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THE OVIPOSITION BEHAVIOUR OF PIERIS RAPAE:

A Study in the Ecological Interpretation of Insects' Egg Distributions

by

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ERRATA

Figure 3.02.	(Immediately before p. 133). Vertical axis should read, "Mean no. of eggs per leaf" not "per plant".
Figure 3.03.	Applies to (a) and (b) (p. 130)
Figure 3.04	Applies to (c) and (d) (p. 130)
Figure 3.05	Applies to (e), (f) and (g) (p. 130)

SUMMARY

The behaviour of the cabbage white butterfly, Pieris rapae, ovipositing on brussels sprouts plants was studied in the laboratory in an attempt to assess the sorts of studies required if one is to interpret the distribution of an insect's eggs in terms of the behaviour which generates it.

A. The butterflies responses to stimuli from the following components of their environment were tested:

1. Members of the same species.

   (a) The presence of eggs and larvae (the latter at densities of about one third to two thirds of the maximum carrying capacity of the plants) were each tested. Neither influenced the distribution of subsequent eggs.

   (b) The presence of other adults (both male and female) in the cage did not appear to influence the oviposition behaviour of the butterflies in specific tests for such interactions. But the sample of butterflies tested was too small to discount the possibility that interactions between adults may sometimes influence the distribution of eggs.

2. Host plants.

   The butterflies discriminated between young and old plants, and between plants grown in low and high light intensity, laying significantly more eggs on the former plants in each case. They

did not, however, discriminate (in terms of the numbers or position of eggs laid) between plants grown in complete and sulphur-deficient nutrient solutions. The reasons for this are discussed, as is the evidence that butterflies were also influenced by certain physical characteristics of the plants.

3. Micro-weather.

There was indirect evidence that the intensity of light at, or reflected off, the leaf surfaces influences the females' choice of plants on which to lay. An experiment designed to test the butterflies' response to light intensity failed to show any discrimination. It was concluded that the method used did not adequately test the hypothesis, as it did not measure reflected light which is probably most influential. It was demonstrated that the butterflies have a circadian rhythm of oviposition, laying most of their eggs in the late morning to midday.

B. The distribution patterns of eggs and the frequency distributions of visits to plants and oviposition on them were analysed statistically in an attempt to determine whether internal stimuli also influence how a female distributes her eggs. In most experiments in which the sample size was large enough for a rigorous test, the distribution was found not to differ significantly from a negative binomial distribution. The distributions of eggs per visit, eggs per plant, settles per plant and per unit time, were analysed and

discussed in relation to Iwao and Kuno's m^*-m regression method and Morisita's indices of dispersion. It was concluded that internal stimuli also do influence the pattern in which a female distributes her eggs. The distribution during a short time interval, especially, is influenced by the level of activity of the female. Thus it seems that further studies of these sorts of components of oviposition behaviour, and further development of these analytical methods, which could enable the relative importance of internal and external stimuli to be assessed, would contribute to the interpretation of insects' egg distributions.

DECLARATION

To the best of my knowledge and belief this thesis contains no work previously published or written by another person except when due reference is made in the text, nor has any of this work been previously published or submitted to this or any other University for the award of any degree.

P. M. Ives

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CHAPTER I

1.1 Introduction

Interpretation of the distribution of an insect's eggs in ecological terms involves two stages:

(i) elucidation of the behaviour by which the distribution is generated,

(ii) assessment of the adaptive value, to the population as a whole, of the observed distribution, or of the behaviour that generates it.

Behaviour that may be involved in generating the distribution falls into two main categories:

(a) Responses to stimuli from the environment. The following components of an insect's environment seem to be the most likely to provide such stimuli:

(i) members of the same species; either progeny or other adults

(ii) heterogeneity in the condition of the oviposition substrates (i.e. of the host-plants in the case of phytophagous insects)

(iii) heterogeneity in the condition of the micro-weather, e.g. in light intensity, wind, pockets of or gradients in humidity, heat, etc.

(b) Responses to internal (physiological) stimuli.

The origins of such stimuli could be changes in restlessness, or in the rates of physiological processes, such as maturation of eggs. As well as internal stimuli there could be internal inhibition of responses to stimuli from the environment.

Probably both (a) and (b) are involved, for most insects, but their relative importance probably varies from species to species or even between populations within the same species.

Most studies attempting an ecological interpretation of the distribution of insects' eggs have followed one or other of two main approaches:

(1) Experimental studies of behaviour in which the ability of certain stimuli from the environment to inhibit or stimulate oviposition is measured by the number of eggs laid during a fixed time of exposure to the stimulus. Sometimes the females being tested may be given a choice between two alternative treatments, only one of which provides the stimulus being tested, or between different "concentrations" of the stimulus. A naturally occurring stimulus may be experimentally exaggerated to accentuate any response to it. The insects' receptors are also sometimes experimentally inhibited to determine, for example, whether the stimulus from a known source is visual, olfactory or tactile. But generally in all these experiments it is the relative <u>number</u> of eggs gained by

a given treatment that is used to assess its effectiveness.

(2) Observational studies concerned with statistical analysis of the distribution of eggs among units of the oviposition substrate. The component distributions such as those of eggs per visit and visits per substrate (i.e. per plant or part) may also be analysed. Clump size and distribution in aggregated populations and even things such as frequency of occurrence of "overloading" or "sparing" of plants may also be measured. The assumption that data are normally distributed underlies most parametric methods of estimating population density by sampling; also for analysis of variance, remainder variances must be homogeneous. Thus much of the data on non-normal distributions of insects' eggs required transformation. In the past, most statistical analysis of the distribution of insects' eggs has aimed at obtaining an adequate statistical description of a patchily distributed population so that the appropriate transformations could be determined. More recently, however, a few workers have used such analyses as a basis for hypotheses about the egg-laying behaviour of various insects.

Insects distribute their eggs in a wide range of distributions (from significantly more uniform than random, through random, to significantly aggregated distributions) but overall and for phytophagous insects especially, aggregated distributions of eggs are

the most common. If the distribution pattern is not random it may have been selected because (in the particular circumstances in which it occurs) it provides a significant advantage for the progeny compared with any other distribution pattern. Such an advantage could be with respect to the quality, availability, or effective use of food; to protection from predators or from harsh weather, etc. On the other hand, there may be two or three different forms of distribution not significantly different from each other with respect to the advantage they give the progeny. In this case the distribution that occurs has probably been selected because it is advantageous to the ovipositing female. Possibly the behaviour which generates that distribution reduces her exposure, during oviposition, to predators, or the rigors of the weather; or it may require less complex development of her sensory receptors, or less expenditure of energy, than behaviour that generates the alternative distributions.

This project is mainly concerned with the first stage in the interpretation of the distributions: elucidation of the behaviour that generates them. In this study of the behaviour of the cabbage white butterfly, Pieris rapae, ovipositing on brussels sprout plants in the laboratory, I have attempted to combine the two approaches described above. In Chapters 2, 3 and 4 respectively, experiments on behaviour give information about the butterflies' responses to stimuli from components (i), (ii) and (iii) above, of their

environment. In Chapter 5 the statistical approach, combined with observation of the components of behaviour in butterflies ovipositing alone, gives information (though indirect) about their responses to, or inhibition by, internal (physiological) factors. I have attempted to use P. rapae as a tool, to help in assessing the sorts of studies required if one is to interpret the distribution of an insect's eggs in ecological terms. Consequently each chapter begins with a theoretical discussion of the topic in question, followed by evidence from the literature, to provide the background in relation to which my experiments were done. Thus only a brief discussion of the results was necessary when they were as expected.

1.2 General Methods for Whole Project

1.2.1 Materials and Methods Common to Experiments with both Groups of Butterflies and Single Females

1.2.1.1 Plants: Throughout my experiments the host plants provided for my butterflies to lay eggs on were small plants of brussels spouts (Brassica oleracea var. gemmifera) growing in 4 in. pots inside a flywire mesh cage 36 ins. wide and 44 ins. long. The cage was 36 ins. high for experiments up to and including those described in Sections 2.2.1.1 and 2.2.1.2; for all subsequent experiments the cage was 30 ins. high. (The cage had to be made smaller so that it would fit through the doorway of the constant temperature room where most of the latter experiments were done).

Alyssum flowers (either growing in pots or picked but in either case containing nectar) were used as a source of food for the butterflies. Generally they were distributed regularly among the sprout plants but when there was not enough space some of the Alyssum was placed at each end and side of the cage, outside the array of spouts. In most of the later experiments the Alyssum was supplemented by small vials 1" x 2" containing cottonwool soaked in honey solution (15% honey, with one teaspoon of sugar per 100 mls solution). On one or two occasions when Alyssum containing nectar could not be obtained at all honey solution alone was used.

1.2.1.2 Butterflies

Butterflies were both caught in the field and reared in the laboratory; the origin of the butterflies used for each particular experiment is specified in the methods section for that experiment.

Whether caught in the field or reared in the laboratory, all but two groups of butterflies were stored at 10°C when they were not actually being used in an experiment. This slowed their ageing and reduced the need to feed them so often. The exceptions were:

(i) Butterflies used for the experiments in Section 2.2.1.2 and the experiment testing whether butterflies discriminate between plants grown indoors and out of doors, described briefly at end of 3.1 (p.124). They were stored at 15°C

after they emerged until they were fed (out in the sun), and again after they had fed until the experiment began.

(ii) The butterflies used for the experiments in Sections 2.2.2.3 and 2.2.3.2. They were reared entirely on growing plants in a controlled temperature room (22 → 26°C, mostly 24°C daytime; 12.5°C at night) and remained there between experiments.

Most of the eggs from which the laboratory population of butterflies were reared, had been laid during experiments (and therefore on brussels sprout plants). Sometimes, however, the females were given access to other plants of the genus Brassica, when the eggs were needed only for rearing more butterflies. In either case, the larvae were allowed to develop on the growing plants (either under a cage on the open roof of the building, or in a room air-conditioned at 21.4°C, 52 - 70% relative humidity range) until at least early third instar. They were then transferred to large glass or plastic containers (22 → 23 cms in diameter) covered with gauze and kept in constant temperature cabinets. Most batches of larvae were reared at either 20°C or 25°C (more at 25°C than 20°C), one or two batches at 27°C, with either continuous light or a long day photo-period (sixteen or more hours of light). They were fed on outer cabbage leaves obtained from the market twice a week and stored at 5°C till used or replaced by fresher leaves. The larvae were given "fresh" food (i.e. up to four days

old, from the refrigerator) every one to two days depending on the rate at which they fouled the food or it appeared to deteriorate markedly e.g. become limp instead of crisp. Occasionally the larvae were also given some freshly picked brussels sprout leaves.

On the whole, therefore, the larval food was rather poor quality and probably occasionally it would have had little nutritional value at all. So long as butterflies were not reared in the laboratory for more than one generation, however they mostly seemed to be normal healthy individuals. The first part of the project was done only in the late spring, summer and autumn (I was occupied with another topic in the winter and early spring) when butterflies could easily be collected in the field at most times, so that few of the butterflies used for experiments were from stock reared in the laboratory for more than one generation. Later, however, when the project had to be continued throughout the winter, an increasing proportion of the butterflies from the second or third generation reared in the laboratory were lethargic and abnormal in their behaviour - they did not mate or lay eggs readily and often died with their abdomens packed full of eggs.

Although the lack of health and vigour was probably partly due to the poor quality of the larval food, even butterflies of a first and second generation reared entirely on growing plants in the laboratory and kept in a regular 14 hours light: 10 hours dark photoperiod throughout their development, included many lethargic and

slightly aberrant individuals. A higher proportion of the butterflies were more-or-less normal, however, than when reared on pre-picked food. But they were the progeny of a very few individuals that emerged in the field in mid-winter due to fortuitous weather terminating their diapause, so perhaps inbreeding could account for some loss of vigour by the second generation. An alternative hypothesis is that although diapause in Pieris rapae is apparently facultative, the progeny of butterflies caught in the field were in some way physiologically influenced by their parents' experience of a few short days and cold nights after emergence before they were collected and brought into the laboratory.

Until the experiment testing for periodicity in egg-laying (Section 4.2.2), no attempt was made to standardize the light regime in which the butterflies were stored. The majority were kept between experiments in a 14 hours light: 10 hours dark, regime, more-or-less synchronized with natural daylight, but they were sometimes stored temporarily (for less than twelve hours; usually less than eight) in complete darkness at 10°C, regardless of the time of day. Some of the earlier experiments were run with the light on only between 05.00 hours and 21.00 hours, but others were run in continuous light at all hours of the day or night. Also butterflies were generally pre-conditioned without reference to the time of day or night. The experiments described in the following sections were done before the experiment testing for a circadian rhythm in oviposition:

(i) Sections 2.2.1.1 - 2.2.1.3

(ii) All sections 3.2

(iii) Section 5.2.1

For reasons discussed in Section 4.2.2, it was not until the experiment described in Section 3.2.3.1 that I suspected periodicity in the oviposition of P. rapae. The experiment in Section 4.2.2 confirmed the presence of a circadian rhythm, so all subsequent experiments were kept more-or-less within a 14 hours light period that was roughly synchronized with natural daylight.

1.2.1.3 Methods of Estimating Leaf and Plant Area and Volume

Leaf area was "measured" by approximating the leaf to one or more circles or parts thereof, of which the circumference was measured by a "sliding circle" device. This device was made from a strip of stiff plastic 2 cms wide and 50 cms long attached at one end to a small flat metal loop through which the remainder of the strip could be slid to make a circle of variable size. Millimetre graph paper was glued to the outside of the plastic strip so that the circumference of the circle could be read off it directly.

The majority of the leaves of brussels sprout plants are approximately circular which made this a relatively quick and convenient method of obtaining a rough measure of their size

without detaching or otherwise damaging them. Few leaves are truly circular however, and some, especially those that have been partly eaten, may be very irregularly shaped. Consequently the circumference of the circle was generally read to the nearest 0.5 cms, or for leaves that were very difficult to fit, within a range of 1 cm. Approximate leaf area was then taken as the area of that circle or part thereof.

As described in Appendix 1, the use of two different methods of measuring the area of leaves whose volumes were also measured led to two different equations for the relationship between leaf area and leaf volume; probably neither equation is very accurate but at least they show the order of size of leaf volume. The estimate of a plant's total volume used for assessing the density of larvae or a butterfly's response to the size of plants, was simply the sum of the estimated volumes of all leaves on that plant. As the larvae do not feed, nor the butterflies lay their eggs, on the stems or petioles, to any significant extent, omission of the latter parts from the estimate of total volume is not likely to reduce its usefulness, unless the butterflies are especially attracted by the height of plants, as distinct from their overall size. As discussed in Sections 3.1.2.3, the evidence about the importance of height per se is equivocal. I did not test the butterflies' response to the height of plants in my experiment set-up, but in some experiments (including the one discussed in Section 3.2.4 (p.173)) in which I noted at least that certain

plants were taller than others, there was no evidence that the taller plants were consistently more attractive than the shorter ones.

The two estimates (by the two different methods) of the total leaf volume of the same plant sometimes differ markedly, as the difference between, and errors in, the estimates for individual leaves are summed. Both estimates are therefore given, or used to calculate approximate larval density. Estimates of the total leaf area of plants are given as well, as they have the advantage of a single, rather more accurate, value. Nevertheless, they still contain the summed errors from each leaf. Consequently the estimate of total leaf area for a plant with many leaves has a much greater error component than that for one with few leaves, so that the method does not give the relative size of plants very accurately. But rough, and more-or-less relative, estimates of plant volume and area are better than none, and probably sufficient for the purposes of this project - that is to be able to relate the butterflies' behaviour to certain approximate levels of density of larvae, or to certain approximate plant or leaf sizes.

1.2.1.4 The Concept of "Unit" Larvae

In experiments testing the butterflies' responses to larvae the densities of larvae used are expressed as "unit larvae"/c.c. in an attempt to achieve a standard measure of density

related to the "maximum carrying capacity" of plants for P. rapae. By "maximum carrying capacity" I mean that number of larvae (of any one age, from hatching 1st instars to mid 5th instars) per c.c. of leaf (in the field) at which, in spite of natural mortality throughout the remainder of their development, the larvae manage to consume all of the plant that is edible. Thus at any density higher than the maximum carrying capacity some larvae will die of starvation. The maximum carrying capacity (m.c.c.) of plants will, of course, be strongly dependent on the prevailing weather through its influence on:

(a) the rates of larval development and feeding, and, to a lesser extent, the mortality, of larvae, and

(b) the rate at which plants grow (or die, as a result of defoliation in dry weather).

But, as in (a), the influence of weather on mortality is much less likely to be important (in determining the m.c.c.). Unless the relative humidity is extremely high or low (e.g. rain drowning young larvae, or severe wilting of plants) then it is unlikely to be very influential, but clearly, the temperature at which the m.c.c. has been estimated is a necessary part of any statement the m.c.c. I was unable to find any estimate in the literature of the m.c.c. for P. rapae on brassicae; nor did I have time to determine it experimentally, so I was limited to calculation of a rather crude estimate from data in the literature on food consumption and mortality of P. rapae larvae in S.A. (For this estimate I had to

make the unrealistic assumption that plants did not grow during the larval feeding period).

Rahman (1966) measured the mortality during each stage of 7 generations (from the 2nd generation in the summer of 1963-64 to the 4th generation of summer 1964-65) of experimental populations of P. rapae in the field. He gave weather and parasitism as main causes of morality in the 1st and 2nd larval instars, and parasitism as the only major mortality factor in the later instars. But he did not give the duration of instars, nor the date, other than to say which generation the data refer to, and only mean monthly temperatures for 1963. The summers of 1963-64 and 1964-65 were a little cooler than average (Pomeroy 1966)so that even for the third generation of P. rapae each summer (which would probably usually experience the highest temperatures, in December and January) the mean temperature was less than 22.5°C - the lower of the two temperatures at which Rahman measured food consumption. But differences of 2 or 3°C in mean temperature (except near the extremes of P. rapae's tolerance) seem much less likely to influence the mortality of larvae than their rates of feeding and development. Although Rahman measured daily food consumption at constant temperature, he did not indicate which instar the larvae were in on any day (except to mention that at 24.3°C the 2nd instar began on the 7th day). Again I could not find any report of the duration of each larval instar of P. rapae, at controlled temperatures, in the literature. Consequently I determined the approximate duration

of larval instars experimentally in a controlled temperature regime with 14 hours light (of which larvae were at 27°C for about 9 hours and 20°C for the other 5 hours) and 10 hours dark (all at 20°C) which gave a mean temperature of 22.6°C. As the experiment was begun with larvae that had already hatched, I could not determine the duration of the 1st instar experimentally. But Rahman has shown that the amount of food eaten in all but the last two to three days of the 1st instar is negligible, so there seemed no need for an accurate determination of the length of the 1st instar for the purpose of calculating the m.c.c. The 2nd, 3rd, 4th and feeding part of the 5th instars lasted about 4.5, 3.4, 3.5 and 3 to 6.5 days respectively. Rahman's figures for food consumption at 22.5°C included separate figures for feeding by larvae parasitized by Apantoles rubecula and A. glomeratus, which emerge in the 4th and 5th instar of the host, respectively. Therefore when calculating concurrent feeding and mortality I assumed that all larvae that died in the 5th instar had been parasitized by A. glomeratus for the previous 15 days (length of larval period of A. glomeratus at 22.5°C (Rahman, 1966)) and adjusted the figures for feeding of that proportion of the population accordingly. Parasitism by A. rubecula was similarly accounted for. Rahman did not describe how larval deaths are distributed in time, within any one instar, so for simplicity I have assumed (though it is unlikely to be true) that larvae die at a constant rate throughout the instar. Thus if one is estimating density half way through an

instar, the total number of live larvae includes half of all those that will die during that instar. The effect on food consumption of a constant death rate will be the same as the effect of all the larvae dying at once, half-way through the instar; as it simplifies the calculation food consumption has been estimated for the latter distribution of mortality.

The second and third columns of Table 1.01 illustrate what happens to a theoretical cohort of one hundred larvae and their food supply throughout their development, according to the calculations described above. Thus if 100 larvae hatch a total of 8.7 ccs of food will have been consumed by the time the survivors cease feeding in the fifth instar. Similarly if 32 larvae in the middle of the third instar (q.v. in Table 1.01) are transferred to a new host plant, then approximately 4.7 ccs of that plant (8.714 - 3.957 = 4.757 ccs for 32.5 larvae) will be consumed before the survivors cease feeding in the fifth instar. Thus if larvae in their mid-third instar are transferred to a plant, at a density of 32.5 larvae per 4.76 ccs of edible plant (and assuming that the plant does not grow), all of the plant that is edible will have been eaten by the time the survivors cease feeding to pupate; i.e. $32.52 \div 4.76 = 6.84$ larvae/c.c is the maximum carrying capacity for mid-third instar larvae. Thus the maximum carrying capacity for each stage of development (column 4) was estimated by dividing the number of larvae alive at that stage by the amount of food they

would eat during the remainder of their development, i.e. the m.c.c. of any given stage depends on the mean potential of larvae in that stage for consumption of food.

TABLE 1.01

Mortality and food consumption throughout their development, of a theoretical population of one hundred hatching larvae of Pieris rapae

Stage of Development (Instar)	Percent Surviving	Food Consumed (cumulative) ccs/100 Hatching larvae	Maximum Carrying Capacity	Unit Larvae
Hatching - start of 1st	100.00	0	11.48	0.28
End 1st - start of 2nd	74.85	0.175	8.77	0.37
Late 2nd - start of 3rd	36.34	2.133	5.52	0.59
Mid 3rd	32.52	3.957	6.84	0.47
Late 3rd - start of 4th	28.71	5.422	8.72	0.37
Mid 4th	17.5	5.985	6.42	0.50
Late 4th - start of 5th	7.09	6.557	3.24	1.00
Mid 5th	4.85	7.568	4.23	0.76
End of Feeding	2.60	8.714	-	-

One way to relate statements about the density of larvae to the m.c.c., is to express the former in terms of the potential for consumption rather than the actual numbers of larvae. This can be done by means of the concept of "unit" larvae. The influence of a single larva at that stage of development (for which the mean

potential for consumption is at a maximum (i.e. when the m.c.c. is at a minimum) was arbitrarily equated to unity. As shown in Table 1.01 this stage was reached at the late 4th or start of the 5th instar. It would take nearly four times as many hatching first instar larvae per c.c., as late 4th to early 5th instar larvae, to eat all that is edible of the plant, so that as Table 1.01 shows, one hatching first instar larva equals 0.28 of a unit larva.

1.2.2 Methods Common to Most Experiments with Groups of Butterflies

With the exception of the experiments testing whether the presence of other adults influence the egg-laying behaviour of a particular female (Section 2.2.3), in experiments with groups of butterflies, the experimental brussells sprouts were arranged in a tray 29" x 33", filled to a depth of 3" → 4" with damp sawdust into which the flowerpots were sunk. For the indoor experiments the tray and cage covering it (Section 1.2.1.1) were under a movable bank of twelve 4 ft. fluorescent lights, ten 40-watt white, with two "Grolux" tubes evenly spaced among them. (A high proportion of the light emitted by "Grolux" tubes is ultra-violet). The lights were approximately 35" above the surface of the sawdust.

A time switch was attached so that they could either be run on a pre-set photoperiod or on continuous light. Unless otherwise specified in the text, the room where indoor experiments were done was air-conditioned with only gentle air movements, but there may have been a slight temperature gradient from under the centre of the bank of lights to the edges (especially up higher, near the tops of the plants) as the temperature at 2" above the floor under the bank of lights was 21°C with the lights off and 22°C with them on.

With the exception of the experiment testing for a response to the presence of larvae (Section 2.2.2.1), leaves were not individually marked in experiments with groups of butterflies. Before the start of an experiment the oldest mature leaf (not one that was obviously senescent - senescent leaves were removed) was marked with red paint at the junction of its petiole with the main stem. Even if the marked leaf subsequently fell off, the node from which it had grown was still marked and could be used as the datum point from which all younger leaves were counted. Thus although only one leaf was marked, each leaf on a plant could be identified when the eggs on it were counted, throughout a series of ovipositions periods (O.P.s.). They could not, however, be identified immediately on sight, but that was not necessary in the experiments with groups of females.

The term "duration of O.P." used as a column heading in tables of methods or results has different meanings for experiments with groups of butterflies and experiments with single females. For the former it means the total time in which the butterflies were in the cage with the plants, so long as the lights were on, regardless of whether the butterflies were active or not. For the latter the meaning is as defined in the next section.

1.2.3 Methods Common to Most Experiments with Single Females

Experiments and observations on the oviposition behaviour of single females involved, among other things, determination of how many eggs a female laid at each visit to a plant. To make this possible:

(a) each leaf of each plant must be identifiable on sight.

(b) the identifying marks on all leaves of all plants present in any one O.P. must be visible without disturbance to the experimental set-up.

(c) the butterfly must be visible at all times throughout an O.P., also without disturbance to the experimental set-up.

To fulfill pre-requisite (a), the leaves were marked on each surface with a two-spot colour-code. Up to thirty-five serial colour combinations were used, so that although so many leaves

were rarely present on a plant at once, the colour spots did not need to be changed whenever old leaves dropped and new ones grew.

