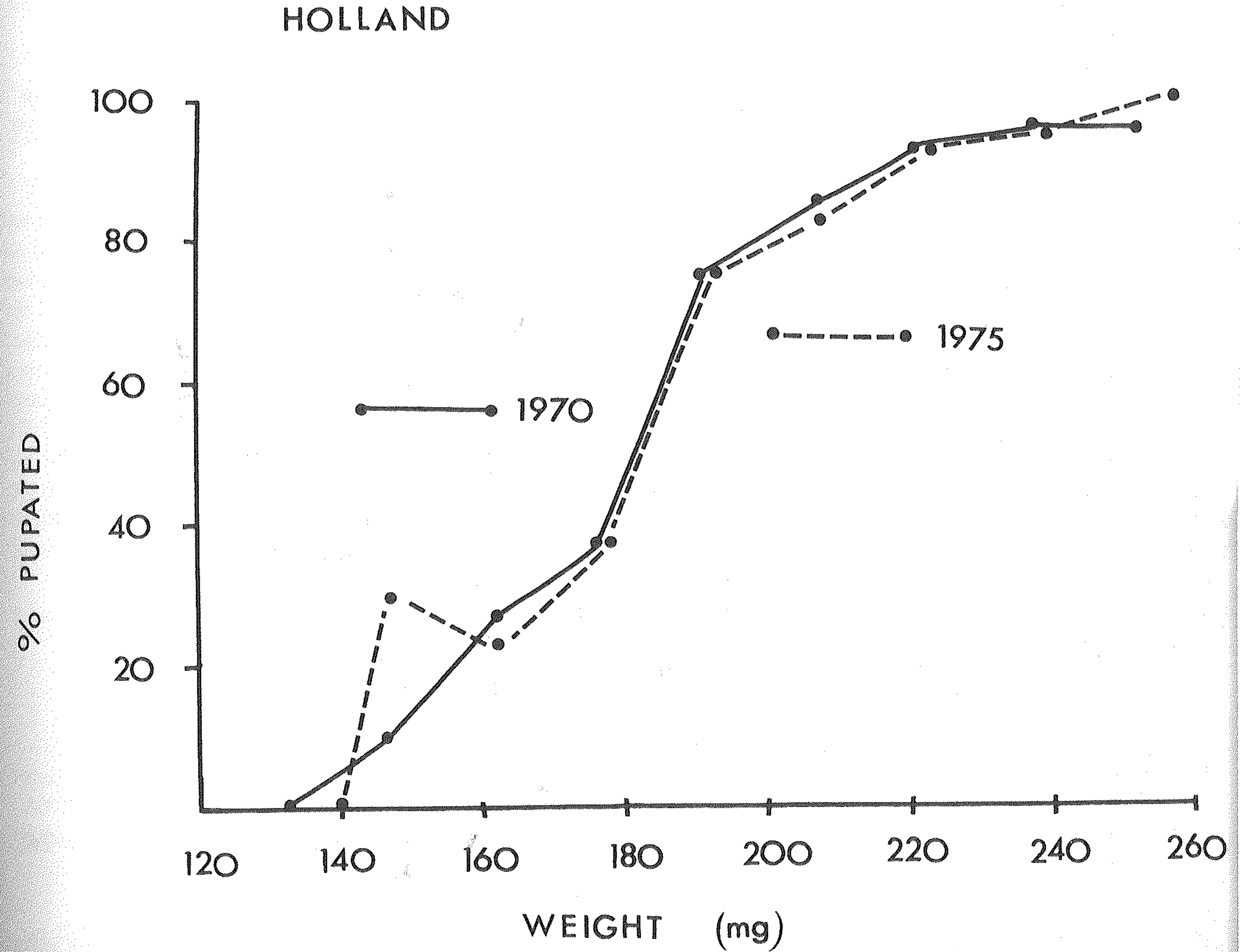

Figure 2. **The relation between the weight of fifth instar larvae when removed from food and the percent which pupate successfully. Tests were made on larvae from the sand dunes near Leiden but 5 or 6 years apart.**

Table 7. Pupation weight threshold measured as the lightest fifth instar weight category in which some individuals pupated successfully and the weight category at which 50% or more of the larvae successfuly pupated. Mean pupal weights are from field samples (Linn and Cultus) or from fifth instar larvae collected in the field and reared to pupation (Ft. Bragg). Weights in mgs.

| Area | Lightest Weight Category with some Pupation | Weight Category with at least 50% Pupation | Mean Pupal Weight |
|---|---|---|---|
| Ft. Bragg (stable) | 110-124 | 140-154 | 122 |
| Linn Co. (stable) | 140-154 | 155-159 | 126 |
| Cultus Lake 1976 (unstable) | 140-154 | 170-184 | 140 |
| Dutch Dunes (unstable) | 140-154 | 185-199 | — |

indicate that the more stable populations have lower pupation weight thresholds and smaller average pupal size than populations undergoing severe fluctuations in population size. From this observation we can hypothesize that a reduction in pupation weight threshold is a mechanism which allows population stabilization. This can be tested by continued studies of pupation weight thresholds of these populations and further threshold determinations for other populations which can be classified into "stable" or "fluctuating" categories.