Pre-requisite (b) was met by using only a small number of plants (five to eight, depending on the experiment) arranged in an approximate circle around a central clump of Alyssum flowers.

To fulfill (c), the plants were placed on metal trays about a foot above the floor, so that a butterfly was less likely to be hidden by the plants themselves when laying eggs on the under surfaces of leaves. (The lighter coloured, smoother surface of the metal trays, as compared with the damp sawdust under the plants in the experiments with groups of butterflies, probably increased the level of reflected light at the under surfaces of the leaves). To avoid losing sight of the butterfly for even a few seconds (in which she could lay an egg) I described her behaviour straight into a tape recorder. Nevertheless it was not always possible to see for sure whether a butterfly had laid an egg when she put her abdomen up to the under surface of a leaf. Consequently after each O:P. it was necessary to count all eggs on the plants and then interpret the tape record according to the number and position of eggs actually found on each leaf. Tape recording the observations had the added advantage that (if I recorded how long the tape recorder was switched off when a female was inactive) the tape also gave a record of the duration of any particular sort of

behaviour. (e.g. it gave the frequency of visits per unit time, as well as per plant). Because of this, the term "duration of O.P. (e.g. in Table 2.20) has a different meaning from that which it had for experiments with groups of butterflies. Here it is the sum of times spent sitting, flying, and laying eggs, excluding a long period of immobility (a) after the butterfly was put in the cage, before her first flight among the plants, and or (b) after her last flight among plants, before collection, if either or both of these occurred. (They frequently did, in experiments with single females).

The first observations on the behaviour of single females (described in Section 5.2.1) were done in the same air-conditioned room (temperature about 21.5^{o}C, R.H. 50-70%) and under the same bank of lights as the experiments with groups of butterflies. All subsequent experiments and observations on single females were done in the same controlled temperature room in which the last butterflies were reared (see Sections 2.2.2.3, 2.3.2 and 4.2.1). (The temperature, when the lights were on, was constant for any one experiment, but the setting varied between 22^{o}C and 26^{o}C from experiment to experiment; for most it was 24^{o}C. When the lights were off the temperature was 12.5^{o}C). There were two banks of lights in the room; one over the experimental cage, the other over the rearing cage. They were set on a 14 hrs light/10 hrs dark photoperiod, light between 07 hours and 21 hours. Each bank

comprised thirtysix 4 foot fluorescent tubes (twenty-eight arranged roofwise, with four across each end of the "roof") and twenty incandescent bulbs (ten along each side of the "roof" below the lowest fluorescent tube). Over the rearing cage all fluorescent tubes were 40 watt white and the incandescent bulbs, 60 watt. Over the experimental cage the "roof" consisted of eleven 40 watt white and three "Grolux" tubes per side, and there were three 40 watt white, and one "Grolux" tube at each end. 100 watt incandescent bulbs were used for the experimental cage, over which the lowest lights were 30 ins, and the highest, 47 ins. above the trays on which the plants stood. There was a high level of reflected light as the room was only 8 ft. x 11 ft, with shiny white walls and ceiling and an unpainted galvanized iron floor.

CHAPTER 2

RESPONSES TO STIMULI FROM MEMBERS OF THE SAME SPECIES

2.1.1 Responses to Other Adults

2.1.1.1 Species with a Single Characteristic Egg Distribution Pattern

If a particular pattern of distribution of eggs is characteristic of a species and is predictable for a range of densities (of both adults and eggs), then, unless

(a) the pre-imaginal stages occur in strongly localized sub-populations within which the emergence of adults is well synchronized and those adults are either gregarious or not very dispersive, or

(b) even though the juveniles do not occur in strongly localized sub-populations the newly emerged adults are very highly mobile and either attracted to a common focus or behave in such a way that they are carried to such a focus by air-currents, as, for example, in those species that show "hilltopping" behaviour (Shapiro, 1970); and if thereafter they are gregarious or non-dispersive,

it is unlikely that stimuli from other adults are important for generation of the distribution pattern.

Only if one of these prerequisites is fulfilled will there be a high enough probability of interactions between ovipositing

females and other adults for such interactions to be influential even at low population densities.

Two species which seem to fulfill prerequisite (a) are the psyllid Cardiaspina densitexta (at least in the summer and autumn generations - spring adults disperse a few hours after emerging (White, 1970 and 1973)) and the desert locust Schistocerca gregaria. Although females of the summer and autumn generations of C. densitexta seem to have the potential to develop an egg distribution strongly influenced (or perhaps even primarily determined) by interactions between adults, they have not done so, as an ovipositing female is able to perceive other stimuli which are more closely related to the chance her progeny will have of surviving to reproduce (White, 1970). In the desert locust, however, it appears that the perpetuation of gregariousness is of prime importance to the species, so that behaviour which ensures that young hoppers will be in close proximity to each other at hatching has been selected, even at the risk of some egg-pods being laid in soil too dry for them to survive till hatching. Both field (Popov, 1958, and Stower, Popov and Greathead, 1958, cited by Norris, 1963) and laboratory observations (Norris, 1963) have shown that female S. gregaria have a strong tendency to lay their egg-pods close to one another, even in an artificial environment where light, temperature and soil conditions are uniform. Norris (1963) found that when female locusts ready to oviposit were given a choice of sites for

oviposition, one with and one without a group of tethered locusts as decoys, the great majority oviposited near the decoys. She showed that the stimulus from the decoys has visual, chemical (partly olfactory, but mainly chemotactile) and mechanical components and that it acts more by causing females that wander near to or into the group by chance to stay there and by stimulating them to probe the soil and lay, than by attracting them from a distance. Such behaviour may facilitate location of possible oviposition sites when locusts are in a patchy environment where such sites are small and sparse. The first females to find them will remain to oviposit and act as natural decoys for later arrivals in the vicinity. Conversely non-laying individuals aggregate with little reference to soil moisture and Norris found that the tendency to oviposit with the group is capable to overriding the females' usual soil moisture preferences.

2.1.1.2 Species with Variable Egg Distribution Patterns

In many species, however, a female will distribute her eggs in one of several pattern depending on stimuli she receives from the environment; these may include stimuli relating to the number of other individuals of the same species that are in her vicinity. In such species responses to other adults may significantly influence the generation of one or some of the possible patterns.

(a) Example of Negative Interactions

Monro (1967) reported that oviposition holes ("stings") were distributed significantly more evenly among fruit than expected on the hypothesis of random stinging by the adults of Dacus tryoni ovipositing in loquats during spring. He attributed this partly to detection of pre-existing stings by females about to oviposit and consequent inhibition of further oviposition on that fruit, and partly to fighting between females. Pritchard (1969), who studied the egg-laying behaviour of D. tryoni on a variety of fruits (including loquats), concluded however that there was no evidence that the presence of an oviposition hole in a fruit had any inhibitory influence on an individual female's oviposition behaviour. On the contrary, in some of the larger fruits with hard, shiny surfaces, even in the field, females tended to lay predominantly in pre-existing holes, whether made by other female D. tryoni, codlin moths, or an experimenter with a pin. He found that when females were each allowed to oviposit once (alone) on pin-pricked apples, their preference for the lower half of the fruit led to a contagious distribution of use of the holes. When he analysed the distribution among the lower ring of holes only, however, Pritchard found that it was effectively random. Apparently such physical factors are not so important in smaller, softer fruits; females introduced singly into a laboratory cage containing one hundred and twenty-six intact (i.e. not pin-pricked) loquats distributed their ovipositions randomly among the loquats. Thus it appears that the

relatively even distribution Monro found in field samples (Pritchard also found one such field sample but the numbers were too low to be statistically significant) probably resulted from aggressive interactions between adults.

Kobayashi (1965) also concluded that some sort of negative interaction between adults was "spacing out" ovipositing females of Pieris rapae crucivora, as he reported that the distribution of eggs became less aggregated as parental density increased. Although such interactions may possibly be influential at very high densities, evidence from my experiments (Section 2.2.3.2 and Section 5.3.1)does not support his conclusion and an alternative explanation of his results is possible.

(b) Example of Positive Interactions

Positive interactions between adults may be one of the factors contributing to the patchy distribution of eggs (not significantly different from a Negative Binomial distribution) of Drosophila melanogaster found by Del Solar and Palomino (1966 (a) and (b)). Although they apparently considered that oviposition on the medium made it more attractive for further oviposition, their results do not exclude the possibility that gregarious behaviour has some influence on the distribution of eggs. Unfortunately they do not discuss the behaviour of gravid females at all except to say that they do not oviposit simultaneously. In a later paper, Del

Solar (1968) did mention that social interactions between adults could possibly be involved, but he still did not give any evidence. Zwolfer found that females of Altica carduorum, feeding and laying eggs on Cirsium arvense in western France, distributed their eggs so non-randomly that some plants were overloaded to the extent that many of the larvae on them died of starvation, while other plants bore very few larvae or escaped attack altogether. He reported (in the discussion at the end of Birch (1971)) that this distribution resulted from "semi-gregarious" behaviour of adult insects.

Pieris rapae does not fulfill either of prerequisites (i) (a) or (b) above, but a number of studies (Utida et al. (1952) Kobayashi (1957, 1960, 1965) and Harcourt (1961)) have shown that the pattern in which eggs are distributed differs in different environments and especially at different densities (category (ii)) so that interactions between butterflies could contribute significantly to the distribution in some cases.

The experiments described in Sections 2.2.3.1 and 2.2.3.2 were designed to test the null hypothesis that there is no difference between the egg laying behaviour of a female Pieris rapae when she is in the presence of other adults and when she is alone.

2.1.2 Responses to Eggs and Larvae

As in the case of responses to other adults, the literature contains reports that females of some species respond positively, others negatively, to the presence of eggs or larvae, and that they disperse their eggs accordingly. There have been instances however, when the simplest explanation of an egg-distribution has seemed to be that females are responding to eggs or larvae yet it has been shown that this is not the true cause.

2.1.2.1 Examples of Responses

Monro (1967) found that in areas favourable to both the moth Cactoblastis cactorum and its host plant Optunia inermis female moths clustered their egg-sticks preferentially on some plants instead of spreading them evenly or at random among the available plants. His initial hypothesis implied that the moths were probably clumping their egg-sticks in response to existing egg-sticks, but he now has evidence (personal communication, and Birch (1971)) that this is not so, environmental stimuli other than members of the same species apparently being the cause of clumping.

On the other hand Clark (1963) found that the presence of eggs of the psyllid Cardiaspina albitextura increased the attractiveness of leaves of Eucalyptus blakelyi to ovipositing females of that species, in proportion to the number of eggs laid on them. White (1970) studied a related psyllid, C. densitexta, on the pink gum

E. fasiculosa. He found that individual females of this species, if undisturbed or if isolated in organdie bags, usually laid their eggs in groups with a characteristic spacing between eggs, so that even at very low population density eggs seldon if ever occurred in isolation. There was one exception. On the broad "juvenile" form of leaf produced by epicormic or sucker growth eggs frequently occurred singly and widely scattered over the entire surface of the leaf. But on the mature leaves on which most eggs were laid, if there were a pit, gall, old lerp, or a few eggs of another species of psyllid on the middle or tip third of the leaf, where C. densitexta eggs are not usually found, such an irregularity on the surface provided a nucleus around which large groups of eggs were laid. The protrusion of the mid-rib of the leaf above the blade at the base of the leaf (where eggs were usually concentrated), seemed to provide a similar tactile stimulus (as would C. densitexta eggs themselves, once present). Possibly females of C. albitextura were also responding to the tactile stinulus of surface irregularities when they laid preferentially on those leaves already bearing more eggs. There is probably an advantage in aggregation of the early instar nymphs (due to the effect their feeding has on local tissue) (White (1970a)) so that the response to surface irregularities has probably been selected because they give the same tactile stimulus as eggs. There is a slight parallel here with the oviposition behaviour of Schistocerca described above in which the female's

response to the group stimulus could override her choice of suitably moist soil. The response of female Cardiaspina to a "false" tactile stimuls (i.e. not from eggs) may cause her to lay on the middle or tip third of the leaf which White (1970) suggested may not be quite as favourable for the larvae as the basal third, where eggs are usually found.

But there is an important difference between the behaviour of females of Cardiaspina and Schistocerca. Although the psyllids' response to physico-tactile stimuli (and hence to eggs) may influence the distribution of eggs on a particular leaf, another response also appears to be involved. White gave evidence that the latter is more likely to be a nutritional stimulus from the leaf itself, on which the ovipositing females, as well as their progeny, feed. This would explain the distribution of eggs on "juvenile" form leaves whose physiological condition is probably more homogeneous over the entire leaf. The latter response is the primary determinant of how the psyllids' eggs are distributed among the available leaves.

The stimuli that an ovipositing female receives from eggs or larvae may also be indirect. If the substrate on which the females of a species normally lay (e.g. the specific host plant, in the case of a phytophagous insect) varies in quality so that some samples are more and some less favourable for oviposition, the presence of eggs or larvae may cause a change, perceptible to a gravid female,

in the quality of the sample itself. Alternatively there may be a "token" stimulus (e.g. a pheromone) associated with the presence of eggs or larvae, which makes a sample more or less attractive than its own intrinsic quality (whether or not this has been changed by the presence of eggs or larvae).

It is well known that when they insert their eggs, some parasitic hymenoptera mark the host insect with a pheromone that inhibits further oviposition into the same host, so that the resulting distribution of parasite eggs among hosts tends to be more even than random.

As mentioned earlier Del Solar and Palomino (1966a) concluded that oviposition by D. melanogaster had somehow "conditioned" the substrate on which eggs had been laid so that it was more favourable for subsequent oviposition than substrates not bearing eggs. They also found that if larvae of the same or a related species (D. funebris) were present in only some of the vials available for oviposition, the vials containing larvae were chosen preferentially by ovipositing females. Although feeding by Drosophila larvae is known to "condition" the medium so that it becomes more favourable for other larvae (Weisbrot 1966, cited by Del Solar 1968) it does not necessarily follow that ovipositing females are responding to such a chemical or physical change in the medium itself. Alternatively they may be responding directly to the eggs or larvae, or

to a pheromone on (or released by) them. (Del Solar and Palomino 1966b).

2.1.2.2 Responses in Pieris rapae

Harcourt (1961), working on P. rapae, studied distributions whose mean densities ranged from 0.14 to 29.92 eggs/plant. He found that at densities higher than about 2 eggs/plant the frequency distribution of eggs/plant was significantly more patchy than random and did not differ significantly from a negative binomial distribution. At the lower densities (i.e. 0.14 → 2 eggs per plant) the distribution of eggs did not differ significantly from either a Poisson (i.e. random) distribution or a negative binomial distribution, but at such low densities the sampling variation is so great that one cannot expect a significant difference from random unless the distribution is really extremely clumped. Kobayashi (1965, 1966) also found that in most of his experiments with P. rapae, the distribution of eggs fitted a negative binomial and not a Poisson distribution, even for mean densities as low as 0.14 - 0.62 eggs/plant, laid in each case by a single female.

Section 2.2.1 describes the experiments in which I sought to determine whether this patchiness results from a positive response to eggs, and Section 2.2.2 the experiments to determine whether it results from a response to larvae (either directly or as a response

to the effect on plants of larval feeding) by ovipositing females. Three distinct hypotheses are involved:

(i) The ovipositing females respond positively to eggs on a plant.

(ii) They respond to other variables in their environment less strongly than they respond to eggs (except in extreme situations e.g. strong winds or strong sun/shade contrasts).

(iii) Under normal conditions, when both sorts of stimuli are present, the stronger response alone accounts for the observed distribution of eggs. (When considering responses to larvae, substitute "larvae" for "eggs" in (i) and (ii) and omit the word "positively" from (i)). Ideally it would be best to use separate experiments to test these hypotheses but in practice it was almost impossible to separate them.

2.2.1 Experiments on the Females' Egg-laying Response to Eggs

2.2.1.1 Pilot Experiment on Responses by Groups of Females

If the butterflies are behaving as predicted by the above hypotheses; then one would expect them to lay more of their eggs on plants that already bear eggs than on others which do not, when given simultaneous access to both. To test whether they do

I started with a pilot experiment on the open roof of the Zoology building.

Method:

Sixteen brussels sprout plants (each with 11 to 18 leaves) were arranged in a 4 x 4 latin square in the tray and cage described in Section 1.2.2, and 5 pots of Alyssum were distributed among them. The pilot experiment consisted of two replicates, in each of which (as shown in Table 2.01) the butterflies were put in the cage, to lay eggs on the plants, twice; that is, there were two "oviposition periods" (O.P.'s) per replicate).

TABLE 2.01

The number of butterflies released, duration of O.P.'s and range of ambient temperature during the pilot experiment

Rep.	O.P.	No.of Butterflies[#]		Released		Recollected		Temp. °C	
		Females	Males	Time	Date	Time	Date	Max.	Min.
1	(a)	17(4*)	12(4)	15.30	13/4	15.30	14/4	32.2	24.0
	(b)	20(9)	15(12)	15.40	16/4	13.00	17/4	35.6	17.8
2	(a)	5(0)	5(2)	12.30	28/4	17.45	30/4	23.3	10.4
	(b)	24(3)	18(2)	16.30	1/5	16.55	2/5	25.6	12.2

*Number that died during O.P. given in parenthesis.

[#]All butterflies used in this experiment had been caught in the field.

During the first O.P. of each replicate eight of the plants were covered with individual gauze cylinders so that there were two covered and two uncovered plants in each row and each column of the latin square. After the first O.P. the eggs laid on the uncovered plants were counted and the covers were removed from the remaining plants. All plants were then re-randomized among positions (still retaining two plants from each treatment in each row and each column) before introducing the butterflies into the cage again - this time with all plants accessible to them.

Results:

TABLE 2.02

Number of eggs laid per plant, their treatment and position in O.P. (b) of Replicate 1

51 C*	55 U	33 C	190 U
44 U	48 U	102 C	66 C
55 U	28 C	23 U	109 C
54 C	22 C	25 U	81 U

N ↑

*C = "covered" treatment

U = "uncovered" treatment

TABLE 2.03

Number of eggs laid per plant, their treatment and position in O.P. (b) of replicate 2

9 U	10 C	23 C	30 U
2 U	2 U	13 C	15 C
4 C	5 U	2 U	30 C
5 C	2 C	6 U	3 U

N ↑

Analysis of variance of the results of first replicate (Table 2.02) showed that there was no significant difference between the numbers of eggs added in O.P. (b) to plants already bearing eggs from the first O.P. and to those that had been covered, but the residual variance was very high.

In the second O.P. of the second replicate (Table 2.03) significantly more eggs were laid on plants in the "covered" treatment - that is on those <u>not</u> bearing eggs at the start of the O.P. ($F^{1}_{8} = 7.136$, $0.01 < P < 0.05$). Tables 2.02 and 2.03 show, however, that in both replicates plants in the north-east and east parts of the cage gained the most eggs, and in the second replicate, those

five of the eight "covered" plants which bore more eggs were in the most north-easterly and easterly positions in the cage. During the experiment winds were predominantly from the west and sometimes south-west and although released in the early to mid-afternoon, the butterflies took no notice of the plants (just fluttered against the cage) until next morning. So I concluded that the apparently significant effect of the treatment was probably spurious.

Hovanitz and Chang (1964) had reported that the sun and wind can influence Pieris rapae's distribution of eggs; these results show that their influence may be so strong that the females' responses to them may be, at least temporarily, dominant over any other preferences they may have when the environment is more nearly uniform. Consequently I did all subsequent experiments indoors.

2.2.1.2 First Indoor Experiment on Responses by Groups of Females

Method:

Twenty-four plants were prepared for the experiment as follows:

(i) The leaves of all twenty-four plants (most plants had between ten and twenty-two leaves) were measured.

(ii) Butterflies were allowed to lay on fifteen of the plants, in cages on the roof, so that as in the pilot experiment, a wide range in the numbers of eggs per plant resulted. The eggs were counted and left on the plants.

All twenty-four plants were then randomized among twenty-four positions (in an array of four rows of four pots per row, alternating with three rows of three pots per row, with the central position of the central row of three, occupied by a pot of Alyssum, in the set-up described in Section 1.2.2). Six more pots of Alyssum were added, two at each end and one at each side outside the tray but within the cage. The lights were set on a 16 hours light/8 hours dark photoperiod, light between 05.00 hours and 21.00 hours.

Butterflies (as shown in Table 2.04) were allowed to lay on the plants (O.P.(a)) and the eggs counted, but once again, left on the plants, which were then re-randomized among positions before being exposed to the butterflies again (O.P. (b)). After O.P. (b) all eggs and young larvae (most of the eggs laid before the experiment had hatched before the end of O.P. (b)) were counted, then all leaves were measured again. (By then most plants had 20 to 32 leaves).

TABLE 2.04

The number of butterflies tested for a response to eggs. (First Experiment).

O.P.	No. of Butterflies#		Released		Recollected	
	Females	Males	Time	Date	Time	Date
(a)	30(2*)	16(6)	<05.00	3/6	18.15	3/6
(b)	36(24)	19(16)	01.45	5/6	15.30	6/6

*Number that died during O.P. given in parenthesis. (In O.P.(b)

at least half of the butterflies appeared to have died by 19.00 hours on 5/6).

#All butterflies used in this experiment were first generation reared in the laboratory.

Results and Discussion:

The results were analysed first at the level of additions of eggs to individual leaves. Table 2.05 shows that there was a significant positive association between the presence of eggs on a leaf and the likelihood of eggs being added to it, in O.P. (a).

TABLE 2.05

Association between the presence of eggs on a leaf and addition of more eggs to it, in O.P. (a)

Number of Leaves with			Initial Eggs: None 0	Few 1-4	Many >5	Total
Eggs Added	None	E*	278.6	66.0	25.4	370
		0	303	54	13	
	Some	E	105.4	25.0	9.6	140
		0	81	37	22	
Total			384	91	35	510
X^2_2	37.8		P		<0.001	

*E - expected number of leaves
0 = observed number of leaves

If it is postulated that females are responding to eggs as such, but only if they encounter them by chance when fluttering

around plants, rather than actively searching for them, then at any one time a butterfly is only likely to receive stimuli from one surface of any particular leaf. Re-analysis of the results for eggs present on, and added to, individual surfaces in O.P. (a) shows (Table 2.06) that there is still a significant association.

TABLE 2.06

Association between the presence of eggs on a leaf surface and addition of more eggs to it, in O.P. (a)

Number of surfaces with			Initial Eggs None	Initial Eggs Some	Total
Eggs Added	None	E	813.9	133.1	
		O	842	105	947
	Some	E	140.1	22.9	
		O	112	51	163
Total			954	156	1110
χ^2_1	47.0		P	<0.001	

In O.P. (b) there were only five plants (three "controls", i.e. those that did not receive eggs till (a), and two which had been laid on before O.P. (a)) on which no eggs had hatched by the time eggs were counted after O.P. (b). Consequently, only the results from these five were able to be analysed in the same way as the O.P. (a) results, as the larvae had often moved from the surface on which they had hatched; also there was no way of knowing whether they had hatched during or after O.P. (b), and it is not

valid to assume that the influence (if any) of larvae, is the same as that (if any) of eggs.

Because the results from only five plants were analysed, the total number of surfaces bearing eggs from O.P. (a) or earlier, and of surfaces gaining eggs in (b), were so low that the number of surfaces bearing eggs that would be expected to have gained eggs in (b) was less than five. (See Table 2.07). But, as the total number of observations (surfaces) is greater than 40, calculation of X^2 by the usual method is valid so long as Yates' correction for continuity is used (Cochran 1952).

TABLE 2.07

Association between the presence of eggs on a leaf surface and addition of more eggs to it, in O.P. (b)

Number of Surfaces with			Initial eggs plus Eggs Laid in O.P.(a) None	Some	Total
Eggs Added in O.P.(b)	None	E	168.30	29.70	198
		O	175	23	
	Some	E	18.70	3.3	22
		O	12	10	
Total			187	33	220
X^2	15.23		P	<0.001	

Table 2.07 shows that in O.P. (b) there was a significant association between the presence of eggs on a surface and the likeliehood

of more eggs being added to it.

If these associations were simply the result of a direct response to the presence of eggs then such an association would also be expected at the level of whole plants. But analysis of the distribution of eggs added in both O.P.'s (Tables 2.08 and 2.09) showed that there was no evidence of any association at all at the level of whole plants. In other words a butterfly was no more likely to lay an egg on a plant with eggs already on it than on one without any, but if she laid an egg on a plant that already had eggs on it, she was likely to choose a leaf that already had an egg on it.

TABLE 2.08

Independence of the addition of eggs to plants from the prior presence of eggs on the plants, in O.P. (a)

Number of Plants with			Initial Eggs None	Initial Eggs Some	Total
Eggs Added	1 → 10	E	4.5	7.5	12
		O	4	8	
	≥ 11	E	4.5	7.5	
		O	5	7	12
Total			9	15	24

TABLE 2.09

Independence of the addition of eggs to plants from the number of eggs already on the plants, in O.P. (b)

Number of Plants with			Initial Eggs Plus Eggs Laid in O.P. (a)		Total
			1→20	≥ 21	
Eggs Added	1 → 8	E	6.3	5.7	12
		O	7	5	
	≥ 9	E	5.7	5.3	
		O	5	6	11
Total			12	11	23*
χ^2_1	< 1.0		P	> 0.3 i.e. NS	

*One plant was omitted from this analysis as loss of eggs during O.P. (b) made it impossible to determine to which class it belonged.

The association found between the presence of eggs on, and their addition to, individual leaves and surfaces of leaves can be interpreted therefore, as evidence of differences in the intrinsic favourableness for oviposition of the leaves and surfaces, independent of the presence of eggs on them. (Although a leaf's favourableness as a site for the oviposition does not depend on whether there are eggs on the leaf or not, it does not necessarily follow that stimuli from eggs on leaves do not influence the butterflies' choice at all). Although the number of eggs added to a plant was independent of how many eggs were already present, the distribution of added eggs, among plants, was neither random, nor more even than

random, in either O.P., but significantly patchy (χ^2_{23}= 113.46, $P < 0.001$, for O.P. (a); $\chi^2_{23} = 99.07$, $P < 0.001$, for O.P. (b)).

Two mutually exclusive explanations of these results are possible:

(1) That in a moderately homogeneous environment such as in the experiment, (although there was spatial variation in light intensity and probably also in temperature and relative humidity, at least, if not other components of the environment, it was not extreme variation as in the pilot experiment done on the roof), a patchy distribution of eggs among plants is not the result of a butterfly's preferences for some plants over others, but a consequence of an innate irregularity in the egg-laying behaviour of the butterfly. Kobayashi (1966) found that although females of Pieris rapae crucivora visited plants at random the distribution of eggs per plant that resulted was patchy, not differing significantly from a negative binomial distribution. This was because the number of eggs a butterfly laid per visit was also distributed non-randomly, fitting a logarithmic distribution.

(2) That in each O.P. the females did prefer some plants to others, but they did not prefer the same plants in both O.P.'s. This could occur for any of the following reasons (which are not mutually exclusive):

(i) If the individual females in a group of butterflies differ with regard to the stimuli they prefer to receive from a site for oviposition, (e.g. stimuli related to light intensity, relative humidity, amount of wind or shelter, physical or physiological condition of the host plant, presence of eggs, larvae, or other adults), then the pattern in which the eggs laid by that group are distributed will probably change as the composition of the group changes. All the females that had already been used in O.P. (a) died during O.P. (b) (being laboratory-reared, they were all more or less the same age), so that the majority of eggs laid in O.P. (b) were probably laid by the twelve younger females that had not been used before. Thus differences in preferences between different females may have been partly or wholly responsible for the difference in which plants gained more eggs in the two O.P.s.

(ii) If, on the other hand, most female P. rapae prefer the same stimuli from a site for oviposition the results could be explained by a change, between O.P.s, in either or both of:

(a) The relative attractiveness of plants themselves. Such a change could only result from a change in the relative physiological states of the plants, either alone or associated with a change in their relative

rates of growth (and hence relative sizes, amounts of new foliage, etc.). The plants' relative rates of growth did change during the experiment (if they are ranked in order of size for the measurements taken before the experiment and again afterward, nine of the twenty-four plants changed their relative size by four or more ranks, two of these by six, and one each by seven and eight ranks). Nevertheless, as the two measurements were taken twelve to seventeen days apart and the interval between O.P.s (a) and (b) was less than one and a half days (31h. 30m.) and less than the duration of O.P. (b) (37h. 45m.), it seems most unlikely that there was any significant change either in relative growth or physiological condition, between O.P.s (a) and (b).

(b) The quality of the micro-environment of each plant. As plants occupied different positions in the array in the two O.P.'s and measurement of light intensity under the bank of lights showed that there were gradients from higher light intensities near the centre to lower light intensities at the ends and sides, this alternative seems more likely than (a). But there was no tendency for plants near the centre of the array to gain more eggs than those near the edges, and although there seemed to be a slight tendency for positions favoured in O.P. (a) to be favoured again in O.P. (b), it was not significant ($\chi_1^2 = 1.51, P>0.2$).

If, however, the butterflies were responding to a combination of stimuli of different strengths from the plants, their micro-environment, and possibly even from eggs, sometimes the different stimuli would conflict with, and at other times reinforce, each other so that results such as those obtained could occur.

If the butterflies were responding to eggs their response could either be:

(a) A density dependent response (i.e. the more eggs she encountered on a leaf surface, the stronger would be her response - in terms of the number of eggs she would lay on that leaf surface). Or,

(b) An all-or-nothing response independent of density (i.e. whether a butterfly encountered only one egg, or many, on a leaf surface, she would respond with the same intensity.

If a female's response to the eggs on each leaf surface that she encounters depends on the number of eggs on that surface, then apart from differences in the frequency with which she visits different plants, her total response to the eggs on a whole plant would depend on both (i) the proportion of all leaves on that plant that bear eggs, and (ii) the mean number of eggs per leaf that bears eggs. So, if she were exposed to a group of plants, some with many eggs and others with only a few on them, and if, for the moment, we consider only that component of her response to

the plants that is due to eggs, rather than her total response, it is likely that her response to the plants with many eggs would be stronger than that to those with fewer eggs; and the response would be largely independent of how the eggs were distributed among the leaves of each plant.

On the other hand, if a female's response to the eggs on each leaf surface that she encounters is independent of the number of eggs on that surface, then her total response to the eggs on a whole plant would depend only on (i) above, that is, only on the proportion of all leaves on that plant that bear eggs. Thus, if she were exposed to plants with many and few eggs, as above, her responses to the plants would be influenced more by the distribution of eggs among the leaves of each plant than by the total number of eggs on it - except perhaps when the total number of eggs on a plant is so high that even in spite of a patchy distribution most leaves bear at least one egg, or so low that in spite of a random or more even distribution, only a low proportion of all leaves on the plant bear eggs.

In summary, then, the results of this experiment do not distinguish between alternative hypotheses at several levels as shown in Table 2.10. Even if the arrangement of plants and their relative physical and physiological condition, and all "micro-weather" conditions of the environment, all remained constant, and the same butterflies were laying eggs each time, the probability that the

TABLE 2.10

Alternative hypotheses to explain the results of the first indoor egg experiment

Level	Alternatives			
(a)	Butterflies behave according to Kobayashi's description of P.rapae crucivora	Butterflies prefer some plants to others in each O.P.		
(b)		Females' preferences differ widely i.e. they are not normally distributed	Normal distribution of females' preferences; variance not very large. Preferences may change between O.P.s because they depend on interactions between responses to plants, their micro-environment and perhaps eggs	
(c)			No response to eggs	Response to eggs
(d)				Density Dependent / Density Independent

same plants (and even mostly the same leaves) would receive most eggs in each of a number of replicates, would be extremely low if the butterflies were behaving as Kobayashi reported.

At level (b), even if the left-hand alternative is true, it is likely that the population of butterflies, whose preferences are not normally distributed, will comprise several sub-populations (divided only with respect to their preferences) with different

mean preferences. Within any one of these sub-populations there is likely to be a normal distribution of preferences. Thus although at each of the other levels, ((a), (c) and (d)) the two alternatives are mutually exclusive, at (b) they are not; the question really being whether the right hand alternative is applicable to the total population under consideration, or just to each of the sub-populations from it, separately.

As shown in Appendix 2.1 the same experimental method can be used to test both levels (a) and (d) of Table 2.10, and though not a critical test of level (c) it may give some indication of whether the butterflies respond to eggs. So I did another experiment, with three oviposition periods, throughout which the same butterflies were used and the plants remained in the same positions.

2.2.1.3 Second Indoor Experiment on Responses by Groups of Females

As shown in Table 2.11, not many butterflies died during the experiment so there was no need to replace those that did.

The twenty sprout plants used were randomized among the positions of a 4 x 5 array in the set-up described in Section 1.2.2 (except that the cage was now only 30" high).

TABLE 2.11

The number of butterflies tested for a response to eggs (second experiment).

O.P.	No. of Butterflies*		Released		Recollected	
	Females	Males	Time	Date	Time	Date
(a)	18(3)	22(10)	01.05	2/4	22.00	3/4
(b)	15(2)	12(2)	13.15	5/4	13.40	6/4
(c)	13(2)	10(2)	03.25	7/4	11.35	8/4

*All butterflies used in this experiment had been caught in the field.

The tray was very carefully centred under the bank of light and the position of each marked on the floor to ensure that although the lights were wheeled away between O.P.'s to give access to the plants, they would always be in exactly the same position relative to the tray during O.P.'s. The eggs were counted after each O.P.; after (a) they were removed when counted, but after (b) they were left on the plants for (c).

Results and Discussion:

TABLE 2.12

Numbers of eggs laid during the three oviposition periods of the second experiment

Plant No.	O.P. When eggs Laid			Plant No.	O.P. when Eggs Laid		
	(a)	(b)	(c)		(a)	(b)	(c)
1	19	6	13	11	11	4	14
2	15	3	18	12	10	6	27
3	4	8	9	13	4	7	25
4	2	2	8	14	4	6	8
5	6	7	17	15	4	5	6
6	10	9	4	16	51	9	28
7	16	19	39	17	3	3	4
8	36	8	11	18	25	3	8
9	2	4	5	19	48	8	5
10	2	4	6	20	7	6	8

If plants were ranked in each O.P. according to the number of eggs laid on them there was a significant concordance between their ranks in the three O.P.'s. ($W = 0.598$, $0.01 < P < 0.02$, where W = Kendall's coefficient of concordance (Siegel, 1956)). Thus the butterflies were laying predominantly on the same plants in all three O.P.'s showing that the left-hand hypothesis in Table 2.10, level (a), that an intrinsic behaviour pattern independent of stimuli from the plants causes them to lay their eggs in a contagious distribution, is highly improbable. The concordance is better explained by the alternative hypothesis that on the whole the butterflies preferred the same plants in all three O.P.'s - although at the start of O.P. (b) there were no eggs on the plants, while in (c) there were eggs remaining from (b).

Also when the plants were ranked in each O.P. according to the number of eggs they received (as for the concordance test) there was a significant correlation between their ranks in O.P. (a) and O.P. (b) ($T = 0.363$, $z = 2.237$, $P < 0.01$, where T = Kendall's rank correlation coefficient (Siegel, 1956)), but the correlation between their ranks in O.P. (b) and O.P. (c) is not significant ($T = 0.243$, $z = 1.499$, $P > 0.06$). Thus in O.P. (c) it was not always the plants with the highest number of eggs on them (from O.P. (b)) to which the butterflies responded most strongly. If part of the butterflies' total response was a response to eggs, therefore, these results suggest (for the reasons given in Appendix 2.1) that it was an all-or-nothing response, independent of

the number of eggs on the leaves encountered, stimulated only by the presence of an egg on a leaf. But when analysed this way, the results are equally consistent with the hypothesis that the butterflies do not respond to eggs at all - the only hypothesis they have eliminated is that of a density-dependent response to eggs by the butterflies.

The results were also analysed at the level of additions to individual leaf surfaces; any leaves that were not present in all three O.P.'s were excluded from the analysis. (Such leaves belonged to one of two categories - old leaves that died and fell off before O.P.'s (b) or (c), some of which had gained eggs while present, and new leaves that uncurled from the central sprout during the experiment. There were usually only three or four such leaves per plant at the most, and only two of all such leaves gained any eggs - one each - so that their omission seems unlikely to bias the results). The results from one whole plant also had to be omitted from this analysis as it was not possible to determine the relationship between leaf numbers in the records for egg-counts after(a), (b) and (c) as an unknown number of leaves had been lost between O.P.'s.

As in the first experiment, there was a significant association between the presence of eggs on a leaf surface (remaining from O.P. (b)) and the likelihood of more eggs being added to it in O.P. (c). (Table 2.14). But, as would be expected from the analysis at

the level of whole plants, there was also a significant association between a leaf being preferred (i.e. gaining eggs) in O.P. (a) and in O.P. (b) (Table 2.13).

TABLE 2.13

Association between the addition of eggs to a leaf surface in O.P. (a) and in O.P. (b)*

Number of Surfaces with			Laid in O.P.(a) Eggs	No Eggs	Total
Laid in O.P.(b)	Eggs	E	13.51	79.49	93
		O	24	69	
	No eggs	E	131.49	773.51	905
		O	121	784	
Total			145	853	998
X_1^2	10.51		$0.001 < P < 0.01$		

*Eggs laid in (a) were removed before (b).

TABLE 2.14

Association between the addition of eggs to a leaf surface in O.P. (b) and O.P. (c)*

Number of Surfaces with			Laid in O.P. (b) Eggs	No Eggs	Total
Laid in O.P.(c)	Eggs	E	13.98	136.02	150
		O	31	119	
	No Eggs	E	79.02	768.98	848
		O	62	786	
Total			93	905	998
X_1^2	26.89		P	< 0.001	

*Eggs laid in (b) were left on leaves through (c).

The strength of associations shown by different contingency tables can be compared using the "mean square contingency" (χ^2/n, where n is the total number of observations) divided by the number of degrees of freedom. But n, and the number of degrees of freedom, both have the same values in Table 2.12 as they have in Table 2.13, so that direct comparison of the χ^2 values is valid. Thus the association shown in Table 2.13 is stronger than that shown in Table 2.12. Does this mean that, although the association is not dependent on a response to eggs, it is being significantly reinforced by such a response in (c)?

Alternatively it is possible that the difference between these two χ^2 values is simply due to sampling variation (of the χ^2 values themselves) and therefore does not indicate a significant influence from eggs. It is possible to determine whether two values of χ^2 differ significantly by comparing their mean squares by a two-tailed variance ratio test. As both values of χ^2 have only one degree of freedom the variance ratio is simply the ratio of the two χ^2values, that is, 2.56. But the probability of a "two-tailed" F value of less than 9.47 is greater than 40%, when each mean square has only one degree of freedom. Thus there is certainly no <u>significant</u> difference between the strength of the association when eggs were already present on the plants at the start of the O.P. and that when they were not.

The degree of association between O.P.s (b) and (c) is probably

stronger than that between O.P.s (a) and (b) simply because more eggs were laid in O.P. (c) than in O.P. (b), so that, as mentioned in Appendix 2.1, the increased number of visits at which eggs were laid increased the influence of choice on the distribution of eggs, and decreased that of chance, in O.P. (c) compared with O.P. (b). There is therefore no reason to believe that the butterflies were responding to the presence of eggs on some of the leaves, in O.P. (c), they were simply responding to the same stimuli that made them choose those leaves in O.P. (b), when eggs were not initially present.

2.2.1.4 Experiment on the Response to the Presence of Eggs by a Lone Female

Introduction:

When the distribution of a population of eggs is being studied, it may sometimes happen that all the eggs were laid by only a very few females, each alone when laying, or more rarely, all by the same female with no other adults present. Under such circumstances an interpretation based on the experiments with groups of butterflies may be misleading, as the hypothesis at level (b) of Table 2.10, that females differ in their preferences for certain stimuli from a site for oviposition, was not tested by those experiments. Thus it is not completely justifiable to assess the responses of an individual (even one in the group - let alone a female on her own) only by those of a group.

Females should be tested individually, to assess their responses when alone, and then the behaviour of individual females observed and tested when they are in company, to determine whether their individual responses are modified by the group. Experiments on the latter topic - interactions between butterflies in groups - are described in Section 2.3. As for the former - ideally a large number of females should be tested individually, but due to a shortage of time and animals I was unable to do the planned experiment and have only the results of a pilot experiment done before I had learnt the techniques of working with single females. It is not justifiable, therefore, to extrapolate from these results; they are included only because the butterfly's apparently extremely atypical behaviour gives an indication of how widely the butterflies' responses may perhaps differ, whether or not they are distributed in a single normal distribution, and even if only an extremely small proportion of the population behave as this female appears to.

Method:

Ten brussels sprout plants were arranged in four rows with two and three plants per row alternately in the tray and cage as described in Section 1.2.2, with eight pots of flowering Alyssum placed regularly among them. As the female seemed rather sluggish and inactive when put in the cage to feed before the sprout plants were put in, two extra lights (200W incandescent) were added, one

each side of the usual bank of lights, shining more horizontally than vertically into the cage. They were put in as nearly as possible the same position for each oviposition period. These lights would have introduced quite strong differences in heat and light intensity of different wavelengths giving the plants a much more heterogeneous environment than in other indoor experiments.

The butterfly was put in the cage for four oviposition periods (a), (b), (c) and (d)) of 4 hours, 3/4 hour, 8 1/4 hours and 16 hours, duration respectively (this last was not continuous light but 6 hours light, 4 hours dark and 10 hours light). As in the second indoor experiment with a group of butterflies (Section 2.2.1.3) the eggs were removed when counted after the first O.P. but left on the plants after the second, through O.P. (c).

On some plants the under surfaces of the leaves were predominantly concave, on others, convex. Previous observations had suggested that butterflies generally found it easier to lay eggs on under surfaces that were concave, so after counting and removing the eggs after O.P. (c) I changed the predominant direction of curvature of the leaves on some of the plants. (It was not possible to change the curvature of some leaves and others grew so vertically that upper and under surfaces were effectively inner and outer surfaces). The plants occupied the same positions in all four O.P.'s.

Results and Discussion:

In O.P. (a) only two of the 101 upper surfaces of leaves received eggs - only one egg each - the other 42 eggs being laid on 24 of the 101 under surfaces. In O.P.'s (b) and (c) none of the upper surfaces of leaves received eggs, and in O.P. (d), when many more eggs were laid, only two upper surfaces received eggs - again only one each. In general female Pieris rapae tend to lay more of their eggs on under than upper surfaces of leaves, but not so exclusively as this female. In an analysis (as for the experiments in Sections 2.2.1.2 and 2.2.1.3)to determine whether the butterfly preferred the same leaf surfaces in several O.P.s, inclusion of results from all available surfaces, both upper and under, would only tell us what we already know - that she did prefer under surfaces and avoid upper surfaces each time. But to determine whether she preferred the same under surfaces in each O.P. it is necessary to omit the upper surfaces from the analysis, altogether.

Table 2.15 shows that unlike the females inthe group of butterflies used for the second experiment, the female used in this experiment did not prefer the same leaf surfaces in two consecutive O.P.'s when there were no eggs on the plants at the start of either. (Analysis of Experiment II (Section 2.2.1.3) results for O.P.'s (a) and (b) using only under surfaces did not change the level of significance from that shown in Table 2.13). Table 2.16 shows, however, that when eggs were already present on some leaves from a previous O.P., the

butterfly apparently preferred the leaves already bearing eggs, as she laid eggs on significantly more of them and less of the "empty" leaves than would be expected if she were distributing her eggs at random.

TABLE 2.15

Independence of a lone female's choices of leaf surfaces on which to lay eggs in two consecutive O.P.'s, when eggs were removed between O.P.'s

Number of Surfaces with			Laid in O.P. (a) Eggs	Laid in O.P. (a) No Eggs	Total
Laid in O.P. (b)	Eggs	E	4.5*	14.5	19
		O	6	13	
	No Eggs	E	19.5	62.5	82
		O	18	64	
	Totals		24	77	101
X_1^2	0.36		P	> 0.5 i.e. N.S.	

*Expected value <5 does not invalidate analysis for reasons given with Table 2.07.

Also unlike the second experiment with a group of females, analysis of the distribution of eggs added to whole plants showed a similar trend to that shown by analysis at the level of leaf surfaces. Although neither correlation was significant, the correlation between the rank of a plant in O.P. (b) and its rank in O.P. (c), when plants were ranked according to the number of eggs added

TABLE 2.16

Association between a lone female's choices of leaf surfaces on which to lay eggs in two consecutive O.P.s when eggs from the first O.P. were left on the plants through the second

Number of Surfaces with			Laid in O.P.(b) Eggs	No Eggs	Totals
Laid in O.P. (c)	Eggs	E	3.0*	13.0	
		O	8	8	16
	No Eggs	E	16.0	69.0	
		O	11	74	85
	Totals		19	82	101
χ^2_1	9.87		0.001 < P < 0.01		

* See footnote to Table 2.15.

to them in each O.P., was very nearly significant (T = 0.528, S = +19, and from Table Q in Siegel 1956, P = 0.054) and much greater than the correlation between their ranks in O.P. (a) and O.P. (b), which was far from significant (T = 0.178, S = +7 and .˙. P = 0.3).

The female's apparent lack of preferences for, or consistent responses to, certain plants or leaves (in the absence of eggs) which these results show, would, if it is true, make the test on the effect of changing the shape of leaves irrelevant to a study of this particular female's egg-laying behaviour. As for the first two O.P.'s, the addition of eggs to leaf surfaces in O.P. (d) was independent of whether they had received them in O.P. (a) or O.P.

(b) ($\chi^2_1 = 2.44$, $0.1 < P < 0.2$, for the test of association between O.P.'s (a) and (d); and $\chi^2_1 = 1.06$, $0.3 < P < 0.5$, for the test between O.P.s (b) and (d)). But it would be circular reasoning to take the results from O.P. (d) as further evidence that the butterfly did not respond consistently to stimuli from the plants or their leaves, as the stimuli themselves were changed when the direction of curvature of the leaves was changed. Thus there was only one valid comparison each, testing for a response to the plants (or their individual leaves) and their micro-environment, on the one hand (the comparison of O.P. (a) with O.P. (b)), and for a response to eggs, on the other (the comparison of O.P. (b) to O.P. (c)). These tests would have to be replicated before it would be valid to draw firm conclusions about the behaviour of even this individual female, especially as her behaviour seems to be so atypical. It seems rather unlikely that there could be selection for butterflies that did not discriminate between plants (so long as they were of the host species) and yet would respond positively to eggs, as with such responses many eggs could be laid on plants that were inadequate for larval development.

2.2.1.5 Conclusions from all Experiments testing Whether Ovipositing Females Respond to Eggs

The only really critical way to test whether the butterflies respond to eggs per se would be for the experimenter (rather

than the butterfly) to attach eggs completely at random on the plants, not only on those plants or plant-parts on which the butterflies chose to lay. In this way a response to eggs could be completely distinguished from a response to plants. But, even apart from the technical difficulties (such as side effects from the glue, wax, or whatever is used to attach the eggs in their new position, on (i) the eggs, (ii) the plant - perhaps altering the attractiveness of both of these to the butterfly - or (iii) on the butterflies themselves) of this method, it is testing a situation that never occurs naturally, so that the errors involved in extrapolating from such results to a real situation are probably no less than those due to having two related variable sources of stimuli (eggs and plants) in the same experiment. In nature, females of Pieris rapae lay their eggs almost exclusively on their host plants, so that eggs would always be associated with a plant, or plants-part that either was, or at least had been acceptable fo oviposition (in that an ovipositing female had either chosen it actively or at least not avoided it when laying).

In the first experiment (Section 2.2.1.2), although plants that had apparently been most favourable (both intrinsically and due to their position in the array) in O.P. (a) had received most eggs then, and the eggs had been left on them, for O.P. (b), the presence of those eggs did not enable the plants to receive the majority of eggs in O.P. (b), once they had been moved into less

favourable positions. Thus the butterflies do not have a response to eggs that is capable of overriding opposite responses to stimuli from the plants or their micro-environment. Nevertheless a response to eggs could still affect the distribution of eggs if it was strong enough even to partially counteract such responses, and to reinforce parallel responses. But there is no evidence of significant reinforcement of the butterflies' responses to favourable leaves and plants, in the second experiment. Therefore it seems reasonable to conclude that in general ovipositing females of Pieris rapae do not respond to eggs, although, because of the inherent variability of biological populations, there may be rare individuals that do respond to eggs. But even if such a small minority exists, their behaviour would have little effect on the distribution patterns of the eggs of P. rapae in general.

2.2.2 Experiments on the Females' Egg-laying Response to Larvae

2.2.2.1 Experiment on Responses by Groups of Females

Materials and Methods

The leaves of 12 young sprout plants were measured and the plants randomized into a 3 x 4 array. Positions in the array were classified into 3 strata

(1) the four corner)
)
(2) the six edge) positions
)
(3) the 2 central)

and larvae were placed on half of the plants in each stratum immediately before the butterflies were introduced into the cage. There were four replicates. In the second, the larvae were placed on those plants which had not borne them in replicate 1. Similarly, larvae were placed on half of the plants available for oviposition in replicate 3, and then on the other half in replicate 4. Using each group of plants for both treatment and control (alternately) will cancel any biases in the results that could otherwise have occurred if by chance either group of plants was intrinsically more attractive than the other.

The leaves were re-measured and the plants re-randomized among positions before the third replicate, and three plants that had become very sickly after replicates 1 and 2 were replaced. (Replacements are signified by n' instead of n).

Because the leaves were measured as near as possible to the start of the experiment I did not have time to calculate the actual total plant volumes before releasing the larvae, so, to give as nearly as possible densities of the same order on each plant, I estimated approximate plant size by eye (judging by past measurements) and added enough larvae to give about 1 unit larvae per c.c. (range $\simeq 0.5 \rightarrow 1.5$ unit larvae/c.c.; unit larvae are defined in Section 1.2.1.4) in the first two replicates, and about 1 to 2 unit larvae/c.c. in the third and fourth replicates. Table 1 in Appendix 2.2 shows the numbers of larvae (expressed as unit larvae)

put on each plant, the resulting approximate densities (see Section 1.2.1.3) and the leaf area available per unit larva. As Table 2.1.6 shows all but seven of the females used in replicate 1 died before replicate 2, but more butterflies had emerged and been brought into breeding condition by the time I did replicates 3 and 4.