## HETEROGENEITY OF EMERGENCE TIME

Another characteristic of moth populations which might be under strong selection is the time of emergence of adults. If food is limited, the offspring of moths which emerged early in the spring might be the only ones to find sufficient food for

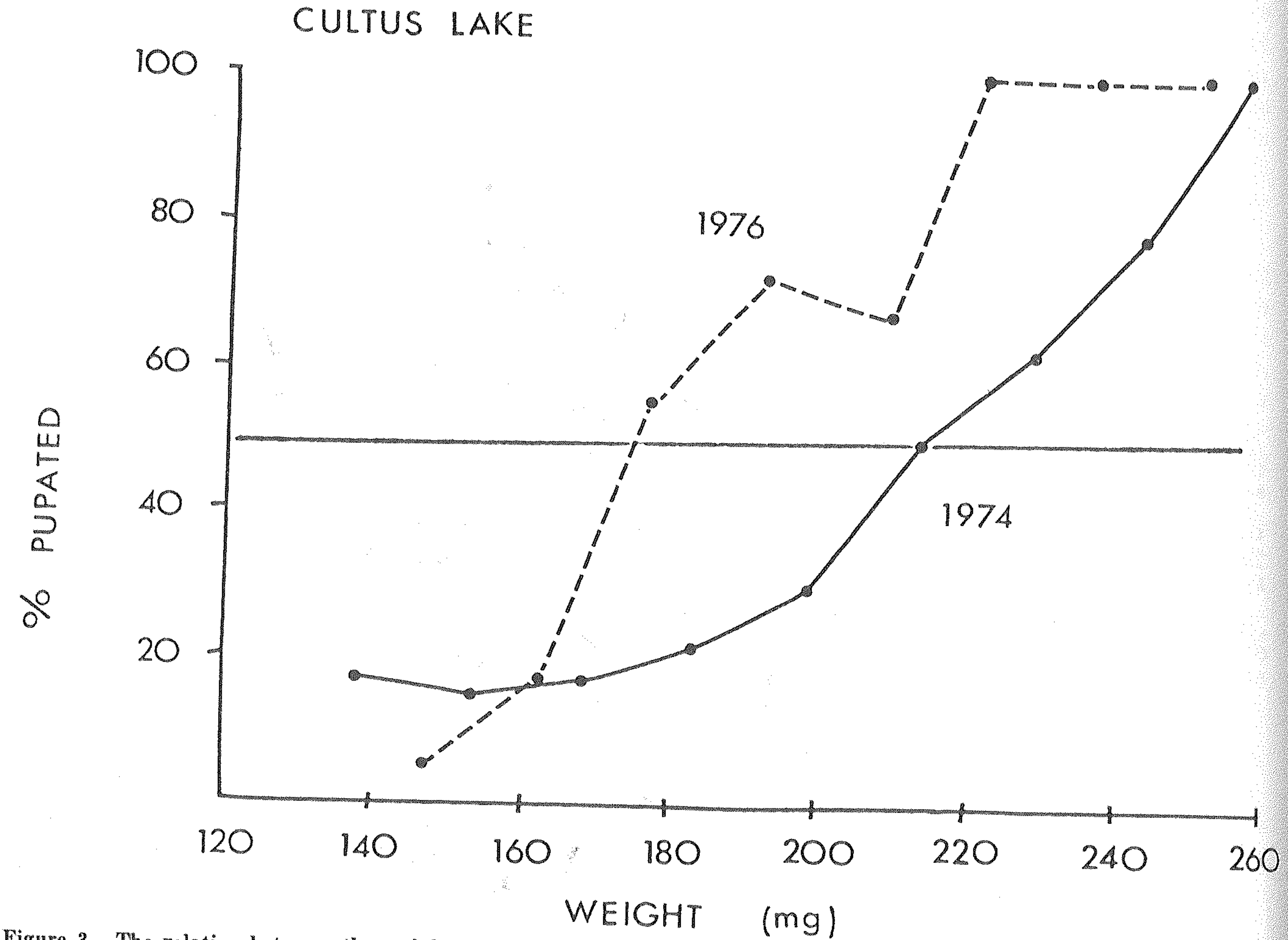

**Figure 3.** The relation between the weight of fifth instar larvae when removed from food and the percent which pupate successfully for the Cultus Lake population. In 1974 this population which was introduced in 1971, reached peak densities and caused severe food plant defoliation. In 1976 the population was still relatively low.

development; thus selection for early emergence occurs. The most obvious way to quantify emergence time is to make field observations on just when the moths begin emerging. This is impossible if you are trying to study several widely separated populations at the same time. However, rates of development and developmental temperature thresholds can be quantified readily. For this, pupae are collected from the field and divided into groups which are exposed to three different temperatures. The time until emergence is recorded. Using techniques described by Campbell *et al* (1974), the temperature thresholds and development time and standard errors can be determined. Selection for early emergency should result in a reduction in the temperature threshold or development time, as well as reduced variation of these. Characterization and populations in this way has just begun but I feel it will give us another method for studying adaptation of introduced populations.