TABLE 2.16

The numbers of butterflies tested for a response to larvae.

Replicate	No. of Butterflies		Origin of	Duration	Between
	Females	Males	Butterflies	of O.P.	(Times of day)
1	31	18	Mixed labora-	10h.30m	09.30 hrs
2	7	7	tory reared and wild caught - mainly the latter	9h.00m.	+ 21.15 hrs of same day
3	43	30	laboratory reared (1st generation)	11h.00m.	
4	40	28		11h.15m.	

Because the total numbers of eggs laid in the replicates differ so, the variances of results from the different replicates differ significantly so that analysis of variance would not be valid unless the data were first transformed. The results were therefore analysed using the simpler non-parametric Mann-Whitney U test. Despite consistent reduction in the numbers of eggs laid on plants bearing larvae the statistical test showed that none of the differences were significant (Table 2.17).

Results and Discussion

TABLE 2.17

Numbers of eggs laid per plant by groups of females, when half the plants bore larvae

Plant No.	Replicate 1	2	Plant No.	Replicate 3	4
	Larvae	No Larvae		Larvae	No Larvae
1	12	5	2	66	22
3	13	1	3	22	18
4	13	3	4	51	71
6	9	7	8'	56	58
7	11	9	9	31	22
12	88	9	11	71	22
	No Larvae	Larvae		No Larvae	Larvae
2	0	0	1'	54	9
5	12	5	5	37	12
8	43	3	6	35	17
9	64	9	7	105	34
10	19	2	10	63	15
11	33	0	12'	65	45
	TREATMENT TOTALS				
Larvae	146	19		297	132
No Larvae	171	34		359	213
Replicate Totals	317	53		656	345
Analysis by Mann-Whitney U-test					
U	13.5	10		15	8
Probability	0.54	0.24		.0.70	0.13

Nevertheless, if the addition of eggs to plants already occupied by larvae and to empty plants is equally likely, then the probability that on all four occasions the plants bearing larvae would gain less eggs than the empty plants is only 1 in 16, i.e. 6.25%. Although not significant, a probability as low as this suggests that it is not resonable simply to conclude from the non-significant values of U that the presence of larvae on plants does not influence the egg-distribution of female P. rapae, without further investigation. Three alternative hypotheses that are also more or less consistent with the results must be tested before drawing any definite conclusions. The alternative hypotheses are:

(i) That the females respond, not to the presence of larvae per se, but to the effect of larval feeding on a plant.

In the experiment just described, larvae were placed alternately on half the plants available for oviposition and then on the other half, in consecutive replicates, so that only in the first replicate had only half the plants been fed upon by larvae. Thereafter there may have been relatively little difference (depending on how recently and how heavily eaten) between plants in the two treatments. So to test hypothesis (i) I did the experiment described next in Section 2.2.2.2.

(ii) That individual females differ in the extent to which they are inhibited from laying on a plant by the presence of

larvae on it, and consequently also in the extent to which their responses to attractive stimuli from the plant are able to override their inhibition.

(iii) That (whether they constitute the entire population of females or only a portion of it) females with a tendency to respond to other individuals of the same species, as larvae, also respond to them as adults, and that their response to adults (whether attraction or repulsion) will override their response to larvae.

It is possible to test whether either of hypotheses (ii) and (iii) could account for the results shown in Table 2.17 simply by introducing only one female into the cage with the plants in any one replicate of an experiment in which larvae are present on half the plants, as before, and in which as many different females as practically possible are tested. Such an experiment is described in Section 2.2.2.3.

2.2.2.2 Experiment on responses by Females (Tested as a Group) to the Effect of Larval Feeding on Plants

Method:

Twenty young sprout plants which differed widely in size and shape were paired by eye so that the members of each pair differed less from each other than from any of the other plants. Four to ten (depending on the size of the plant) mid-fourth to

fifth instar larvae were put on one member of each pair of plants, to feed. The plants were not measured but even the largest of them were much smaller than any of the plants used in the experiment described in 2.2.2.3 (the smallest of the latter plants had 10 larvae on it, to give a density of about 1.6 larvae/c.c.) and the smallest plants used in this experiment were little more than seedlings with only four or five very small leaves. After thirteen hours some of the plants did not show very much feeding damage so an extra fifth instar larva was added to each of them so that no plant had less than five larvae on it. Twenty hours later all larvae were removed and all the plants (i.e. including those not exposed to larvae) were arranged in a 4 x 5 array for oviposition. As described in Section 2.2.2.1 the positions in the array were classified into three strata, (but in this case there were 4 corner, 10 edge and 6 central positions) with half the plants in each stratum coming from the treatment and half from the control. In this experiment both members of a pair were in the same stratum. (Within these limitations, plants were randomized among positions). Twenty five laboratory-reared female butterflies were released into the cage for a total period of 31 hours 40 minutes (8 hours light, 10 hours dark, 13 hours 40 minutes light, with the dark period between 21.00 hours and 7.00 hours the next day).

Results:

TABLE 2.18

Numbers of eggs laid in the absence of larvae, when half the plants had been partly eaten by larvae immediately before the butterflies were given access to them.

Pair No	Numbers of Eggs Laid on	
	Plants partly eaten by larvae	Control Plants
1	43	29
2	38	32
3	12	30
4	25	28
5	6	21
6	9	11
7	10	21
8	17	11
9	2	15
10	15	15
Total	177	213

Table 2.18 shows that the butterflies' choice of plants on which to lay was not influenced by whether or not the plants had recently been fed upon by larvae.

2.2.2.3 Experiment on the Egg-laying Responses to the Presence of Larvae by Lone Females

Method:

The central bunch of unopened juvenile leaves was

removed from each of eight plants, chosen originally to be as similar in appearance, age, etc. as possible; on some plants older leaves were also removed (from the bottom) till all plants had 19 or 20 leaves, which were marked with the two-spot colour code, and measured. The plants were divided randomly between two groups, each of four plants, and larvae placed on all plants in one group. As in the first experiment (Section 2.2.2.1), any bias that might result from some plants being intrinsically more attractive than others was cancelled by retaining the same four plants in each group throughout all replicates but swapping the larvae from plants in one group to those in the other, after each pair of replicates. To minimize any biases due to the positions of plants relative either to the food source or to each other, they were more or less evenly spaced in a slightly flattened circle around a central clump of flowering Alyssum in the experimental set up described in Section 1.2.3. Each plant was assigned its position in the circle randomly and remained in that position even when the treatments were reversed. By chance group I and group II plants occupied alternate positions around the circle.

Larvae were added at a slightly lower density than in replicates 3 and 4 of the first experiment with larvae, but a higher density than in its first two replicates - allowing about 25 → 30 sq.cms. of leaf per unit larva. The plants grew during the experiment but it was not possible to measure the leaves again soon enough after the

experiments to be meaningful. Therefore I have not expressed the numbers of larvae used in terms of density, in Table 2 of Appendix 2.2. Nevertheless, as the table shows, the numbers of larvae were increased slightly in the later replicates, and even allowing for growth, the densities are unlikely to have fallen below those used in the first two replicates of the first experiment.

The butterflies used in this experiment were reared throughout their larval life on brussels sprout and cabbage plants growing in a cage in the same controlled temperature room in which the experiment was done. They pupated within the cage and even after they emerged as adults they were kept there at all times except when in use for an experiment. As adults they were provided with Alyssum and honey solution for food and the remaining brussels sprout plants provided them with a substrate for oviposition.

Results and Discussion

It does not seem necessary to analyse the results shown in Table 2.19 statistically.

The total number of eggs laid in each treatment was almost identical, suggesting that females are not influenced by the presence of larvae on a plant. But inspection of the rows labelled Treatment Totals shows that when the plants in group I bore larvae (if the sub-totals, in the Treatment Totals rows, are matched with

TABLE 2.19

Numbers of Eggs laid per plant by lone females, when half the plants bore larvae

Female		1	2	2*	6	Sub Total	3	4	7	8	Sub Total	Total Eggs
Minutes#		35	36	181	134	386	39.5	75	131.5	147	393	
Plant No.		LARVAE					NO LARVAE					
GROUP I	9	1	6	4	1	12	1	4	2	2	9	21
	11	6	9	11	2	28	3	1	0	5	9	37
	12	5	14	13	8	40	4	17	15	17	53	93
	13	7	2	7	9	25	5	5	6	24	40	65
		Sub-Total				105	Sub-Total				111	216
		NO LARVAE					LARVAE					
GROUP II	10	4	1	15	7	27	1	3	8	11	23	50
	14	1	4	4	2	11	0	0	0	1	1	12
	15	6	0	7	2	15	1	5	6	14	26	41
	16	1	0	1	0	2	1	3	2	3	9	11
		Sub-Total				55	Sub-Total				59	114
TREATMENT TOTALS												
Larvae		19	31	35	20	105	3	11	16	29	59	164
No Larvae		12	5	27	11	55	13	27	23	48	111	166
Rep.Totals		31	36	62	31	160	16	38	39	77	170	330

*The female used in the 2nd replicate was used again in the 5th.

#Duration of oviposition period, as defined in Section 1.2.3.

the sub-totals in the table above, they show which group of plants has which treatment) more eggs were laid on the plants in group I than on those in group II in each replicate; the same was true even when the larvae were on the plants in group II.

Obviously the plants in Group I are more favourable for oviposition than those in group II (at least while occupying the positions they did in this experiment. But as the group I and group II plants occupied alternate positions around a ring any heterogeneity in the micro-environment should be experienced more or less equally by plants of both groups, so that the contribution of position effects to the difference in favourability between the two groups is probably small). Thus even if the butterflies were responding to the larvae, their response could be masked by their stronger response to particularly favourable plants.

That this is not happening, is shown by the sub-totals; in almost the same total oviposition time (386 and 393 minutes), two different series of butterflies (the only two replicates in which the same butterfly was used both contribute to the same sub-total) laid almost the same number of eggs on a particular group of plants whether or not the larvae were on that group at the time. Also, the number of eggs laid on plants in group I (in each sub-total, of course) is very nearly double the number laid on plants in group II.

2.2.2.4 Conclusions from all Experiments Testing Whether Ovipositing Females Respond to Larvae

There is no evidence that the egg-laying behaviour of female Pieris rapae is influenced by the presence of larvae. But

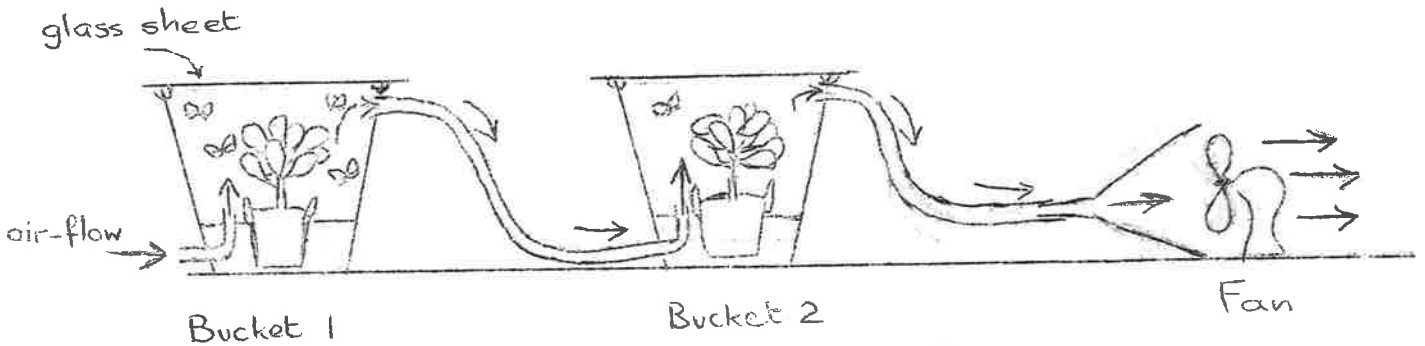
the results in Section 2.2.2.3 give strong support to the hypothesis, suggested by the results of the experiments testing for a response to eggs (Sections 2.2.1.2 and 2.2.1.3), that the pattern in which a female distributes her eggs may be significantly influenced by heterogeneity among the plants available to her. Experiments to test this hypothesis directly are described in Chapter 3.

2.2.3 Preliminary Experiments and Observations on the Females' Egg-laying Response to Other Adults

2.2.3.1 Pilot Experiment to Test Whether Ovipositing Females Release a Pheromone that Stimulates other Females to Lay Eggs

Method: A fan was used to draw a gentle stream of air through two plastic buckets connected in series as shown in the diagram, and sealed with plate glass covers. Each bucket contained a potted brussels sprout plant and one or two small containers of Alyssum flowers. The buckets were stood under the light bank in the constant temperature room described in Section 1.2.2.

A single female was placed in the bucket nearer the fan (bucket 2) and the number of eggs she laid in a measured time was counted: (a) when different numbers of females (4-11) were ovipositing in the further bucket (bucket 1), (b) when there were no butterflies in bucket 1, and (c) when there were six males in bucket 2 with



Apparatus for Pheromone Test.

the single female. (In both tests with males in bucket 2, there were females in bucket 1). A total of eight single females were tested in a total of fourteen tests (8 x (a), 4 x (b) and 2 x (c)). The duration of the test period ranged from four to twenty-five hours, with a mean of ten hours. (The aim of (c) was to determine whether physical contact with other active butterflies would stimulate a female either to lay, or to become more active and responsive to a pheromone, if the females release one).

At the time of this pilot experiment I was still unaware of the butterflies' circadian rhythm of oviposition, so many of the test periods began late at night. Most of them overlapped with the females' natural oviposition period by an hour or two, but as the butterflies had been stored without regard for photoperiod, this might not have been sufficient overlap to ensure normal oviposition.

Results and Discussion:

In only five of the fourteen tests did the female in bucket 2 lay any eggs; three of these were tests in which there were no females in bucket 1. Males were not present in the other two tests when the female in bucket 2 laid eggs; nor were these the occasions when the female in bucket 1 laid the most eggs.

Thus although the results gave only scant information, they do not support the hypothesis that ovipositing female P. rapae release a volatile pheromone which stimulates other gravid females in the vicinity of oviposit.

2.2.3.2 Observations of the Behaviour of Individual Females in the Presence of Other Adults

Methods:

There were six tests altogether:

(i) Female No. 2* with seven other females on 19/9#

(ii) Female No. 2* with seven other females on 21/9.

(iii) Female No. 2* with fifteen other females on 21/9.

(iv) Female No. 8* with fifteen other females on 26/9#.

(v) Female No. 8* with seven other females on 27/9.

(vi) A previously unused female (not dyed) with ten males on 2/10.

The butterflies were observed with the same plants and in the same experimental set-up as in the experiment in Section 2.2.2.3, except that there were no larvae on the plants during these observations. As the presence of larvae in that experiment did not influence the oviposition behaviour of the butterflies significantly the following comparisons between the behaviour of a female alone, and in company, are valid:

Footnote #On 19/9 female 2 did not lay any eggs, nor did female 8 lay eggs on 26/9.

*These are females numbers 2 and 8 from the experiment described in Section 2.2.2.3.

Female No.	Alone (Section 2.2.2.3 Experiment)	Other Females Present
2	Replicate 2 (15/9)	(ii) above (21/9)
	Replicate 5 (18/9)	(iii) above (21/9)
8	Replicate 8 (24/9)	(v) above (27/9)

To get an accurate record of the oviposition behaviour of an individual female in the presence of other ovipositing females one must be able to distinguish her eggs from those laid by the others. Parker (1970) reported feeding Pieris rapae with a dye that not only dyed the adults' internal organs pink, but also caused the females to lay pink eggs. Parker fed the dye (calco oil red N1700; American Cyanamid Co.) to his larvae in an artificial diet, but throughout my project larvae had been reared on either picked or growing leaves of various brassicae, so I painted a suspension of the dye (0.1 gms/4 mls. olive oil) on the upper surfaces of the leaves of growing brussels sprout plants. The dye may have reduced the plants' ability to photosynthesize, thus reducing their food value for the larvae; also quite a number of larvae died, apparently from getting too much oil on them, perhaps blocking the spiracles. Nevertheless the plants, and the majority of larvae, survived. But apparently most of the larvae had not consumed enough dye, as when they emerged as adults relatively few of them were noticeably pink and, when first observed, their eggs did not appear pink at all. As the dye had apparently not taken, the butterflies were put in the

same cage as the normal laboratory population. Later, however, I found that the eggs were distinguishable if observed under incandescent, rather than fluorescent, light, but by then it was not possible to distinguish more than three of the females reared on dyed plants from females of the normal laboratory population. The three dyed females were then used for replicates 1, 2, 5 and 8 of the experiment described in Section 2.2.2.3, with the aim of the comparisons mentioned above. Unfortunately dyed female No. 1 (used for replicate 1 above) was extremely lethargic and inactive and would not lay any eggs when tested in company with other females. Consequently no such comparison is possible for her. Also dyed female No. 8 was accidentally injured at the end of observations on 27/9, so I was unable to test her again with fifteen other females.

Results

During the observations the female butterflies alternated short periods of flight and oviposition among plants, with periods of immobility - most commonly spent sitting on the upper surface of a leaf. The females generally seemed much less responsive during such "sits" (as such periods of immobility were called, for convenience) than at other times. A sudden gust of air would usually disturb a settled female during the flight and laying periods (F.L.P.'s) causing her to fly off the leaf, but during a sit, she would not respond at all, unless it were just to take a tighter grip of the leaf.

But the females' unresponsiveness was a subjective impression, whereas an objective (even though arbitrarily chosen) definition of a "sit" was necessary for analysis of the butterflies' behaviour. A "sit" was therefore defined as any period of immobility longer than one minute. Although the minimum duration of a "sit" was arbitrarily chosen, there was no such arbitrary maximum and the duration of "sits" varied widely. The hypothesis that there was no significant difference between the mean duration of "sits" when a butterfly was alone, and when males, or other gravid females, were present, is tested by the results shown in Table 2.20. So also are the hypotheses that neither the duration of the flight and laying periods (F.L.P.'s) between "sits", nor their duration expressed relative to the duration of the "sits" immediately preceding them, differed significantly when a butterfly was alone, from when she was in company.

As the table shows, whether the same female, or different females were being observed, alone or in company, the mean duration of F.L.P.'s was remarkably constant over most of the observation periods, considering the high degree of variation within any one observation period. There was thus no evidence that the presence of other ovipositing females influences the duration of an individual's flight and laying periods (F.L.P.'s). The duration of "sits" varied much more between observation periods than the duration of F.L.P.'s did. Nevertheless, although the variation within any one

TABLE 2.20(a)

Duration of "sits", and flight and laying periods, and relative duration of each F.L.P. to that of the sit preceding it, when females were alone, and when they were in company with other adults

	Alone			Other Adults Present		
	Date	Mean	s.d.	Date	Mean	s.d.
			Female No. 2 - with other Females			
Duration of sits (mins)	15/9	4.754	4.034	21/9(7)#	6.928	3.632
	18/9	27.425	16.220	21/9(15)	10.680	8.177
Duration of F.L.P.'s (mins)	15/9	1.407	8.857	21/9(7)	1.979	8.627
	18/9	1.767	6.442	21/9(15)	1.733	6.161
Duration of F.L.P. ÷ that of preceding sit	15/9	0.507	0.500	21/9(7)	0.714	0.721
	18/9	0.099	0.110	21/9(15)	0.223	0.154
			Female No. 8 - with other Females			
Duration of F.L.P.'s (mins)	24/9	1.831	6.383	27/9(7)	2.197	8.420
			Undyed Female - with Males			
Duration of sits (mins)	-	-	-		1.431	6.705
Duration of F.L.P.'s (mins)	-	-	-	2/10(10)	4.317	22.907
F.L.P. ÷ sit					3.413	2.882

Number in parenthesis refer to number of other adults present

TABLE 2.20(b)

Testing whether duration of sits etc., differ significantly between different observation periods

Comparison Between	Duration of Sits			Duration of F.L.P.'s			Relative Duration F.L.P. ÷ Preceding Sit		
	t	dfs	P	t	dfs	P	t	dfs	P
15/9 & 18/9	*3.275	6	0.01<P<0.02	0.082	10	>0.9(NS)	*1.606	3	>0.2(NS)
15/9 & 21/9 (7)	1.002	8	>0.3(NS)	0.112	10	>0.9(NS)	0.495	8	>0.6(NS)
15/9 & 21/9 (15)	1.314	7	>0.2(NS)	0.072	9	>0.9(NS)	*1.095	3	>0.3(NS)
15/9 & 2/10	0.379	8	>0.7(NS)	0.267	10	>0.7(NS)	*2.192	5	>0.05(NS)
18/9 & 21/9 (7)	*3.056	5	0.02<P<0.05	0.052	12	>0.9(NS)	*2.066	5	>0.05(NS)
18/9 & 21/9 (15)	2.085	9	>0.05(NS)	0.010	11	>0.9(NS)	1.563	9	>0.1(NS)
18/9 & 2/10	3.628	10	0.001<P<0.01	*0.284	7	>0.7(NS)	*2.586	5	0.02<P<0.05
21/9(7) & 21/9(15)	*0.985	5	>0.3(NS)	0.058	11	>0.9(NS)	*1.623	6	>0.1(NS)
21/9(7) & 2/10	1.845	10	>0.05(NS)	*0.253	8	>0.8(NS)	2.003	6	>0.05(NS)
21/9(15) & 2/10	2.065	9	>0.05(NS)	*0.287	7	>0.8(NS)	*2.478	5	>0.05(NS)
24/9 & 2/10	# -		-	*0.281	7	>0.7(NS)	-		-
27/9 & 2/10	-		-	0.190	10	>0.8(NS)	-		-
24/9 & 27/9	-		-	0.099	15	>0.9(NS)	-		-

* In tests marked thus, the "sits" or F.L.P.'s etc. being compared had significantly different variances, so that a standard t test was not valid. An adjusted t test (Bailey, 1966 p.51) was therefore used.

\# The records of duration of sits on 24/9 and 27/9 were inadequate for analysis.

observation period was not as large for sits as it was for F.L.P.'s, it was large enough to account for all but three of the apparent differences between means. On 18/9 female No. 2 tended to sit for significantly longer periods between F.L.P.'s than she did on 15/9, or on 21/9, when seven other females were present. Thus there is no evidence that the presence of other females influences the duration of sits, nor the relative duration of an F.L.P. to the duration of the preceding sit. As only one butterfly was observed ovipsoting in the presence of males, and as she was not observed either alone, or in company with other females, it is quite possible that any differences observed are simply differences between the behaviour of different individuals, independent of whether they are alone, or in company with either males or females.

But even if the amounts of time a female spends in active flight and oviposition, and in resting, are not significantly influenced by the presence of other butterflies, the frequency with which she visits plants, moves from leaf to leaf, and lays eggs could be. Because the duration of sits varies so widely, it seems that rates of visiting plants, etc., should be expressed in two ways: (a) in terms of visits per total time (i.e. the sum of all the F.L.P.'s and all the sits in an observation period, (b) in terms of F.L.P. time (i.e. the sum of F.L.P's only). Comparisons in terms of total time give information about the effect of different conditions (in which females were ovipositing) on generation of the resulting

distribution of eggs. But comparisons by both (a) and (b) are necessary if one wants information about the means by which different conditions may influence the resulting distribution of eggs, through changes in the behaviour of individual butterflies.

Because of the extreme variability in the duration of sits, and because visits and oviposition only occur within the F.L.P.'s, rates of visiting, oviposition, etc., expressed per total time will have extremely high variances, so that comparisons between them are unavoidably crude. Consequently Table 2.21 gives only the mean rates of visiting, settling and oviposition per total time, and statistical comparisons are restricted to rates measured per minute of F.L.P. time (Table 2.22 (a) and (b)). As the females lay most of their eggs on the under surfaces of leaves, settles on undersurfaces generally involve either oviposition, an attempt to oviposit, or at least what appears to be testing of the surface as an oviposition substrate. (Settles on the edge of leaves, from which the female bent her abdomen up to the under surface, have also been counted as settles on an under surface). Settles on upper surfaces, however, are relatively rarely associated with oviposition. Hence only settles on under surfaces (N settles) are considered in the analyses of Tables 2.21 and 2.22.

The results for female No. 8 are quite consistent with the hypothesis that she was influenced by the presence of other females, her rates of visiting, settling and oviposition, all being

TABLE 2.21

Mean rates per minute of total time, of visiting settling, oviposition, by females when alone and when in company with other gravid females

Female No. 2	Alone		Other Females Present	
Date	15/9	18/9	21/9(7)	21/9(15)
Mean No. Visits/min.	0.269	0.181	0.433	0.219
Mean No. of N Settles/min.	0.998	0.384	0.866	0.564
Mean No. eggs laid/min.	*1.344	0.339	0.451	0.235

* A few of these eggs were laid on P (upper), not under, surfaces.

significantly lower on 27/9 than on 24/9. On the other hand the results could simply reflect a decline in the female's fecundity with age.

The presence of other females certainly did not influence the frequency with which female No. 2 moved from plant to plant (visiting), on leaf to leaf (settling) within an F.L.P. These rates seemed to be influenced more by the mean duration of "sits" - when she rested significantly longer between F.L.P.'s she was significantly more active during them. The longer duration of "sits" on 18/9 than 15/9 appeared to have also increased the oviposition rate during F.L.P.'s slightly, but the increase was not significant. But, as for female No. 8, her mean rates of oviposition per minute

TABLE 2.22 (a)

Rates per minute of F.L.P. time, of visiting, settling and ovipositing by females when alone and when in the company of other gravid females

	Alone			Other Females Present		
	Date	Mean	s.d.	Date	Mean	s.d.
Female No. 2						
Visits per min. of F.L.P.	15/9	0.700	0.571	21/9(7)	1.111	0.792
	18/9	2.000	0.343	21/9(15)	1.000	0.630
N settles per min. of F.L.P.	15/9	2.600	2.205	21/9(7)	2.667	1.645
	18/9	4.250	2.146	21/9(15)	2.571	1.988
Eggs laid per min. of F.L.P.	15/9	2.900	1.969	21/9(7)	1.389	0.979
	18/9	3.750	1.880	21/9(15)	1.143	0.770
Female No. 8						
Visits/min. F.L.P.		2.741	1.155		1.214	0.611
N Settles/min. F.L.P.	24/9	2.889	1.785	27/9(7)	0.714	0.995
Eggs/min. F.L.P.		2.704	1.540		0.714	0.825

of F.L.P. were significantly lower in both tests on 21/9 than on 15/9 or 18/9. But Table 2.21 shows that in spite of the significantly higher rate of oviposition per minute of F.L.P. on 18/9 than on 21/9, the mean number of eggs laid per minute of total time was about the same on both these days (mean of pooled results for both tests on 21/9 = 0.344 eggs/min). Thus even if the presence of other females does influence the rate of oviposition within an F.L.P., whether or not it will influence the overall rate of laying eggs, and so, perhaps their distribution, depends very much on the

TABLE 2.22 (b)

Testing whether rates of visiting, etc. differ significantly between different observation periods

Comparison Between	Visits/min.of F.L.P.			N Settles/min.of F.L.P.			Eggs laid/min. of F.L.P.		
	t	dfs	P	t	dfs	P	t	dfs	P
15/9 & 18/9	7.291	24	<0.001	1.888	24	>0.05(NS)	1.102	24	>0.2(NS)
15/9 & 21/9 (7)	1.441	26	>0.1(NS)	0.091	26	>0.9(NS)	2.275*	12	0,02<P<0.05
15/9 & 21/9 (15)	1.195	22	>0.2(NS)	0.033	22	>0.9(NS)	2.679*	11	0.02<P<0.05
18/9 & 21/9 (7)	4.328*	24	<0.001	2.430	32	0.02<P<0.05	4.510*	22	<0.001
18/9 & 21/9 (15)	5.110*	20	<0.001	2.212	28	0.02<P<0.05	5.082*	20	<0.001
21/9 (7) & 21/9(15)	0.429	30	>0.6(NS)	0.148	30	>0.8(NS)	0.772	30	>0.4(NS)
24/9 & 27/9 (7)	5.532*	39	<0.001	5.006*	39	<0.001	5.384*	39	<0.001

* In tests marked thus, the rates being compared had significantly different variances so that as in Table 2.20(b) an adjusted t test had to be substituted for the standard t test.

female's own level of activity (which in the butterflies observed, was independent of the presence of other females).

Investigation of the mean number of eggs per visit, settles per visit, etc. (Table 2.23) shows that the fecundity of both females did decrease throughout the series of tests, but the results do not contradict the hypothesis that the presence of other females somehow inhibits oviposition as well. But one conclusion that can be drawn from Table 2.23 without doubt, is that the decrease in fecundity occurred in quite different ways in the two females. Thus to get clear information on the effect (if any) of other butterflies on the rate of oviposition of individual females, one would need to do many more replicates, testing each of a number of females several times, alternatively in company and alone.

But whether or not other butterflies influence the rate at which a female lays eggs, they could influence the distribution of her eggs among plants either by:

(a) influencing which plants she chooses to visit, or

(b) interrupting her visits and attempts to ovipost, causing her to move to a different plant before she could finish laying.

The distribution of other butterflies each time the marked female settled, was not adeuately recorded to give any information on (a). But for each of the six tests (i.e. even those at which the marked

TABLE 2.23

Mean number of eggs per visit, eggs per settle and settles per visit when a female is alone and in company with other gravid females.

		Date and Test	Mean No. of eggs per visit (all surfaces)	Mean No. of eggs on N surfaces per visit	Mean No.of settles (on all surfaces per visit)	Mean No.of settles (on N surfaces) per visit	Mean No. of eggs/settle (settles and eggs on N surfaces only)
Female No. 2	Alone	15/9	5.000	4.143	5.429	3.714	1.115
		18/9	1.875	1.875	2.469	2.125	0.882
	With other Females	21/9(7)	1.042	1.042	2.417	2.000	0.521
		21/9(15)	1.143	1.143	3.143	2.571	0.444
Female No.8	Alone	24/9	0.987	0.946	1.297	1.054	0.897
	With other Females	27/9(7)	0.588	0.529	1.118	0.588	0.900

butterfly did not lay any eggs) I had recorded any apparent attempts by the other butterflies to cause the marked females to fly (challenges) and whether or not they were successful. Sometimes a butterfly would fly up to and actually bump the female when she was sitting on a leaf, usually bumping her several times, as if attempting to forcibly knock her off the leaf. At other times the butterflies would simply fly low over the top of the female, persistently, as if attempting to disturb her, but without physical contact. Table 2.24 shows the records of such challenges and their consequences; also the circumstances of each departure from a plant by the marked female.

Although over all of the six tests there were a total of 107 challenges, less than ten percent of all departures occurred in conjunction with any sort of challenge, and 88 of the challenges (including ten involving actual bumping) received no response. Thus it seems that females No. 2 and No. 8 were influenced very little, if at all, by the challengesof other females.

Although the males did not challenge the female on 2/10 by bumping (nor were any males seen attempting to mate with her at any time during the test period), she departed in conjunction with - perhaps in response to - a higher proportion of their challenges than either of the other two females did in any of the other five tests. The possibly slightly higher responsiveness of the undyed female to challenges may perhaps lend more weight to the differences

TABLE 2.24

Relative independence (from the challenges of other butterflies) of departures from plants by the marked butterflies

Female No.	Date	Other Butterflies	Departures occurring with			Total Departures	Unsuccessful Challenges		Total Challenges
			Bumping	Near Flight	Others Absent		Bumping	Near Flight	
2	19/9	7	0	1	3	4	3	7	11
	21/9	7	0	2	59	61	1	9	12
	21/9	15	1	6	41	48	2	26	35
8	26/9	15	2	1	4	7	2	20	25
	27/9	7	0	0	16	16	2	5	7
Undyed	2/10	10	0	6	49	55	0	11	17
TOTALS			3	16	172	191	10	78	107

between her behaviour and that of females No. 2 and No. 8, shown in Table 2.20 (a) (p84). Although only six of her fifty-five departures from plants actually occurred in conjunction with challenges, the possibility that her responsiveness to challenges is related to her general level of activity, cannot be completely dismissed. Table 2.20(a) shows that although the extreme variability in duration of sits and F.L.P.'s within the same observation period caused most of the differences to fall short of significance, the undyed female had the shortest observed mean duration of sits and the longest observed mean duration of F.L.P.'s. As well as this, her F.L.P.'s were, on average, four times as long as the sits immediately preceding them, while in all four tests with female No. 2 the duration of F.L.P.'s tended to be less than that of the sits preceding them. Whether the undyed female's apparently higher levels of activity and responsiveness are real, and if so, whether they are cause and effect, or both effects of a common cause - the presence of males - cannot be determined from so little data.

2.2.3.3. Conclusions from all Experiments and Observations on the Females' Responses to Other Adults

The sample of butterflies tested was too small to draw general conclusions from the results, but some specific ones are worth mentioning, perhaps as a starting point for any further experiments.

There was clear evidence that some of the differences in behaviour between different observation periods were not responses to the presence of other adults but the result, instead of differences in the physiological condition of different butterflies, or of the same butterfly at different times. Most other differences in the components of oviposition behaviour could be explained equally as well by differences in physiology as by responses to other adults. More extensive experiments would be required to determine whether the latter are ever very important.

Throughout the observation periods on 21/9 and 27/9 I also gained the strong (though subjective) impression that the marked females were behaving largely independently of the other butterflies.

CHAPTER 3

RESPONSES TO STIMULI FROM HOST PLANTS

3.1 Introduction

3.1.1 Theoretical Background

If the host plants of a monophagous or oligophagous insect are homogeneous throughout the area in which the distribution of the insect's eggs is being studied, then either they will all be equally favourable (or unfavourable) for oviposition and larval development (or for larval development alone, in the case of those insects that do not lay their eggs on the larval host plant) so that the quality of the plants will not influence the pattern in which the females distribute their eggs in that area, at all. (This will be true even if the distribution is being studied on a smaller scale, e.g. the distribution among parts of plants such as leaves or fruits, rather than the distribution among whole plants). Thus this chapter is concerned with the question, "How, and how much may a female's egg-laying behaviour be influenced by heterogeneity in the host plant, and what sorts of differences between plants or their parts are likely to influence her?"

Differences in the physical and chemical properties of the tissues of the host plant which make them more or less favourable for larval development often result from differences in the age or stage of development of the plant, or in the conditions of mineral

nutrition, soil and water relations, or the intensity or wavelength of light, under which they have grown.

Whether or not they themselves influence larval survival, the characteristics of a plant (or plant-part) such as size, shape, growth form, colour, surface-texture and flexibility or rigidity of leaves, and the level of production of secondary plant substances, will be related to each other and to the properties that do influence larval survival in a fairly well defined way according to whether they reflect the age, stage of development, or particular conditions under which the plant grew (allowing, of course, for a certain amount of genetic variability). Ovipositing females of many insects have evolved responses to stimuli from these relatively obvious characters of the plant; it is postulated that because of the relationship between these characters and the properties that influence larval survival, the former are able to act as token stimuli. Selection has probably favoured those responses to the former that are also appropriate to the latter, when the female is unable to monitor the latter properties herself.

For there to be a selective advantage in a female distributing her eggs in response to the pattern of stimuli she receives from host plants, however, the incubation period must be less than the time within which the quantity, distribution or quality of the host plants (or plant parts) is likely to change significantly. For instance, although adults of the grasshopper Austroicetes cruciata

No p. 99.

feed on the same range of host plants in the same sorts of condition as do the nymphs, stimuli from the host plants do not influence the females' distribution of their eggs, which they lay in holes they bore in the ground. Nevertheless the eggs are not distributed randomly but apparently in response to the soil and topography (Andrewartha 1939), showing that the females do have the capacity to respond to stimuli from the environment. Why, therefore, do they not respond to stimuli from the plants? The example of the cabbage root fly, discussed below, shows that just because an insect does not lay her eggs on the larval host plant this does not necessarily mean that she does not respond to stimuli from the plants. It seems instead that there has been no selection for females of Austroicetes that distribute their eggs in response to stimuli from the plants because the eggs are laid in October or November but do not hatch until the following August or early September. As most of the host plants are annuals, and even those that are not (e.g. Stipa spp.; speargrass) change condition according to the season (Andrewartha 1944), the quality and quantity of suitable food available at particular locations when the females are ovipositing are unlikely to be mirrored the following year when the nymphs emerge. Thus selection has favoured the females' response to soil in which the eggs can survive and the nymphs' ability to withstand enforced dispersal soon after emergence (even if they have not fed at all).

Although oligophagous insects differ in their degree of

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oligophagy - that is, according to whether their host plants comprise a group of genera or species (whether related phylogenetically or just by some common characteristic), or just one species, or only certain sub-species or varieties of a species - Nishijima (1960) pointed out that in general, the factors influencing an insect's selection of, or preference for, a host plant, depend on the relationship of the insect to its host plant. He gave four possible categories of relationship:

(i) Adults' food = larval food = oviposition site

(ii) " " = " " ≠ " "

(iii) " " ≠ " " = " "

(iv) " " ≠ " " ≠ " "

Similarly the degree to which stimuli from the larval host plant can influence the pattern in which females distribute their eggs, compared with stimuli from other components of the females' environment (e.g. other members of the same species, or micro-weather) or even internal physiological stimuli, tends to be greater for those insects whose relationship with their host plants falls into categories (i) and (iii) than for those to which categories (ii) and (iv) apply.

In some cases the extent to which a female's egg-laying behaviour can be influenced by the larval host plant may be more closely related to the ability of larvae to migrate relatively long distances without food and to recognize host plants when they encounter

them. For example, Traynier (1965) found that females of the cabbage root-fly, Erioischia brassicae, laid more eggs in sand surrounding a tube of juice squeezed from the root of swede (a favoured larval host plant) than in sand around tubes of certain concentrations of aqueous solutions of mustard oil glucosides and their derivatives, and more around these than in sand around tubes of distilled water. Thus although this insect falls into Nishijima's category (iv), as the females oviposit in the soil rather than on the larval host plant, the larvae do not need to be able to withstand a long migration before feeding, because the gravid females respond to volatile stimuli (mustard oil glucosides and their derivatives) from suitable host plants and so lay their eggs near them. On the other hand, although females of the butterfly Melitaea harrisii lay their eggs on the leaves of Aster umbellatus (the sole acceptable food plant for their larvae) Dethier (1959b) found that throughout the four years of his study not one of the plants on which eggs were laid was seen to constitute an adequate supply of food for the members of the clutch of eggs laid on it. Eventually defoliation became so extreme that the larvae were forced to wander in search of other asters, with the result that many of them were lost. Such behaviour could not have been perpetuated, however, if M. harrisii larvae had not evolved a fairly good ability to survive migration and to recognize host plants when they encounter them, although such encounters occur mainly by chance. This ability was probably selected because the females, although they respond to

enough stimuli from the asters to recognize them as host plants and lay their eggs on them, are not in any way influenced by the size of a plant, or whether it can provide an adequate food source for their progeny.

In general, however, the larvae of insects in Nishijima's categories (ii) and (iv) have a greater ability to survive migration and recognize host plants than those of insects in categories (i) and (iii), so that Nishijima's classification is probably a simpler index (than ability to survive migration and recognize host plants) to the degree to which the host plant is likely to be able to influence a female's egg-laying behaviour.

3.1.2 Examples of Responses by Insects from Categories (i) and (iii) to Differences among Plants or their Parts in the Following Respects:

3.1.2.1 Age or Stage of Development:

As mentioned in Section 2.1.2.1, females of the psyllid C. densitexta (a species in which the quality of nymphal food may limit the population) distribute their eggs primarily in response to nutritional stimuli from the leaves of Eucalyptus fasciculosa on which they feed. The incubation period ranges from about 14 days at 25°C to about 35 days at 15°C (both measured at constant temperatures). White (1966) found that the nutritive value of a leaf for psyllid nymphs usually depends on its age

(although the extent to which it is water-stressed may also be very important) but changes in favourableness take place slowly relative to the speed of development of the eggs and a significant change in favourableness generally takes much longer than the incubation period. (He reported that leaves that were unfavourable in early February had become favourable for oviposition by the next generation by early May, by which time those favourable in February had become unfavourable. Eggs laid in January and February gave rise to the females that laid eggs in May). When the favourableness of a leaf is mainly the result of water-stress, however, a sudden change may sometimes occur - such as when rains break a drought - with a consequent death of nymphs. The relative rarity of such occurrences would enable the usual selective advantage of the females' ovipositional response to nutrient levels in the leaves to outweigh the selective disadvantage of an occasional "wrong prediction". Nevertheless, the leaves of E. fasciculosa provide the optimum stimulus for psyllids to oviposit on them during only a relatively short period of their life - the first few weeks after they have expanded fully and hardened (White 1970). Infestation of cacao trees by the cacao thrips, Selenothrips rubrocinctus, followed a very similar pattern of age preferences. Fennah (1955) reported that the apical flush of leaves (youngest), as long as the leaves were flaccid, was free from infestation. Leaves of the previous flush which were fully hardened were heavily attacked; those of the next

older flush were either infested more lightly or not at all, and old leaves were not infested.

Both these insects are sap-suckers and in both host trees the level of photosynthesis in a leaf reaches a maximum as the leaf becomes fully expanded then falls slowly as senility sets in; associated with the maximum photosynthetic activity is a great increase in the proportion of soluble nitrogen nutrients in the leaf. (Cameron 1964 and Fennah 1954, cited by White 1970).

Miller and Hibbs (1963) reported a rather similar pattern of distribution of eggs (by the potato leaf-hopper, Empoasca fabae) in relation to the physiological age of leaves of Solanum tuberosum; perhaps the egg distribution is again influenced by the female's ability to monitor the levels of soluble nutrients in the leaves through her own feeding.

On the other hand gravid females of insects in category (iii), which cannot get such direct information about the nutritional suitability of the host plant for the larvae, have evolved responses to visual, olfactory, chemotactile and physicotactile stimuli from a plant, associated with its age or stage of development. For instance although adults of Plutella xylostella and Pyrausta nubilalis do not feed on the larval host plant, and although their larvae are not sap-suckers and are therefore not so dependent on the level of soluble nutrients in their food, the females nevertheless prefer to

lay their eggs on leaves or plants at a certain stage of development.

Gibbs (1970) found that P. xylostella adults, caged on turnip or radish plants laid most eggs on mature or ageing leaves; after hatching most of the larvae moved to young leaves. Some moved to a young leaf straight away without feeding; others fed for a while on the old leaf before moving to a young leaf; others again did not move, but fed and developed on the old leaf. Gibbs remarked on the high variability in the behaviour of young larvae, which apparently is not heritable - he attempted to select for those that stayed on old leaves but this did not alter the proportion of hatchlings with different kinds of behaviour. In this case therefore, it does not seem that the females' choice of mature or ageing leaves as an oviposition substrate results from selection pressure related to providing their progeny with optimal food. Although the stimulus to which the females were apparently responding was associated with mature or ageing leaves in Gibbs' experimental plants, perhaps the moths' response to this stimulus had been selected because the stimulus was a token, not only of the physiological and biochemical state of mature or ageing leaves (when plants were grown under the conditions of these experiments), but also of another physiological or biochemical state (of certain leaves or even of whole plants) that is much more favourable for larval survival and development. Such a physiological state perhaps occurs in nature as a result of different growth conditions or in alternative, slightly more favoured host plants.

In the case of Pyrausta, the stage of development of the corn plant has a profound effect on both the numbers and distribution of the eggs that it receives and the survival of the larvae. At more than ten days before mid-silking, the eggs are distributed among plants in response to the height of a plant rather than its relative stage of development (Everly, 1959) and larval survival is lower than when the eggs are added to plants that have recently silked (Patch,1947). If peak oviposition occurs within 10 days either side of mid-silking, both the stage of development and the height of a plant influence the number of eggs laid on it. Recently-silked plants gain about three times as many eggs as plants that have silked fifteen days to one and a half months earlier; on the younger plants most of the eggs are laid on the leaves, with only about 1% on the ears. About 15% of eggs are laid on the ears of the older plants.

Beck (1965) described cornborer larvae as "essentially polyphagous" and reported that newly hatched larvae were apparently unable to distinguish between different parts of the plant, being equally attracted by extracts from leaf, stem, leaf sheath, or tassel, though not attracted at all from more than a few millimetres away. The main orientation behaviour of newly hatched larvae is simply a combination of a positive thigmotaxis and a negative phototaxis, which, on the plant, tends to take them down into either the plant whorl, or the confined spaces between the stem and leaf sheath

or the ear husks, depending on where the eggs were laid. But Beck (1957) showed that the levels of production, by different parts of the corn plant, of Resistance Factor A (a compound that inhibits survival and growth of cornborer larvae) depend on the stage of development of the plant. So also do the concentrations of sugars in the different tissues of the plant; Beck reported an inverse relationship between Resistance Factor A activity and sugar concentration in the same tissues. He also reported that sugars have a marked influence on the feeding behaviour of later stage borer larvae.

Thus there would be a selective pressure favouring the progeny of moths that laid their eggs predominantly on those parts of a plant that gave the larvae the greatest chance of survival at that stage of the plant's development. But the females are not likely to be able to detect the concentrations of either sugars or Resistance Factor A. Schurr and Holdaway (1970) demonstrate however, that the moths will oviposit in response to the olfactory stimulus of vapours from uninjured host plants. They did not test vapours collected from different parts of the corn plant e.g. ears versus leaves, nor from plants at different stages of development, but it seems possible that like Resistance Factor A, the levels of production of odours by different parts may change as the plants mature, with originally more of the odouriferous substances in the leaves and very little in the ear, gradually increasing through silking. A month or so after silking the ears may have higher levels

of such substances than the leaves. Delaveau (1958) reported changes of this sort in the levels of mustard oil glucosides in different parts of the crucifer Alliaria as it matured.

Takata (1961) reported that when females of the Japanese subspecies of Pieris rapae, P. rapae crucivora, were given access to ten cabbage plants, five of which were young (with only about ten leaves each) and five at the stage when they are usually harvested (i.e. with developed heads), in a large net cage, the butterflies laid more than ten times as many eggs on the young cabbage plants as on those ready for harvest. The behaviour of P. rapae crucivora differs in some respects from that of P. rapae in South Australia (see Chapter 5) so an experiment to determine whether females of the South Australian butterflies discriminate between brussel sprouts plants of different ages is described in Section 3.2.1.1.

In Section 2.2.1.2 it was concluded that some leaves are intrinsically more favourable for oviposition than others; possibly the butterflies prefer to lay on leaves of a certain age. I did not have time to do an experiment that would test this hypothesis directly and rigorously but in Section 3.2.1.2 six distributions of eggs (from the experiments described in Sections 2.2.1.2 and 2.2.2.1) among leaves of different ages are analysed graphically to determine at least whether they would be consistent with the hypothesis.

3.1.2.2 Levels of Secondary Plant Substances that give Olfactory or Chemotactile Stimuli

Fraenkel (1959, 1969), following the terminology of Paech, divided the substances present in, or released by, plants into two groups, primary and secondary plant substances. Those he termed "primary" "are constituents of all living cells, especially..... those of green leaves" and include sugars, essential amino acids, minerals salts, most vitamins of the B group, and a sterol. There are about thirty to forty such substances altogether, which constitute the basic food requirements of insects (and, in fact, of herbivors in general). Those substances Fraenkel termed "secondary" have a rather sporadic occurrence, being specific for certain groups of plants. They comprise the alkaloids, glycosides, saponins, tannins, essential oils and organic acids, plus a few related compounds that Fraenkel did not define more specifcally. He believed it "almost inconceivable" that secondary plant substances have any function in the basic metabolism of plants or are of any direct nutritional value (such as primary substances are) to insects, and so proposed the hypothesis that plants evolved secondary plant substances simply as deterrents to herbivors in general, including of course, phytophagous insects.

Many of these products are indeed toxic or repellent to phytophagous insects (and often also to other herbivors, if the plants containing them constitute too high a proportion of the total diet).

But there is also ample evidence that many phytophagous insects, having evolved the ability to tolerate a particular secondary plant substance (or group of such substances), have found it advantageous (perhaps because of the reduced competition for food when it contains such substances) to confine their feeding and oviposition to plants that contain the particular secondary plant substances that they can tolerate, but most other insects cannot. The former insects have evolved the ability to recognize potential host plants by the secondary plant substances the plants produce, and to oviposit and/or feed in response to these substances; some insects will even respond to the extent of laying their eggs on artificial substrates, if the secondary plant substances are present as vapours, or painted on, or impregnated in, those substrates. (Beck (1965); David and Gardiner (1962a); Fraenkel (1969); Schurr and Holdaway (1970); Yamamoto et al. (1969)).

Nevertheless, there is equally strong evidence (Thorteinson 1960, Kennedy 1965, Schoonhoven 1969), that, contrary to Fraenkel's hypothesis, deterrence of herbivors is not the sole function of secondary plant substances, nor are stimuli from these substances necessarily the only, or even the principle, means by which insects select their host plants, even at the family, genus or species level. As already indicated, in relation to an ovipositing female's choice of individual plants (within the normal host species) on which to lay (Section 3.11), stimuli from secondary plant substances probably

play a more important role in host selection (i.e. at the species, genus, or higher taxonomic level) by ovipositing females when the females belong to the categories in which the adults do not feed on the larval host plant (Nishijima's categories (iii) and (iv)). This is because such stimuli are olfactory or chemotactile more often than those from primary, i.e. nutrient, substances, which tend to be gustatory stimuli.

Beck (1965), Mehta and Saxena (1970) and many others have shown, however, that even for insects in categories (iii) and (iv), physical factors sometimes influence oviposition more than stimuli from secondary plant substances.

Butterflies of the genus Pieris identify their host plants by means of stimuli (probably mainly chemotactile, possibly also olfactory) from the mustard-oil glucosides that the plants produce. The mustard-oil glucosides also stimulate the larvae to feed, while their iso-thiocyanate derivatives (the mustard oils) which are continuously released in small quantities as a result of slow enzymic breakdown of the glucosides within the plant, act as volatile attractants for the larvae and possibly also for the adults, though for longer range attraction at least, visual stimuli appear to be more important to the butterflies.