## POPULATION FLUCTUATIONS AND HABITAT CHARACTERISTICS

We may next ask if there are characteristics of the habitats which seem to be associated with fluctuating population in contrast to stable ones? Using techniques described in Campbell and Meyers (1976) we have measured the average size of tansy ragwort rosettes and stems as well as the average distance between plants for three "fluctuating" populations and two "stable" populations (Table 8). The data show that the more stable populations tend to have larger and more widely spaced plants. Small rosette size seems in particular to be characteristic of fluctuating populations.

## DISCUSSION

In considering a model for the population dynamics of introduced Cinnabar moths, Campbell and I hypothesized that introduced populations of moths would adapt to the characteristics of their new habitats (Myers and Campbell 1976). The genetic divergence between the Linn Co. Oregon population and the Ft. Bragg California population at the PHI locus shows that genetic change can rapidly take place. Introduced populations adapt.

In the model presented in Table 9 two pathways are depicted and we suggest that the plants' reaction to defoliation determines which pathway a population will follow. Small and closely spaced plants allow animals to successfully disperse from plant to plant. This leads to increased innate dispersal but eventual overexploitation of the food resources; thus, the strong population fluctuations. On the other hand if plants are larger but more widely spaced, larval dispersal will be less necessary because each plant will support a greater number of larvae, and will be less successful because of the greater distance between plants. We hypothesize that in this siuation the population will stabilize, since individuals can adapt to a more constant food supply from year to year. Reduction of moth size and egg batch size and increased density dependent dispersal are suggested as means of attaining this stability.

We previously tested the hypothesis that larval dispersal and egg deposition would be adapted to environmental conditions (Myers and Campbell 1976). We now have the data to test the relationship between plant size and spacing and population stability (Table 8) and moth size (pupal size) and population stability. Plants in areas of fluctuating populations tend to be smaller and closer than those in

Table 8. Plant size and spacing in "fluctuating" and "stable" Populations of Cinnabar moth. Standard error in parentheses.

| | Average Biomass/Stem | | Average Biomass/Rosette | | Average Distance Between Plants | |
|---|---|---|---|---|---|---|
| | 1974 | 1975 | 1974 | 1975 | 1974 | 1975 |
| "FLUCTUATING" POPULATIONS | | | | | | |
| Cultus | 10.2 (1.9) | 15.3 (2.1) | 2.6 (.2) | 2.2 (.1) | 10.6 (.6) | 8.1 (.2) |
| Dutch Dunes | | 14.5 (2.2) | | 2.2 (.8) | | 16.5 (3.4) |
| Weeting Heath | | 11.1 (1.6) | | 1.2 (.2) | | 11.8 (.6) |
| "STABLE" POPULATIONS | | | | | | |
| Linn, Oregon | 41.0 (16.2) | 19.3 (4.3) | 8.0 (1.5 | 8.8 (1.5) | 15.3 (1.7) | 11.1 (.6) |
| Ft. Bragg, CA | 19.6 (4.0) | 22.0 (3.3) | 6.1 (1.4) | 8.3 (.6) | 13.6 (2.4) | 14.9 (1.2) |

Table 9. Part of a model of Cinnabar moth—tansy ragwort dynamics presented in Myers and Campbell (1976). The response of the plant to defoliation determines which pathway will be followed. Following pathway 1 leads to greater population stability.

| DEFOLIATION LEADS TO: | |
|---|---|
| 1 | 2 |
| No Reduction in Plant Size and Spacing | Reduction in Plant Size and Spacing |
| Reduced Moth Size | More Successful Dispersal |
| Smaller Egg Batch Size<br>(More Contagious Distribution) | Increased Innate Dispersal |
| Increased Density Dependent Dispersal | |

areas supporting "stable" populations and "stable populations have lower pupation weight thresholds and smaller pupae.

While we can attribute the population fluctuations to the characteristics associated with small closely spaced plants, the mechanism allowing adaptation to smaller groups and lower pupation weight thresholds in "stable" populations but not "fluctuating" ones is not so obvious. Larger female pupae produce more eggs (Dempster 1971b; Van der Meijden 1976; personal observation). Therefore, selection should be pushing towards larger size. Starvation on the other hand selects for lower pupation thresholds and possibly also smaller pupae. "Stable" populations will be able to adapt to a constant level of starvation. But "fluctuating" populations will be subjected to alternating periods of abundant food and limited food. This may prevent adaptation to pupation thresholds as low as those in the "stable" populations.

Future work for testing the model proposed will involve following the stable and fluctuating populations for several more years. In addition new populations will be established to study patterns of adaptation in expanding populations. As suggested in Myers and Campbell (1976) the response of the plants to defoliation may well be the key to the whole picture and this will have to be investigated further.

In conclusion, if the experimental approach is the way of the future in insect population ecology, then biological control agents are the ideal candidates for future study. I think the Cinnabar moth has begun demonstrating the validity of this approach.

## ACKNOWLEDGMENTS

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