Most studies of the role of secondary plant substances as stimulants or inhibitors of oviposition have been concerned only

with the influence of their presence or absence in certain species or varieties of plants, on whether or not eggs are laid on that species or variety, rather than determining whether different levels of production of such substances by plants of the same species or variety, but of different ages, or grown in different conditions of mineral nutrition, etc., can also influence the oviposition behaviour of insects.

Very often the same secondary plant substances that stimulate the adult females to oviposit, also stimulate the larvae to feed, and act as attractants to both adults and larvae.

There are two possible ways (not mutually exclusive) in which an insect's behaviour could be influenced by differences in the level of production of secondary plant substances by different plants:

(i) If the stimuli from some plants are so weak that they are below the insect's threshold of perception, while those from other plants are stronger so the insect can perceive them and respond; this situation will give rise to the single difference of response versus no response.

(ii) If all stimuli are above the insect's threshold of perception but they differ in intensity, then for these differences to influence the insect's behaviour, the insect must (a) have sensory receptors that can detect them, and (b) be able to vary the intensity of its response

to match the intensity of the stimulus, rather than only having an all-or-nothing response that acts like a switch that simply releases oviposition or feeding behaviour once a certain threshold concentration is reached or exceeded.

When a series of concentrations of volatile secondary plant substances are tested for their relative ability to attract larvae or adults, if the frequency of attraction increases with increasing concentration of the attractant, this is not necessarily evidence that the insects can discriminate between a series of different concentrations; the vapours from the more concentrated sources will have wider spheres of influence than those from the more dilute sources, so that the former reach and attract the insect sooner than the latter. Also, if the insect has an all-or-nothing response it will not be distracted from its orientation to the original stimulus by passing through any overlap with the spheres of influence of other sources.

When the most attractive source is not the one with the highest concentration of attractant, however, there are two possible explanations: either the insects <u>can</u> discriminate between a series of concentrations and respond appropriately, or else as well as their lower threshold (or threshold of attraction) they have an upper threshold, (or threshold of repulsion) at which concentration

another receptor is activated, to which the response is negative i.e. repulsion instead of attraction. For any individual insect any concentration of the volatile plant substance above its threshold of repulsion would elicit the same reaction - avoidance of the source, but if a group of insects were being tested, it is possible that different individuals may have different thresholds, both of attraction and repulsion, so that group results may show a graded increase to, and decrease from, the maximum attraction.

Graded responses by individual insects to different concentrations of oviposition or feeding stimulants, either when the stimulus is chemotactile or gustatory instead of olfactory, or when the sources of vapours are so close together that the higher concentration does not have the automatic advantage due to its wider sphere of influence, would be evidence that the insects could discriminate between concentrations.

Hovanitz and Chang (1963) found that larvae of Pieris rapae were differentially attracted by different concentrations of the mustard oil, allyl isothiocyanate, with their preferred concentration depending on the host plant on which they had been reared for several generations. But their results were the sum of choices by fifteen to fortyfive larvae, each usually tested about twenty times, and no individual results are quoted so that it is not possible to determine whether individual larvae were discriminating between the different concentrations of mustard oil. David and

Gardiner (1966b) tested four concentrations of each of eight mustard oil glucosides (incorporated into an artificial diet) as feeding stimulants for the larvae of Pieris brassicae. They found an increase in the total frass count with increasing concentration of the glucosides, brought about by an increase in the number of larvae feeding and also usually by an increase in the average number of frass pellets produced per feeding larva. Yet they commented that "the average number of frass pellets produced by each feeding larva usually did not show the consistent increase in effectiveness which would have been expected if the effect of increasing the concentration of glucoside was primarily to cause progressively more food to be consumed." Thus even a feeding experiment did not give clear unequivocal results. But Ma and Schoonhoven (1973) have recently reported that certain hairs on the tarsi of P. brassicae are associated with contact chemoreceptor cells which are sensitive to mustard-oil glucosides. In serial experiments with increasing concentrations of mustard-oil glucosides (or at least solutions of their salts) the overall electrophysiological response from these "B-hairs" increased with increasing concentration. But in some animals tested the B-hairs were not responsive, suggesting there is variation in sensitivity, perhaps associated with age or physiological condition. Ma and Schoonhoven also discussed evidence that such responsiveness probably varies between different strains of P. brassicae.

Hovanitz and Chang (1964), testing oviposition responses of

P. rapae found that artificial media containing 2, 4 or 6% of a water-soluble extract of ground mustard seeds were more attractive than those containing lower concentrations or no extract at all but they reported no more than two replicates of any of the concentrations tested, and only one test per concentration for most concentrations, so that there was no clear evidence whether the butterflies' response was all-or-nothing, or a graded one, though the former appeared more likely. When they tested the butterflies' response to different concentrations of the mustard oil, allyl isothiocyanate, itself, the medium with the maximum concentration of mustard oil (10 ppm) did not gain a significantly higher proportion of the total eggs laid than the medium with distilled water instead of mustard oil, and the media with lower concentrations received even less eggs than the medium with distilled water. But one of the six artificial substrates available for the butterflies to choose between, contained 2% mustard seed extract, which, as already mentioned, is significantly more attractive than medium with only distilled water. Hovanitz and Chang concluded that the lower concentrations (less than 10 ppm) of mustard oil may be repellent, but as they reported the results of only one test per concentration, this conclusion does not seem to be justified. Gupta and Thorsteinson (1960) painted the surface of artificial substrates with allyl-isothiocyanate at concentrations of 1, 10, 100 and 1000 ppm, to test its effect on the oviposition behaviour of Plutella maculipennis. They found that at 1000 ppm the moths laid significantly

less eggs, and at 10 ppm, significantly more, than on untreated substrates - at 1 ppm and 100 ppm there was no significant difference between the numbers of eggs laid on treated and untreated substrates.

More meaningful results would probably have been obtained for Pieris rapae, therefore, by testing the response to allyl isothiocyanate in the absence of "competition" from the much stronger stimulus from the mustard seed extract, using more replicatas, and also perhaps testing one or two higher concentrations of mustard oil.

Thus Hovanitz and Chang's results did not really indicate whether or not differences in mustard oil glucoside levels between different plants, such as might occur in different varieties or due to differences in the availability of sulphur in the soil, can influence the distribution of eggs among plants by P. rapae. To determine whether they can, I followed the method used by Gupta and Thorsteinson (1960) for white and black mustard plants. They compared the attractiveness for oviposition by Plutella maculipennis of plants grown in a complete nutrient solution with others (of the same species - they tested white mustard first then black mustard) grown in medium deficient in sulphur. The females in their experiments laid more eggs on the plants in the complete nutriant solutions but a high variance in the results made the difference non-significant. My experiments, using brussel sprouts plants, are described in Sections 3.2.2.1 (for responses to olfactory stimuli only) and 3.2.2.2 (for responses to total - i.e. both chemotactile

and olfactory - stimuli).

3.1.2.3 Conditions under which Plants Grew

Like ageing, the conditions of mineral nutrition, soil-water relations, and quality and quantity of light, in which plants have grown, will influence their physiology and will be reflected in their physical characteristics such as size, shape, general growth form, the colour, surface texture and rigidity or flexibility of leaves, and even the production of secondary plant substances, such as just discussed.

Many workers have shown that physical characteristics are important in determining whether or not an insect will attempt to lay an egg or eggs on a particular plant, or part of a plant, or on an artificial oviposition substrate, and if she does attempt to lay, whether or not she will succeed.

For instance Prokopy (1968) and Prokopy and Boller (1971) have shown that for 2 species of Tephritid flies visual, tactile and proprioceptive stimuli are the most important for eliciting oviposition. The colour of artificial substrates in important but it seems that the flies' colour preferences, and their means of colour discrimination, depend also on the size and shape of the objects being tested. When large (30 x 40 cms) rectangles were tested, flies were significantly attracted to yellow ones in preference to

other colours, and when there, they attempted to feed. Prokopy (1968) concluded that yellow rectangles were selectively attractive (because they simulate natural feeding sites) on the basis of true colour discrimination, whereas the flies' preference for dark colours when small spheres (which simulate apples, in the case of apple maggot flies, or cherries, in the case of cherry fruit flies, to which fruits the flies would normally be attracted for mating and oviposition) were tested, depended not on true colour discrimination but on detection of contrast with the surroundings. In the case of the apple maggot flies, as the diameter of the spheres was increased from 7.5 cms to 45 cms the flies' preference changed from dark colours to yellow.

It appears that Pierid butterflies' colour preferences are based on true colour discimination rather than contrast. Ilse (1937) showed that Pieris brassicae were attracted to green and blue-green paper discs, on which they would "drum" with the forelegs in the same way that they do on a leaf, preparatory to ovipositing on it; on papers of other colours, e.g. yellow, red, blue or white, they rarely "drummed" but usually attempted to feed instead. They neither "drummed" nor attempted to feed on (apparently just ignored) grey paper of the same tone, or degree of contrast, as the green or blue-green. Hovanitz and Chang (1964) found that Pieris rapae also preferred green and especially blue-green artificial substrates for oviposition.

Unlike Prokopy's work on the oviposition responses of apple maggot flies to artificial substrates, none of the studies of ovipositional responses in Pieris have considered interaction between different physical stimuli e.g. whether or not colour preferences are influenced by other stimuli such as size, shape, surface texture, etc. or whether although they are independent a response to one stimulus may override that to another. Nevertheless it is unlikely that one physical characteristic of a plant will change as a result of growth conditions, without any change occurring in others, so that although studies of single characteristics are an essential basis for an understanding of the insects' responses, it cannot be assumed that their response to the whole plant will be the sum of their responses to each character, tested individually.

There are conflicting reports about whether female Pieris rapae lay more eggs on larger plants - probably partly because the workers have not allowed for the variety of ways in which a plant may be "large". It may be tall, but with relatively few large leaves, on long petioles and with long internodes; or it may have many leaves either large or small; or, again, a plant may be "large" while only of average height but if it has very many small, closely packed leaves, or fewer, but still a fairly large number, of very large leaves.

Thus butterflies may respond to the height of a plant, or the number of leaves it has, or the size of those leaves, or all three,

and if they do, their responses will vary for different combinations of these factors, so that to try to find relationships with one factor alone without controlling or even considering others, may lead to conflicting results.

Utida et al. (1952 cited by Takata 1961(a)) did not find a significant correlation between the number of eggs and the number of leaves on a cabbage plant, but occasionally they found a significant correlation between the number of eggs laid on a plant, and its height. On the other hand, in 1957 Kobayashi reported that there was occasionally a significant correlation between the number of eggs on a plant and the number of leaves it had; in 1960 he again reported significant correlations, but in 1965 he apparently concluded that such correlations did not occur frequently enough for the butterflies' response to the size of plants to significantly influence the pattern in which they distribute their eggs.

Gilbert (pers. comm. 1973) found that female Pieris rapae are attracted to a cabbage plant over a distance of up to one meter. He concluded that the strength of the attraction is a function of plant size, not merely height, for more eggs were laid on large plants than on small,even when the small plants were propped up on bricks to be taller than the large ones.

Although I did not do an experiment testing the butterflies' responses to the size of plants or leaves, directly, the leaves of

plants were measured for the experiments described in Sections 2.2.1.2 and 2.2.2.1, so that the distribution of eggs has been analysed graphically in relation to leaf area as well as leaf age in Section 3.2.1.2, and to the total area of each plant in Section 3.2.3.2. Plant height was unfortunately not measured so the same criticism applies to these analyses as to those of other workers; that if some factors are neither controlled nor measured the true relationship, if any, may not show up. Section 3.2.3.2 also gives a graphical analysis of the distribution of eggs laid naturally by wild P. rapae on young cabbage plants of different sizes growing in a private garden. These plants were not measured precisely but both the whole plants, and their leaves, were rated (subjectively) according to sizes.

Differences in the susceptibility of different plants, or different varieties of the same plant, to attack by insect pests are sometimes largely determined by the qualities of the surface of the leaves, pods, or which ever part of the plant the female insect oviposits on. For example, Nishijima (1960) found that hairy varieties of soy-bean received many more eggs than hairless ones, and in the former eggs were laid predominantly on the pod, which was the least favoured site in the latter. Mehta and Saxena (1970) showed that the favourable stimulus of a hairy surface was apparently more important to Earis fabia than the presence of chemical oviposition stimulants, as some plants with a hairy surface but without the

chemicals gain eggs, but those with the chemical oviposition stimulants but no hairs, don't. The most favoured plants provide both stimuli.

Similarly the thickness, and texture, penetrability etc. of the wax layer with which Prokopy and Boller (1971) coated their artificial oviposition substrate were critical in determining whether or not the flies would oviposit on it.

Although differences such as the presence or absence of hairs on a surface are only likely to occur between different varieties, rather than as a result of growth conditions, other qualitative differences in the leaf surface, such as, perhaps, differences in the wax layers, smoothness, wrinkles, or lumps in the surface, its degree of moisture, and things like flexibility or rigidity of the leaf, (depending perhaps on how fibrous it is) may result from differences in the light, mineral nutrition and accessibility of water during the plants' growth.

Beck (1965) has reviewed the subject of insects' oviposition responses to physical stimuli (in a review of plants' resistance to oviposition) more fully, but the above examples give a background against which to interpret my results.

Butterflies were tested to determine whether they would discriminate between young brussel sprouts plants grown outside and others grown indoors in artificial light ever since they were potted

as seedlings. Unfortunately this was only six to eight weeks and to the human eye there was very little difference between the appearance of plants in the two treatments; a few of the plants grown outside were taller than any of those grown inside, but otherwise there was about as much variation within treatments as between them, in the general size of plants and the number and colour of leaves. There was a slight tendency for the leaves on plants grown indoors to be smoother and more even in shape, and to grow rather more horizontally than leaves on the plants grown out-of-doors, but the differences were not striking.

As the butterflies did not discriminate between plants in the two treatments, the experiment is not described in detail. Apparently the plants required longer periods of different treatment to induce consistent differences that would be greater than the normal within-treatment variability in their physical characteristics.

Butterflies were also tested to determine whether they would discriminate between plants grown in high intensity, and others grown in low intensity, artificial light. These treatments did appear to induce differences between the plants, so the experiment is described in Section 3.2.3.1.

3.2 Experiments on the Females' Egg-laying Response to Differences Between Plants

3.2.1.1 Experiment to Determine Whether Females Discriminate Between Plants of Different Ages

Method:

10 young (2 months and) since potted as seedlings)
10 old (11 months)

sprouts plants were arranged in a 5 x 4 array, with the positions classified into three strata as described in Section 2.2.2.2, with half the plants in each stratum coming from each treatment. Plants were randomized first into strata and then into positions within each stratum. Twenty-three female and ten male butterflies (caught in the field a few days prior to the experiment) were put in the cage at 17.05 hrs and those surviving (fourteen females and five males) were recollected at 09.35 hrs two days later, so that they had a total of 24 hrs (4, 16 and 4 hrs consecutively in three light periods) for oviposition.

Results and Discussion

The young plants received more than twice as many eggs as the old onces but the number of eggs laid per plant was extremely variable on the young plants. Because the between-plant variance for the young plants was significantly greater than that for the old plants, an analysis of variance (to separate position effects from random variation within each treatment and from treatment effects) would not be valid unless the data were transformed, nor would a

TABLE 3.01

Preference of females for young plants over old ones, for oviposition

Plant No.	Number of Eggs	
	Young Plants	Old Plants
1	35	11
2	164	74
3	54	42
4	45	1
5	77	45
6	119	11
7	69	12
8	187	12
9	114	74
10	14	27
Total	878	309
s^2	3220.6	717.0

F $s_y^2/s_0^2 = 4.49$

$0.02 < P < 0.05$

$\therefore$ Analysis of Variance not valid

Mann-Whitney U test:

$U = 15.5$

$P < 0.01$

simple t test be valid. But the validity of the non-parametric Mann-Whitney U test does not depend on the variances of the two treatments being homogeneous, so this test was used. As Table 3.01 shows, in spite of the extreme variability in the numbers of eggs per plant, young plants did receive significantly more eggs than old ones.

The younger plants tended to be more variable in size and growth form than the older ones, with softer, lighter green leaves; the older plants' leaves tended to be thicker, more stiff and fibrous

and more grey-green in colour and regular in shape. [Brussel sprouts plants do not normally live as long as eleven months after transplanting as seedlings - market gardeners tell me that when grown commercially if they are not harvested they run to seed by about six months after transplanting. My plants had been growing in small (4" top diameter, 4" high) non porous plastic flower pots with four drain holes around the base, ever since they were seedlings, so probably they were root-bound. (For later experiments most plants were grown in porous earthenware pots). They had been intermittently in an air-conditioned room, under artificial lights, being used for experiments, and out on the open roof (i.e. exposed to natural daylight and weather conditions). They were given "Aquasol" liquid fertilizer occasionally and any sprouts that started to grow were removed, as they made counting and analysis of the egg-distribution on a per leaf basis, much more difficult].

Delaveau (1958) reported variations in the mustard-oil glucoside content of different parts of the crucifer Alliaria officinalis at different stages of its development. The mustard-oil glucosides move from the rosette leaves to the upright growth and thence to the seed heads as they develop. Though my older brussel sprouts plants had not run to seed (because of the artificial conditions under which they had been growing) it is still possible that the mustard-oil glucoside levels in the leaves of young and old plants have differed as

(i) In the old plants some of the mustard-oil glucosides may have been transferred into the developing sprouts, which I subsequently removed before the experiment.

(ii) Delaveau reported that even during the rosette stage in Alliaria the total content of mustard-oil glucosides rose, and as at that stage most of it was in the vegetative leaves, their mustard-oil glucoside level rose.

Thus although the butterflies probably chose to lay eggs preferentially on the younger sprout plants in response to the colour, growth form and texture of the leaves, it seemed possible they were also responding to differences in olfactory or chemotactile stimuli from mustard-oil glucosides, from plants of different ages. But the experiments described in Section 3.2.2 showed that even if the plants did differ in this respect it is unlikely that the butterflies were responding to the difference.

3.2.1.2 Analysis of the Numbers of Eggs Laid on Leaves of Different Ages

Methods:

The seven distributions of eggs analysed in this section were taken from the results of experiments reported earlier, in Chapter 2, as set out in the following table (over).

Symbol in Text	O.P. When Eggs Laid	Section Describing Experiment
(a)	Pre-experiment O.P.	2.2.1.2 ("initial eggs")
(b)	O.P. (b)	2.2.1.2
(c)	Replicate 1 (with larvae)	2.2.2.1
(d)*	First replicate without larvae	2.2.2.1 (but this O.P. not described)
(e)	Replicate 3) with	2.2.2.1
(f)	Replicate 4) larvae	2.2.2.1
(g)*	Third replicate without larvae	2.2.2.1 (this O.P. not described)

* The experiment described in Section 2.2.2.1 actually had three more replicates that were not included in the description and analysis as they did not contribute any information relevant to the hypothesis, as there were no larvae on any of the plants in these replicates. The first ((c) above) and second replicates with larvae were each followed, the next day, by a replicate without larvae (the first of these is (d) above), and the third and fourth replicates with larvae ((e) and (f) above) and the third without ((g) above), were done (in that order) on three consecutive days. The replicates without larvae were intended to serve as extra controls for differences in plant attractiveness when no larvae were present, but problems with the analysis led to contradictory results and a waste of potential information. So the extra "control" replicates were omitted and the results re-analysed by a different method - that shown in Section 2.2.2.1.

Leaves were classified according to their position on the plant, such that the youngest leaf unit - the still curled leaves of the central sprout - was numbered 1, the most recently unfolded leaf, as 2, the next one to uncurl before that, as 3, and so on down to the oldest leaf. All leaves numbered 1, or all those numbered 6, etc. on all plants in the same O.P. were considered to be in the same age-class. The mean number of eggs per leaf for each age-class was plotted against that age-class (or position) for each of the seven O.P.'s. (If there were less than 5 leaves in any age-class the result for that age-class was omitted).

If there is any relationship between the position or age-class of leaves and the mean number of eggs they gain, then it could result from a response by the females to either (a) age, or (b) leaf size. So the mean area of leaves in each age-class was calculated for each O.P. and plotted against position or age-class, to determine the approximate distribution of leaf size with age, shown in Figures 3.03 - 3.05. The mean number of eggs per leaf in each age-class was also plotted against the mean area of leaves in that age-class to determine the relationship, if any, between them. [It was to allow the best possible estimate of leaf area that the seven O.P.'s used were chosen - leaves were measured just before (a) and then not measured again throughout that experiment until immediately after (b). For the second experiment from which distributions are used, the leaves were measured immediately before (c), so that as (d) was done

Fig. 3.03

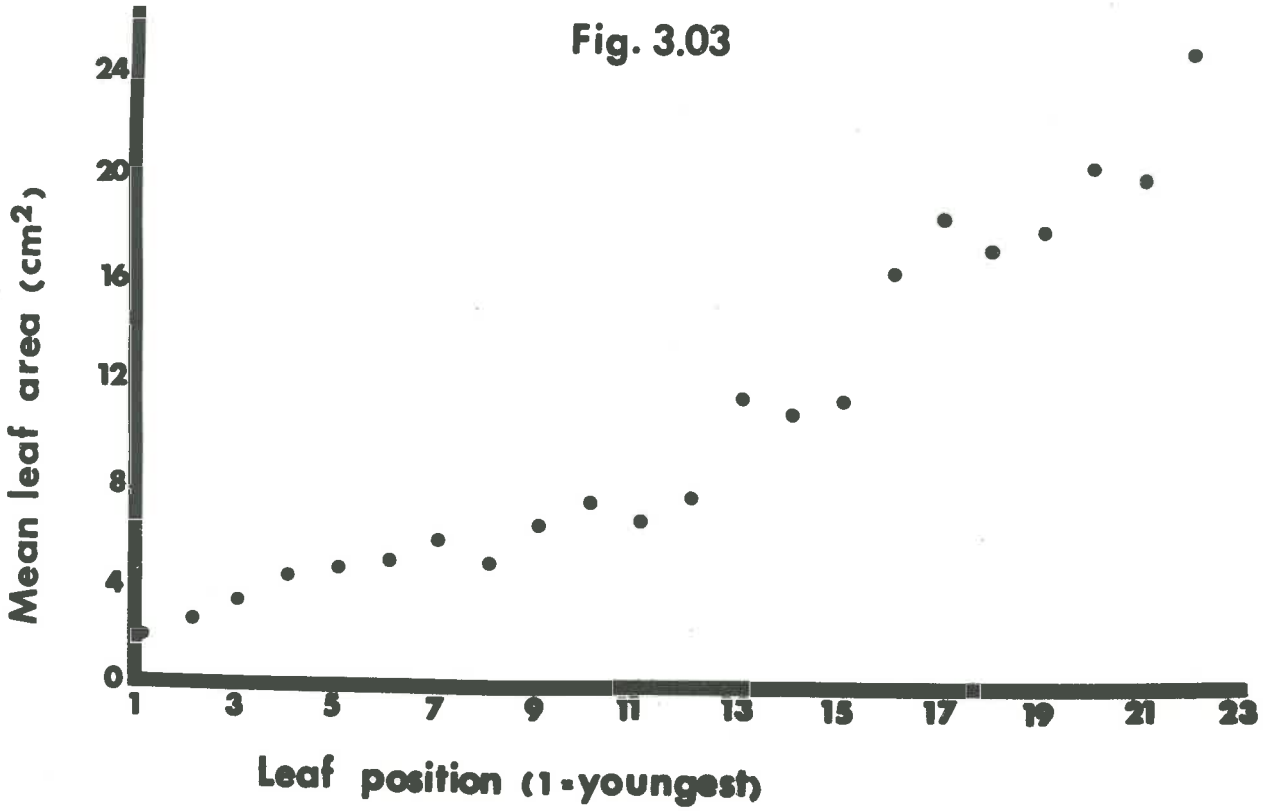

Mean leaf area (cm²)
0
4
8
12
16
20
24
1
3
5
7
9
11
13
15
17
19
21
23
Leaf position (1 = youngest)

Fig. 3.04

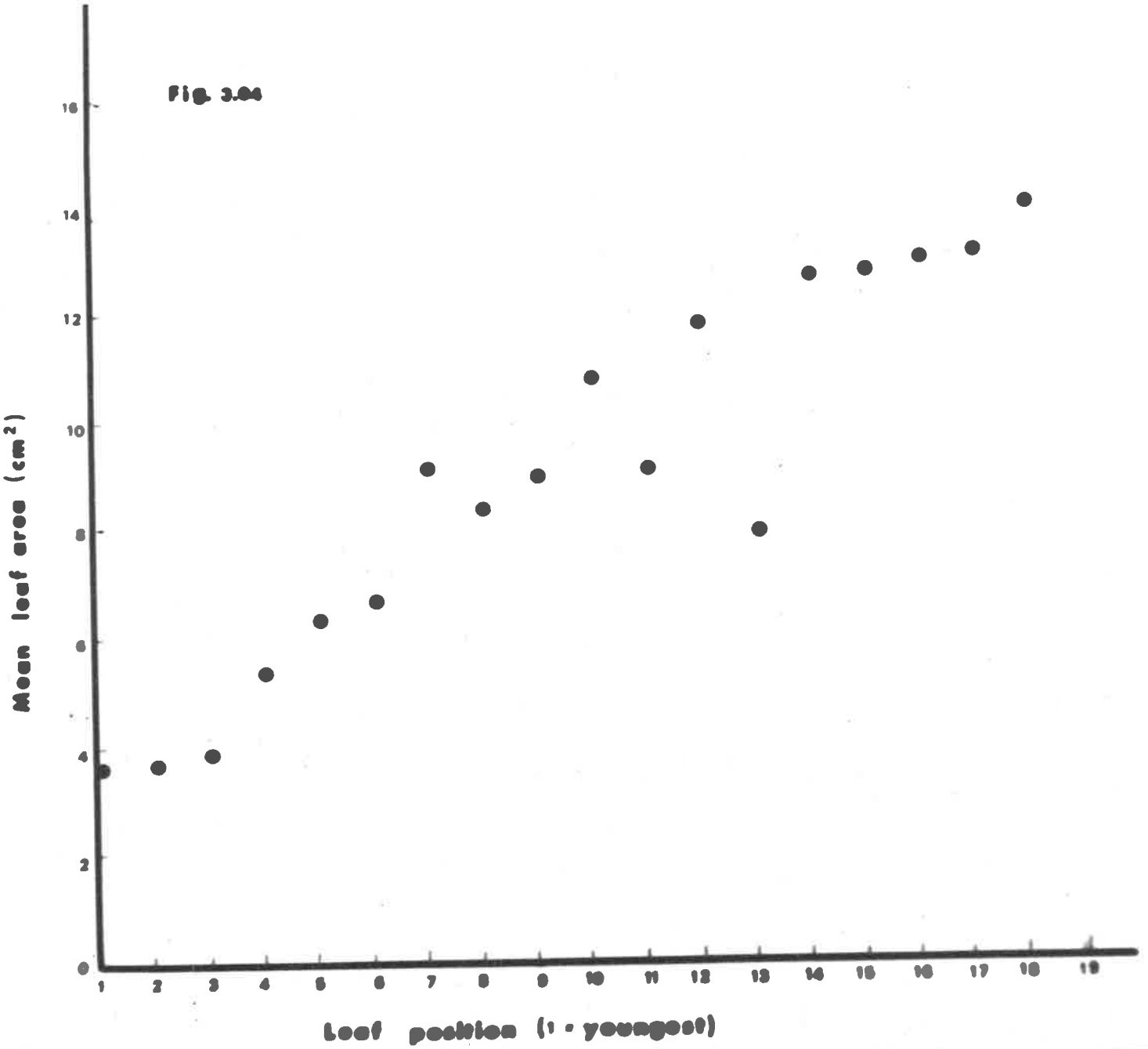
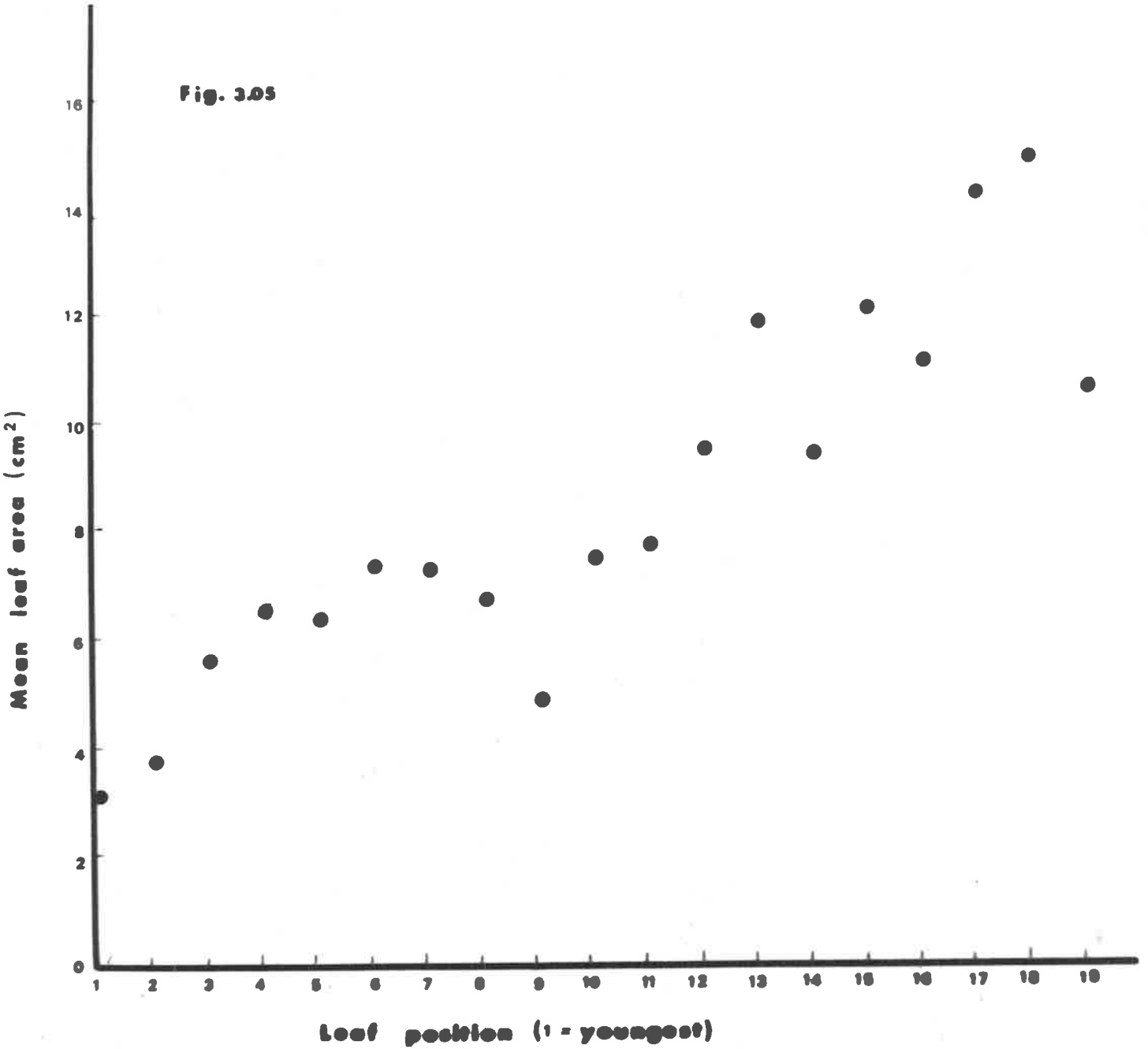

Mean leaf area (cm^2)
0
2
4
6
8
10
12
14
16
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Leaf position (1 = youngest)

Fig. 3.05


Mean leaf area (cm^2)
0
2
4
6
8
10
12
14
16
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Leaf position (1 = youngest)

the following day, there was little enough change in the leaves' classification for it to be valid to use the same leaf measurements for both replicates. By the time of the second replicate with larvae, there would have been a difference between the class occupied by a leaf then, and the class it had been in when its area was measured, for more leaves, and the difference would have been greater, than between (c) and (d); also some leaves would have grown significantly. Consequently it would probably not be valid to use the same estimates of area, etc., for the first and second replicates with larvae so the second replicate was omitted from this analysis. Similarly, the leaves were measured again immediately before (e), so that it was valid to use these estimates for (f) also, as it was done the day after (e). By (g), which was done the day after (f), there was a little more discrepancy, but it was necessary to have another replicate without larvae to compare with (d). The leaves were numbered as for (e)]. Although 24 of the butterflies used in (a) were also used in (b), as mentioned in Section 2.2.1.2 (p. 39) it is unlikely that they laid many of the eggs in (b), most of which were probably laid by the twelve younger females that had not been used before. But for the four replicates of the other experiment, the butterflies that were used for (c) were also used for (d), and the butterflies that were used for (e) were also used for (f) and (g).

Fig. 3.01

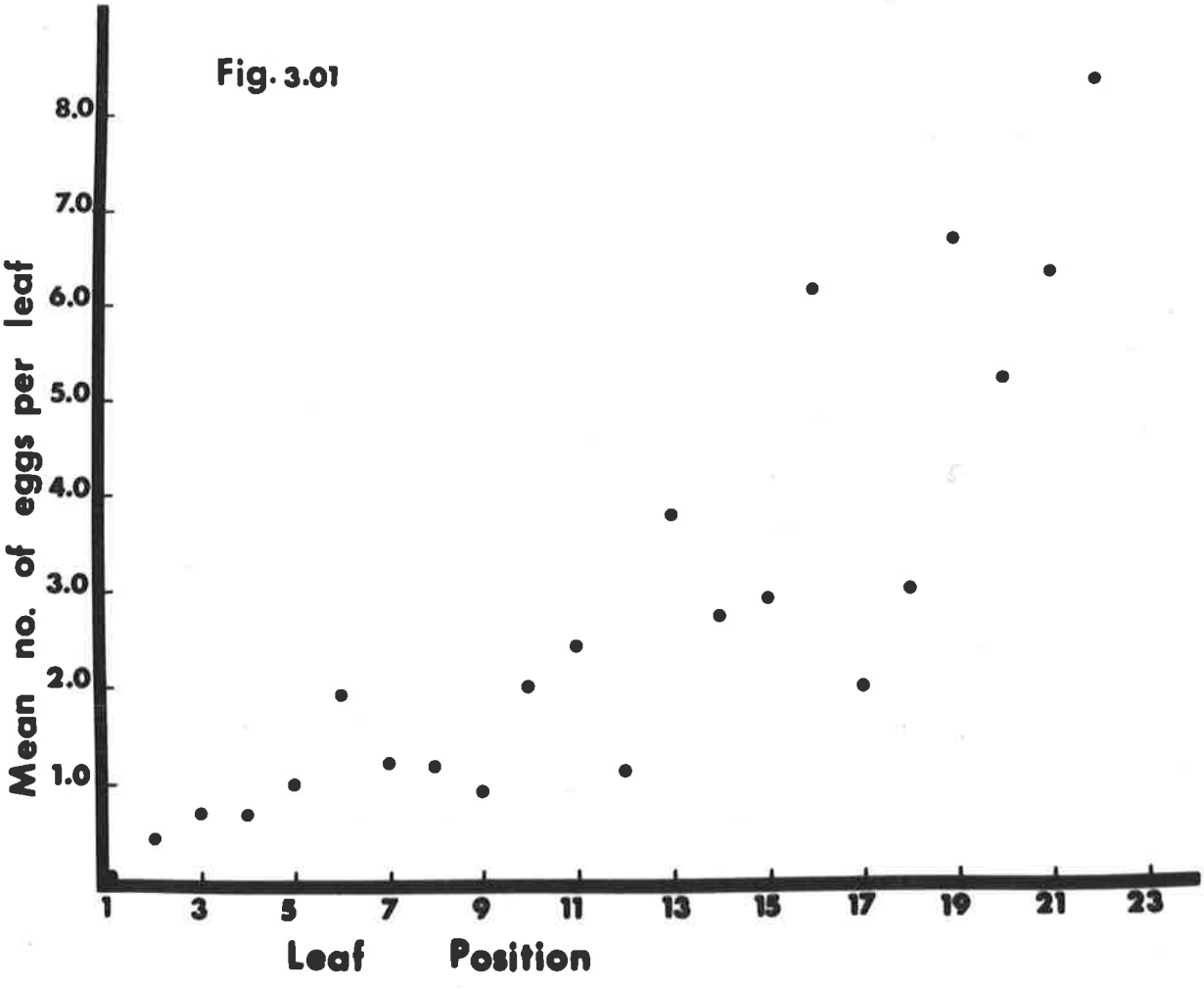
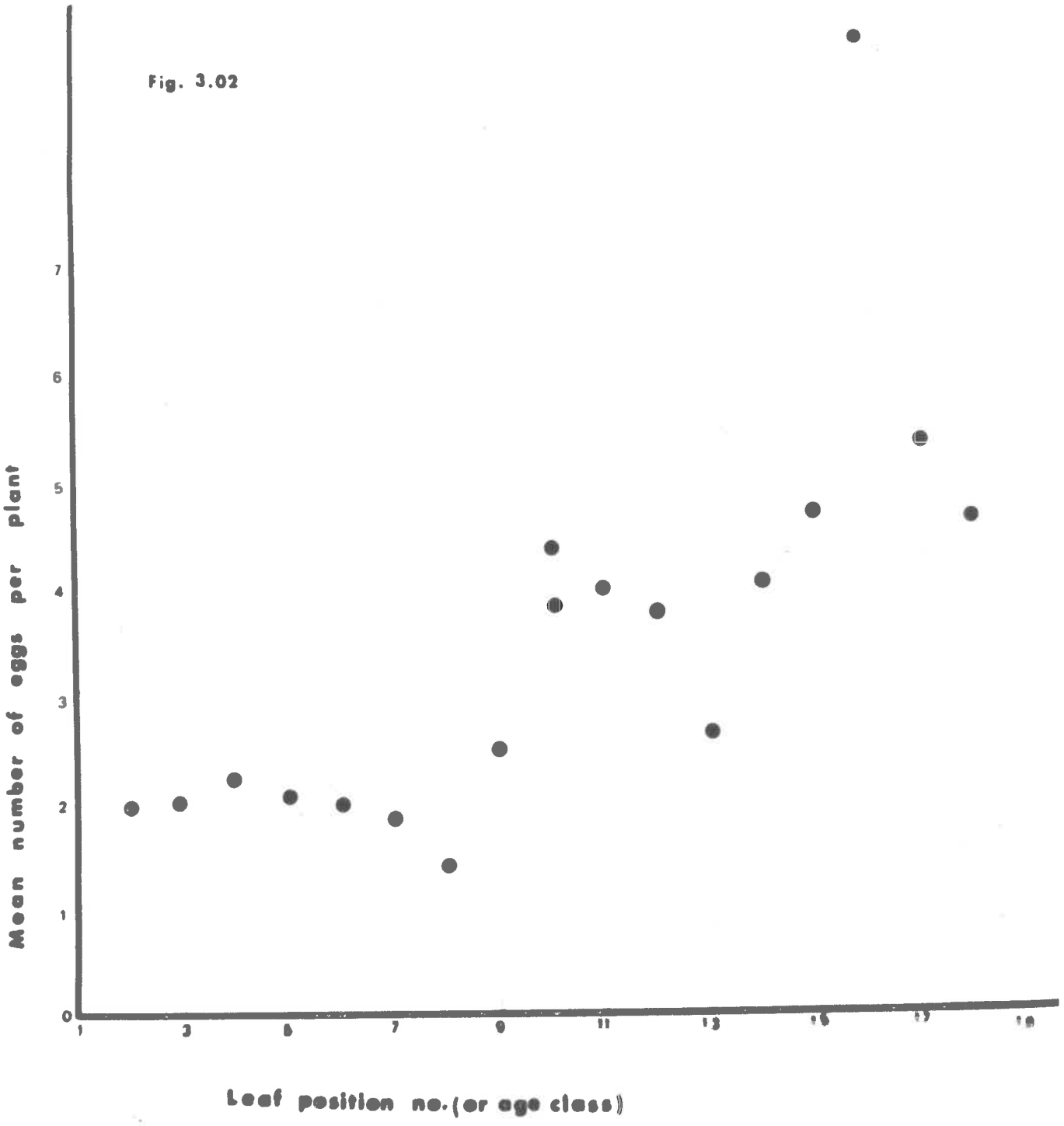

Mean no. of eggs per leaf
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
1
3
5
7
9
11
13
15
17
19
21
23
Leaf Position

Fig. 3.02


Mean number of eggs per plant
0
1
2
3
4
5
6
7
1
3
5
7
9
11
13
15
17
19
Leaf position no.(or age class)

Results and Discussion

As shown in Figures 3.01 and 3.02, in two of the seven distributions (those for (a) - Fig. 3.01, and (d) - Fig. 3.02) there appeared to be a tendency for the mean number of eggs per leaf to increase with increasing age (position number) of the leaves, though the points were rather widely scattered. The other five distributions showed no consistent relationship at all between the mean number of eggs on a leaf and its age-class, just a random scatter, so their plots are not included. For four of these five distributions ((b), (c), (f) and (g)), there was also no relationship between the mean number of eggs per leaf in a given age-class and the mean area of leaves in that age-class, so those graphs also were excluded. Although the same butterflies were used in (c) and (d), in the former there was no sign of a relationship between the mean number of eggs per leaf in an age-class and the mean area of leaves in that age-class, but in the latter there was a significant linear regression (Figure 3.07) with a probability of less than one in a thousand if the butterflies do not really prefer larger leaves. This seems too large a difference between consecutive replicates to simply be the result of different individual members of the group, with different preferences, laying eggs in the two replicates. But nor does it seem likely to be due to the presence of larvae on half of the plants in (c) and not in (d), as although larvae were present in (e) and (f), and absent in (g), some of the butterflies showed a slight (though by no means significant, $0.1 < P < 0.2$) preference for

Fig. 3.06

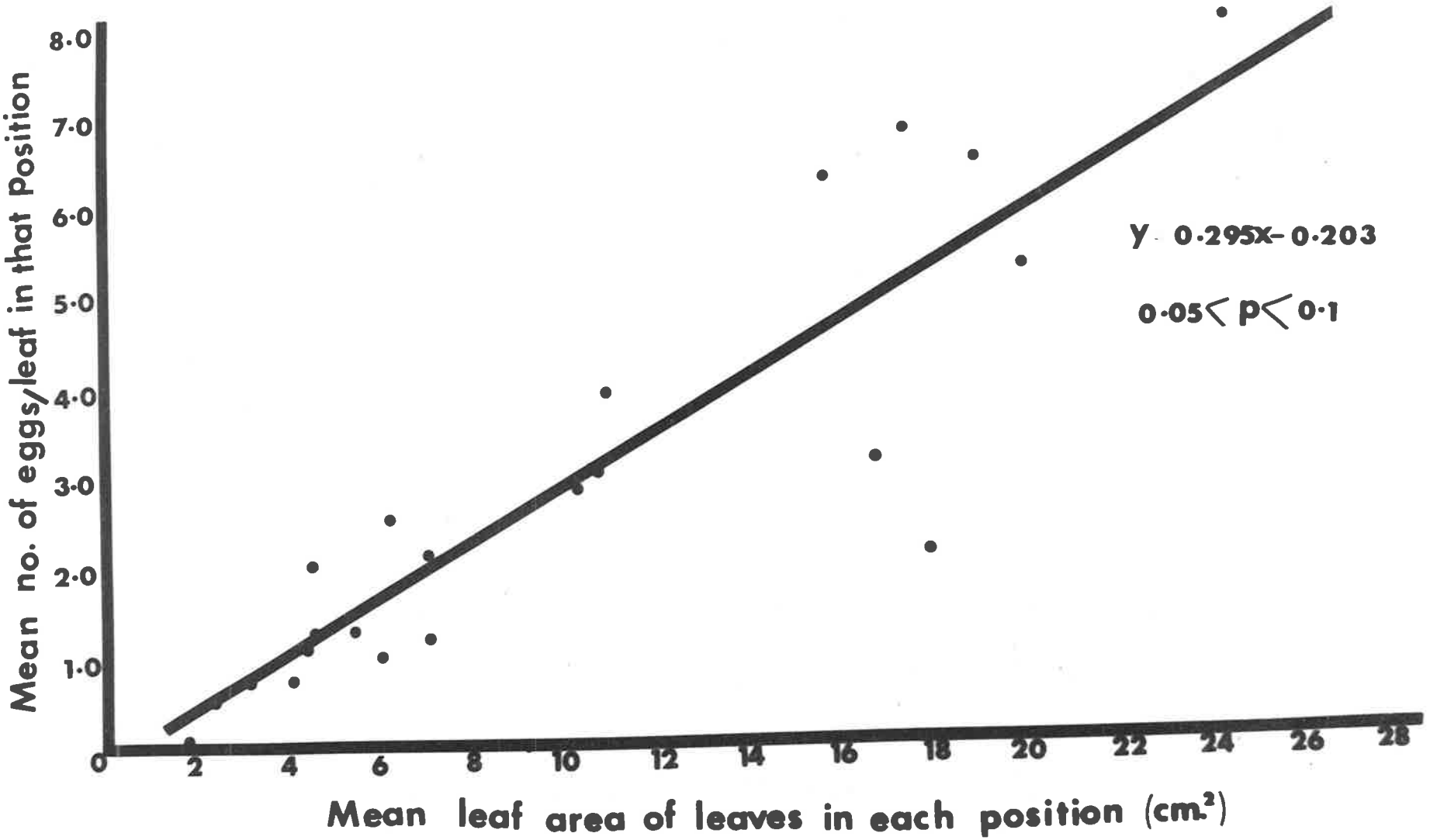
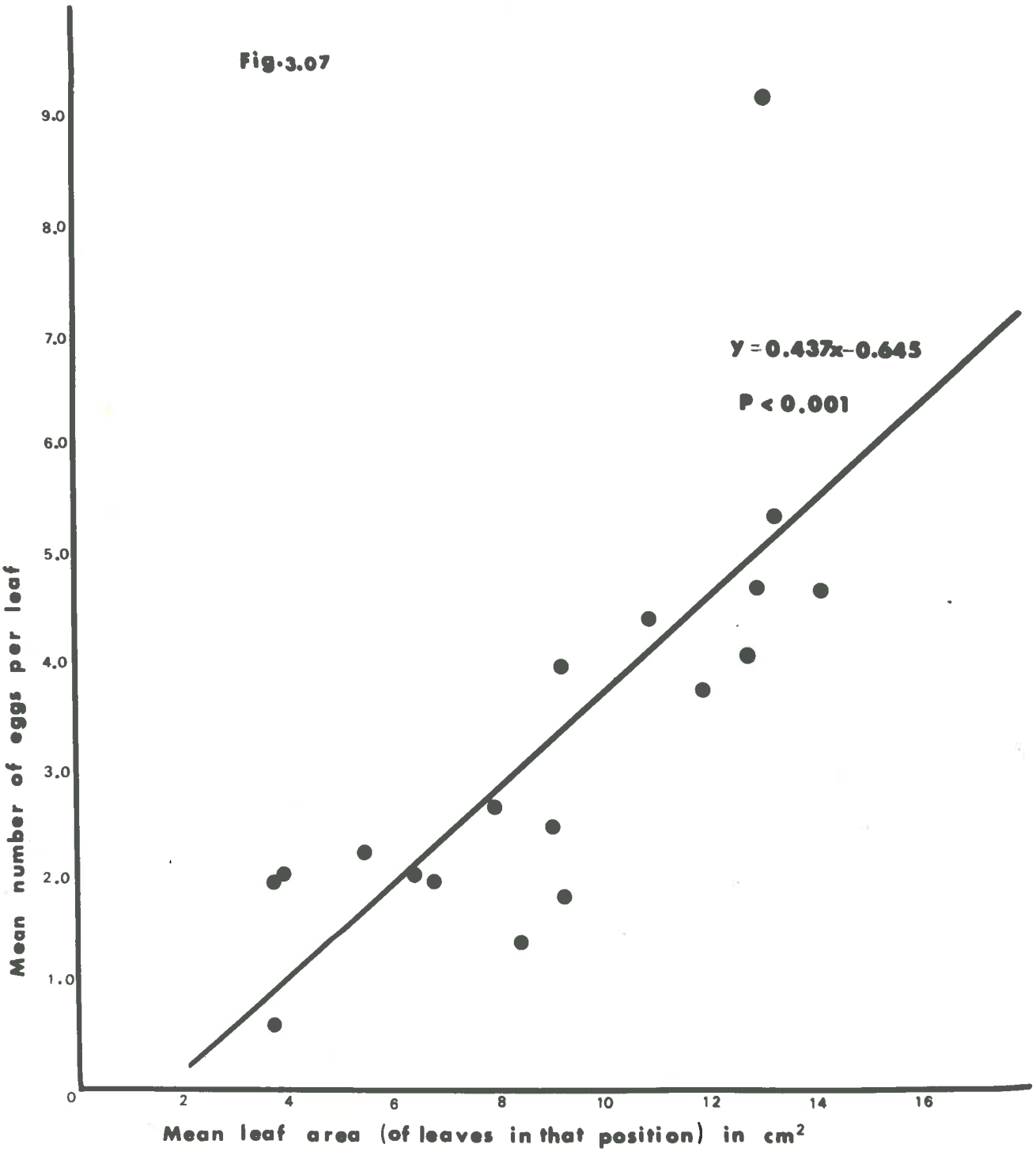

Mean no. of eggs/leaf in that Position
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
Mean leaf area of leaves in each position (cm.²)
y 0·295x - 0·203
0·05 < p < 0·1

Fig.3.07


y = 0.437x-0.645
P < 0.001
Mean number of eggs per leaf
Mean leaf area (of leaves in that position) in cm²
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
2
4
6
8
10
12
14
16

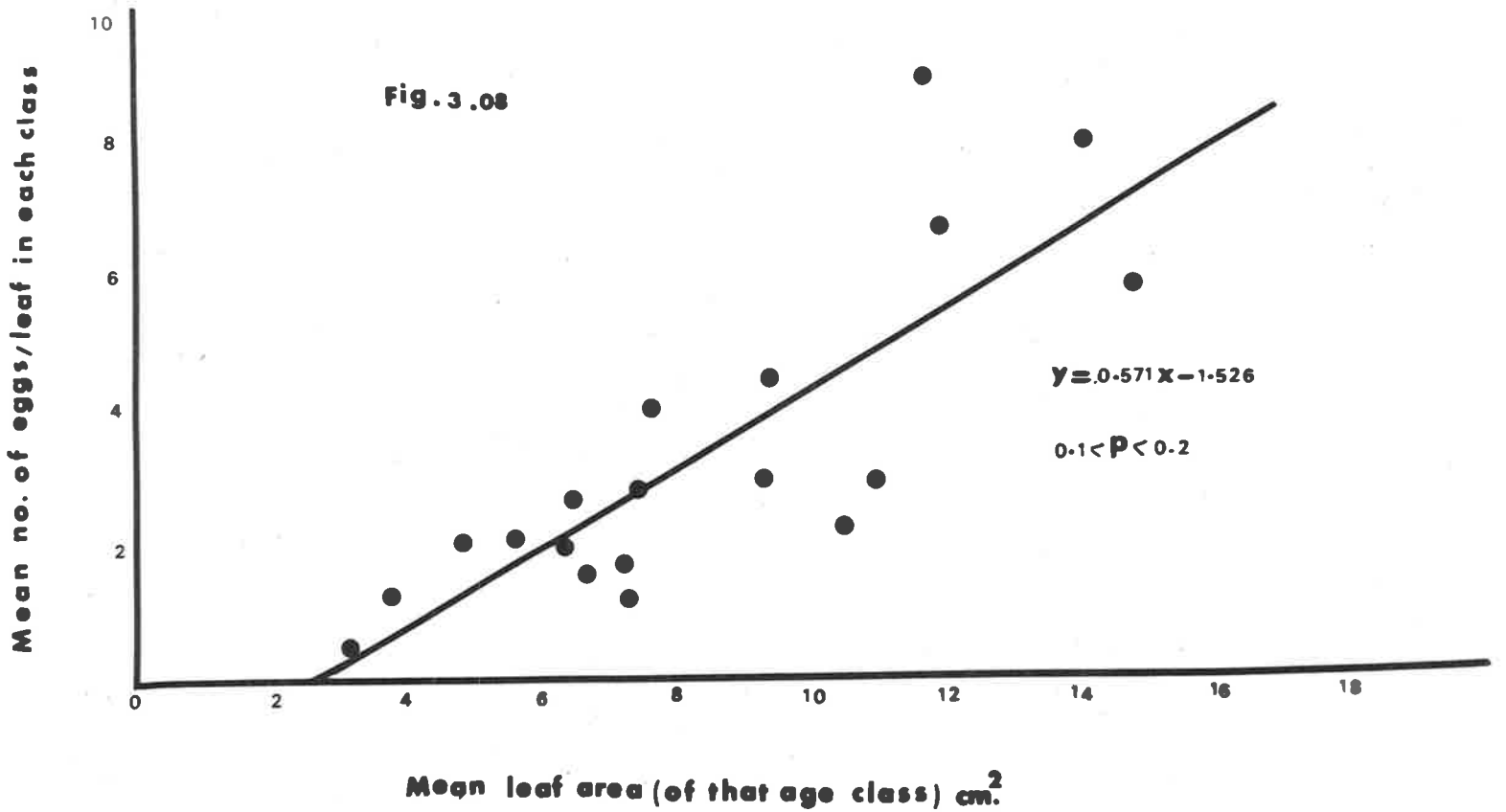


Fig. 3.08
Mean no. of eggs/leaf in each class
Mean leaf area (of that age class) cm.²
y = 0.571 x − 1.526
0.1 < P < 0.2
0
2
4
6
8
10
12
14
16
18

larger leaves (Figure 3.08) in (e), but no preference in either of Replicates (f) and (g) ($0.2 < P < 0.3$ and $0.3 < P < 0.4$, respectively).

It might be suggested that using the mean values of leaf area, or number of eggs, for any given age-class, rather than individual values, to determine the relationship (if any) between these two variables, will give inaccurate results. This may be so, but the inaccuracy is not likely to be large enough to affect the general conclusions; certainly it could not be used to explain the discrepancy between the results from (c) and (d), as the same measurements of leaf area and the same classification of leaves were used for both (c) and (d). If the number of eggs on each leaf had been expressed relative to the area of that leaf and these values plotted for each age-class more information may have been gained. Unfortunately the data had been collected in a way which made such analysis very time consuming, so that by the time I realized the advantages of the latter analysis, there was not time to do it. Nevertheless, not even that analysis could explain the discrepancy between the butterflies behaviour in (c) and (d), as the same measurements of leaf area were used for both. Thus I cannot think of any reasonable explanation for this anomalous behaviour by the butterflies.

The similar, though smaller, discrepancy between (a) and (b) (for (a) there was a weak trend, as shown in Figure 3.06, while

(b) was just a random scatter), possibly could have occurred because probably very few of the same butterflies were laying eggs in both O.P.s - the eggs in (b) being mainly laid by the twelve new butterflies. Apparently some, but not all, of the butterflies that laid eggs in (a) were attracted to the older, larger leaves, as although not significant ($0.05 < P < 0.1$) the trend is more convincing than for (e). The only general conclusions (applicable to caged butterflies) that can be drawn from this analysis therefore, is that sometimes some butterflies prefer to lay their eggs on the larger, older leaves of the plants available to them. The preference may rarely (as for example in one out of seven distributions analysed) significantly influence the overall distribution of eggs among leaves, though I was unable to determine what conditions led the butterflies to be selective on some occasions and not others.

3.2.2 Experiments to Determine Whether Females Discriminate Between Plants Containing Different Levels of Mustard Oil Glucosides

Method of Preparing Plants for Both Experiments

Two dozen brussels sprouts plants were grown hydroponically indoors under artificial lights from the time they were transplanted as seedlings. Twelve of them were grown in a complete nutrient solution and the other twelve in a sulphur deficient nutrient solution. (This was not completely free of sulphur but

contained only 1/23rd of the amount in the complete nutrient solution, i.e. 0.09 mgm-atoms S/litre of S-deficient solution as compared with 2.09 mgm-atoms S/litre of complete nutrient solution). The recipes for these solutions and a description of the method of growing the plants hydroponically are given in Appendix 3.

The plants grown in sulphur-deficient solutions did not show any symptoms of sulphur deficiency but throughout the course of the experiments plants in both treatments were attacked by what appeared to be a latent viral disease (as described in Section 1.2.2). If they were stressed in any way - i.e. if their nutrient solution was not topped up regularly enough, so that it became a little too concentrated or unbalanced, or if the light intensity was too low, or if the supply of air bubbling through their solutions was reduced or cut off even for a short time (and they were very sensitive to this last form of stress) - they began to show signs of the disease. Usually they did not recover even though given ideal conditions. (A very thorough cleaning of the plant and its container and removal of dead or unhealthy roots, leaves or "bark" on the stem, occasionally managed to save such plants).

As I did not know how long it would take for the plants to reach equilibrium with the nutrient solutions it seemed important that all plants should have experienced their respective treatments for as nearly as possible the same length of time. Consequently only plants that died within the first week after culturing were

replaced (before the experiments). The last of the plants in the "sulphur-deficient" treatment died before I had managed to find a satisfactory method (within the limits of the equipment and time available) of analysing the mustard-oil glucoside content of the plants. (Plants that died in the "complete nutrients" treatment were replaced after the experiment, as more of them than plants from the "sulphur deficient" treatment died, and theoretically,at least, the former plants should not take as long to equilibrate as the latter). Thus the mustard-oil glucoside content of the plants was not checked analytically and it must simply be assumed that the treatments did take effect. The plants were used for the following two experiments.

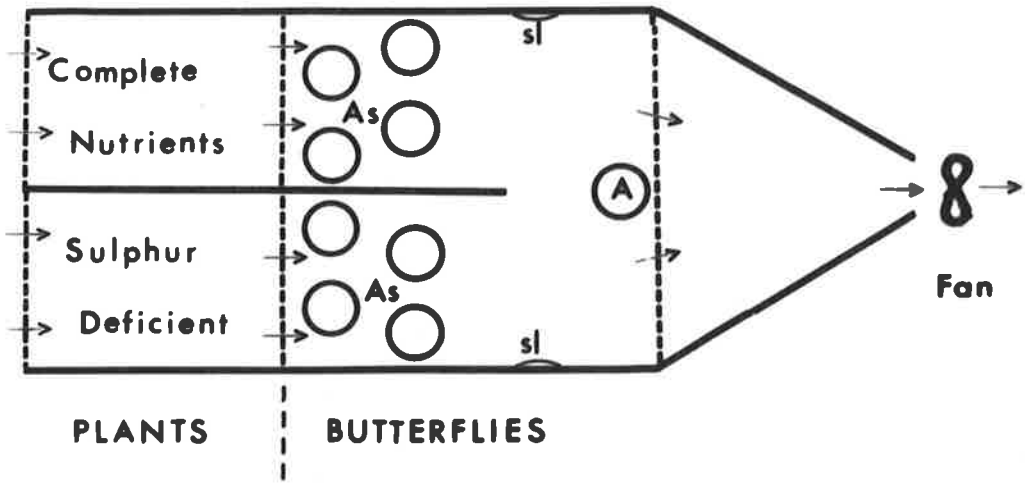
3.2.2.1 Test for a Response to Volatile Attractants

Method

The plants had been growing hydroponically and subject to the treatments for at least nine weeks prior to the first two attempts at this experiment, and for at least ten weeks by the third attempt. Six plants from each treatment were placed in the olfactory choice apparatus shown in Figure 3.09. As the figure shows, a fan 3 1/4 inches from the narrow opening in front of the choice chamber drew a gentle stream of air through the plants and into the choice chamber. The central partition kept the airstreams from plants in the two treatments completely separate until they had passed over the artificial oviposition substrates and were half-way

Fig. 3.09

OLFACTORY CHOICE APPARATUS



A = Alyssum

As = Artificial substrates

sl - sleeve

through the choice chamber. (If the fan draught was too strong the butterflies remained settled all the time). A pot of nectar-bearing Alyssum flowers was provided for the butterflies to feed on, as shown.

The artificial oviposition medium was made more or less according to the recipe given by Hovanitz and Chang (1964). Petri dishes 9 cms in diameter with a capacity of about 80 ccs were filled with a medium consisting of 1.5% agar in water, coloured with Pinnacle powdered food colouring, Apple Green 162; at the second attempt the green was diluted to be paler and for both the second and third attempts a little powdered "Reckitt's" washing blue was added while the solution was still warm and liquid. As the experiment was to test the effect of volatile attractants in the air-stream from the plants, no attractant was added to the medium. For the first attempt, the medium was covered with a thin sheet of polythene perforated with pin-holes, and for the second and third attempts, with a sheet of stretched "Parafilm", also perforated with pinholes. There were four petrie dishes of medium in each side of the choice chamber; they were placed on top of flasks to be at the level of the leaves.

During the first two attempts the choice chamber was under the usual bank of lights in the same air-conditioned room as usual, so the temperature was about 20 → 22°C. The butterflies were not very active and did not lay any eggs. In the hope of increasing the volatility of the mustard oil glucosides and the activity of the

butterflies, the olfactory choice apparatus and the bank of lights over it were moved to a laboratory that was not air conditioned, so that the ambient temperature varied between about 30° and 32°C.

The butterflies were pre-conditioned for the third attempt at the experiment by putting them to feed on Alyssum flowers and to lay eggs on three normal (i.e. growing in soil, not hydroponically) sprouts plants for about five hours before the experiment; then at 23.05 hrs thirty-six females were put into the choice chamber. At 00.20 hrs (i.e. 75 mins. later) another ten females, and eight males were added.

Twelve hours later no eggs had been laid so two dishes of artificial oviposition substrate were removed from each side and replaced by leaves (one leaf per flask) from a plant (brussels sprouts) grown in the sulphur deficient solution, in case either the colour or surface texture of the artificial substrates, or even the odour of the agar itself, was repellent to the butterflies. Although no eggs had been laid, there had appeared to be more butterflies attracted to the side of the choice chamber with the "complete nutrients" treatment each time I had observed them since the apparatus was in the warmer room. So from the time when half the artificial substrates were replaced by leaves, I counted the more-or-less instantaneous distribution (took a minute to count, at the most) of butterflies settled in each part of the choice chamber, on nineteen occasions, separated by intervals of half an

hour, one hour, or several hours, throughout twenty-four and a half hours.

Twenty more females were added (ten introduced through each sleeve, i.e. from each side of the choice chamber) after the second count. After the eighth count the leaves were removed, the eggs on them were counted but left there and the leaves were put back, with four more leaves as well, so that there were then two leaves in each of the four-flasks (two flasks in each side of the choice chamber). After nineteen counts the sixteen surviving butterflies were recollected and the eggs counted.

Results and Discussion

As shown by Table 3.02 almost equal numbers of eggs were laid in the two sides of the choice chamber; also almost all eggs were laid on the leaves. Very few eggs were laid on the artificial oviposition substrates; and as Table 3.03 shows, very few butterflies were observed sitting or settling on them either.

As the butterflies have a circadian rhythm of egg-laying (about which, unfortunately, I did not know when I did these experiments, or they would have been designed to take account of it), it seems quite probable that their general level of activity and responsiveness to attractive sources may also vary throughout twenty-four hours. To allow for this the results were analysed by means

TABLE 3.02

Distribution of eggs laid by females in the olfactory choice chamber

Substrate on which eggs laid	Treatment Side			
	Complete		Sulphur Deficient	
	On Surfaces	Total	On Surfaces	Total
Outer Leaves	11 P*, 14 N*	25	21 P, 12 N	33
	11 P, 24 N	35	5 P, 16 N	21
Inner Leaves	12 P, 5 N	17	12 P, 18 N	30
	11 P, 13 N	24	11 P, 7 N	18
Outer Artificial Substrate	2 on vertical side surface	2	2 on top	2
Inner Artificial Substrate	1 on top, 1 on side	2	2 on top, 1 on side	3
Central Partition		1		1
Knocked off when extra leaves added		-		2
Total		106		110

* P = upper surface of leaf, N = under surface of leaf.

TABLE 3.03

Numbers of butterflies settled in the two sides of the choice chamber

Results Summed over 19 Observations			Paired Comparison t test		
Substrate on which settled	Side of Chamber		(C Compared with S for each observation)		
	Com-plete	S-defi-cient	t	df's	P
Fibre glass mesh end	112	76	4.080	18	< 0.001
Wooden Walls	39	14	4.764	18	< 0.001
Leaves or Cotton wool	52	31	2.483	18	0.02<P<0.05
Sleeve*	8	11	0.644	17	>0.5 ie N.S.
Artificial Oviposi-tion* Substrate	4	9	1.577	15	>0.1 IE N.S.

* Presence or absence of butterflies on sleeve recorded for only 18 observations and on artificial oviposition substrates recorded for only 16 observations.

of paired comparison t tests (comparing C (= complete treatment) counts with S (= sulphur deficient treatment) counts for each observation period.

Contrary to the impression that the butterflies were not discriminating between the treatments, given by the almost equal distribution of eggs between leaves in the two sides of the choice chamber, Table 3.03 shows that significantly more butterflies were observed settled in the "complete nutrients" side than in the "sulphur deficient" side on

(a) the fibre-glass mesh separating the butterflies from the plants

(b) the wooden walls, i.e. the outside walls and central partition, and

(c) the leaves or the cotton wool around their petioles.

On the sleeves and artificial oviposition substrates, although the total number of observed "settles" was higher on the "sulphur-deficient" side, the numbers settling at all were so low that the differences were not significant.

The use of males as well as females in this experiment may have contributed to this anomaly in the results. Counting at each observation had to be very quick so that the risk of butterflies settling on, or leaving, a surface during a count (with the consequent risk of a subjective bias influencing the decision of which

butterflies to count and which to ignore) was kept to a minimum. Consequently it was not possible to sex the butterflies at the same time, so that some of the butterflies contributing to the significantly greater number settling in the "complete" treatment side of the choice chamber may have been males. There is no reason to believe that the males would show a stronger response to mustard oil glucoside levels than would the females - on the contrary, it seems most unlikely - but the possibility has not been disproved. That males were present in the pre-conditioning set-up should have been sufficient, they should have been omitted from the olfactory choice experiment.

The majority of females used in the total 36 1/2 hours (12 hrs with artificial substrates only, 24 1/2 hrs with leaves as well) of this experiment (sixty-six females were used altogether - twenty of which were added about one hour after the leaves) must have been in no fit state to lay eggs as only two hundred and sixteen eggs were laid in the whole experiment and all but sixteen of the butterflies died during it. Also, as already mentioned, female Pieris rapae reared in a natural, or simulated natural, photoperiod have a circadian rhythm of egg-laying (see Section 4.2.2). As described in Section 1.2.1.2 they had been reared, stored, fed and used for the first two attempts at this experiment, with little regard for photoperiod. This may also have reduced their readiness to lay eggs or even feed properly. Also the pre-conditioning period of five hours was probably far too long - because their rhythm may

have been either destroyed altogether or out of phase with daytime, those that had eggs to lay apparently laid most of them in the pre-conditioning period although it was at night (1800 hrs - 2300 hrs). Thus many of the females that contributed to the distribution count of Table 3.03 did not contribute eggs. As shown in Sections 2.2.1.2 - 4, females differ in the stimuli to which they respond most strongly (or at all) so that the distribution of the eggs laid by only a few females may not be as indicative of the response of the majority as the distribution counts of adults themselves.

Ideally the experiment should have been repeated with fresh gravid females and improved artificial oviposition substrates so that it was unnecessary to use real leaves. However, some plants had died and others were dying so, as there were only seven healthy plants remaining in each treatment, I had to cease testing for a response to volatile attractants and test directly whether the butterflies would lay more eggs on plants grown in the complete nutrient solution than on those grown in the sulphur deficient solution, while there were still enough plants for valid statistical analysis of the results.

3.2.2.2 Test for a Response to the Plants Themselves

Method

The plants had been in the treatments for at least eleven weeks when used for the first replicate of this experiment.

The fourteen plants, seven from each treatment, were randomized between fourteen regularly spaced positions in the tray and cage set-up described in Section 1.2.2, in the same laboratory (not air-conditioned) as the previous experiment, so the ambient temperature varied as shown in Table 3.04. The room was kept free of strong draughts or directional light throughout the experiment. As Table 3.04 shows, there were three replicates; plants were re-randomized before the second, but otherwise the method for the first and second replicates was the same. The results for the first two replicates will be discussed before the method for the third replicate which was changed from that for the first and second replicates because of their results.

Butterflies used in all three replicates of this experiment had been reared in the laboratory. All three replicates were run in continuous light.

TABLE 3.04

Numbers of butterflies tested for discrimination between plants grown in complete and sulphur-deficient nutrient solution

Range of Ambient Temperature °C	Replicate	No. of Butterflies Released		Recollected	
		Females	Males	Time	Date	Time	Date
22→26	1	-	-*	12.00	24/2	12.00	25/2
25→28	2	36(3)	18(3)	05.45	27/2	10.30	28/2
28→31	3	28(13)	18(10)	06.25	4/3	11.25	5/3

* The numbers of butterflies used in the first replicate were not recorded, but did record that only four died. Numbers of butterflies that died in the other two replicates are given in parentheses

Results and Discussion of Replicates 1 and 2:

TABLE 3.05

Numbers of eggs laid per plant when half of the plants were growing in sulphur-deficient, and half in complete, nutrient solutions.

First two replicates.

Treatment	Replicate 1		Replicate 2	
Plants Numbered	Complete Nutrients	Sulphur Deficient	Complete Nutrients	Sulphur Deficient
1	7	20	26	22
2	39	25	59	62
3	10	26	20	61
4	39	15	36	56
5	48	13	16	116
6	37	18	58	48
7	55	8	76	117
Total	235	125	291	482

When the number of eggs laid on each plant (as shown in Table 3.05) was written in the position occupied by that plant on a plan of the array, there appeared to be a similarity between Replicates 1 and 2 in the attractiveness to butterflies of any given position. So, for each replicate, each position was ranked according to the number of eggs laid on the plant in it, and the correlation between the rank of a position in Replicate 1 and its rank in Replicate 2, was significant. ($T = 0.751$, $Z = 3.750$, $P < 0.001$; where T = Kendall's rank correlation coefficient). Thus any differential

response by the butterflies to plants in different treatments, may have been masked by their response to whatever caused some positions to be more favourable than others. As mentioned in Section 2.2.1.2 (p. 48) the light intensity differed measurably under different parts of the bank of lights, yet it would have been consistent for different replicates as the lights were very carefully positioned over the tray of plants each time. Although relative humidity and temperature would probably also have differed in different parts of the array, neither of them is likely to have been consistent for different replicates as the sawdust in the tray was not evenly damp and variations in local temperature would have been influenced by evaporation as well as heat from the lights.

As differences in light intensity seemed the most likely cause of the position effect, the light intensity was measured at the upper leaves of the plants, positioned both as for Replicate 1 and as for Replicate 2. The assumed importance of light intensity was confirmed when, for each replicate, the plants were ranked according to the light intensity measurement at their upper leaves, and also according to the number of eggs laid on them; there was a significant correlation between their ranks for the two variables. ($T = 0.589$, $Z = 2.938$, $P < 0.05$, for Replicate 1; $T = 0.429$, $Z = 2.135$, $P < 0.05$, for Replicate 2). Figures 3.10 and 3.11 show the relationship between the light intensity on a plant and the number of eggs it received in Replicate 1 and Replicate 2, respectively.

Fig. 3.10

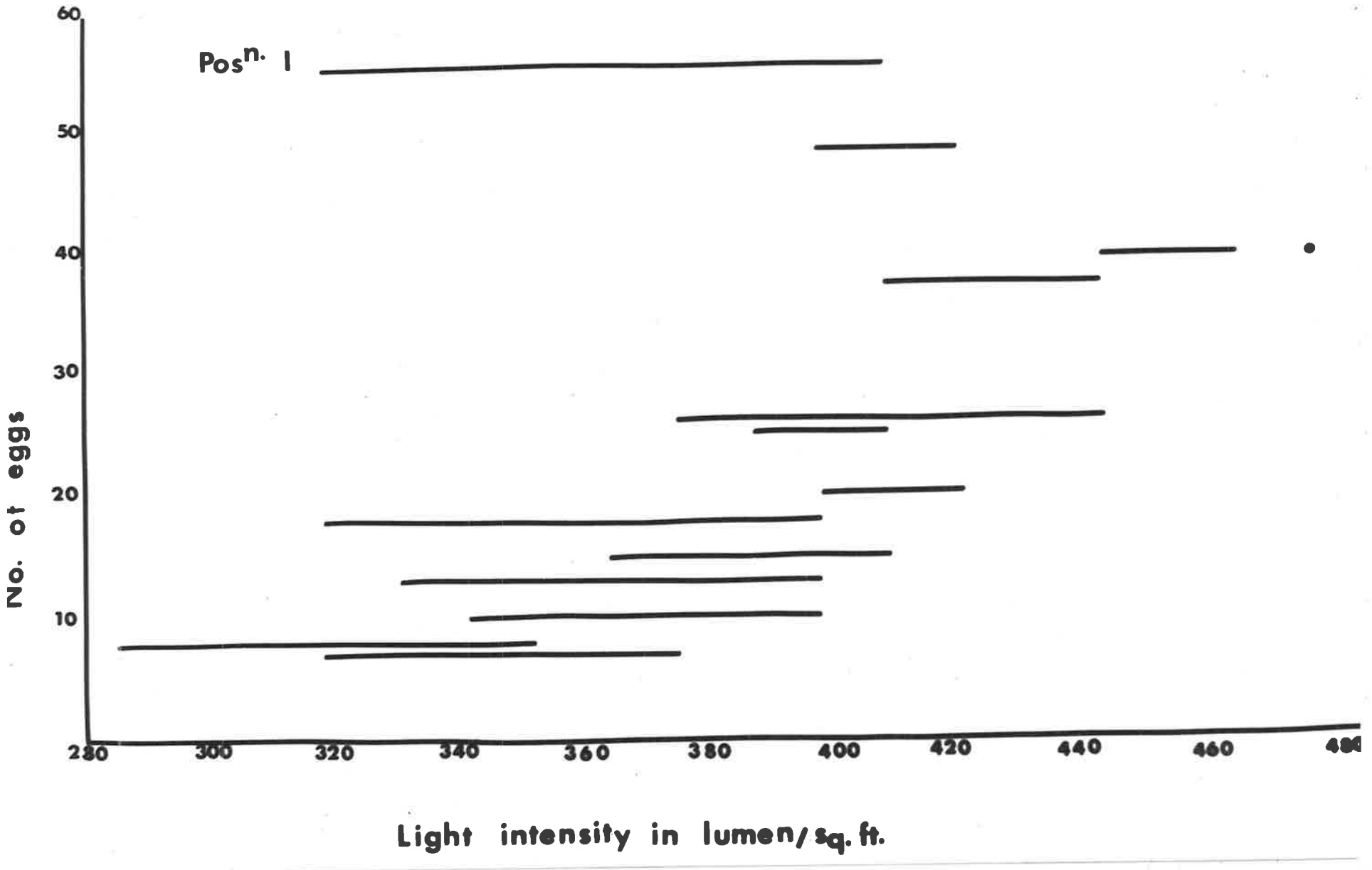
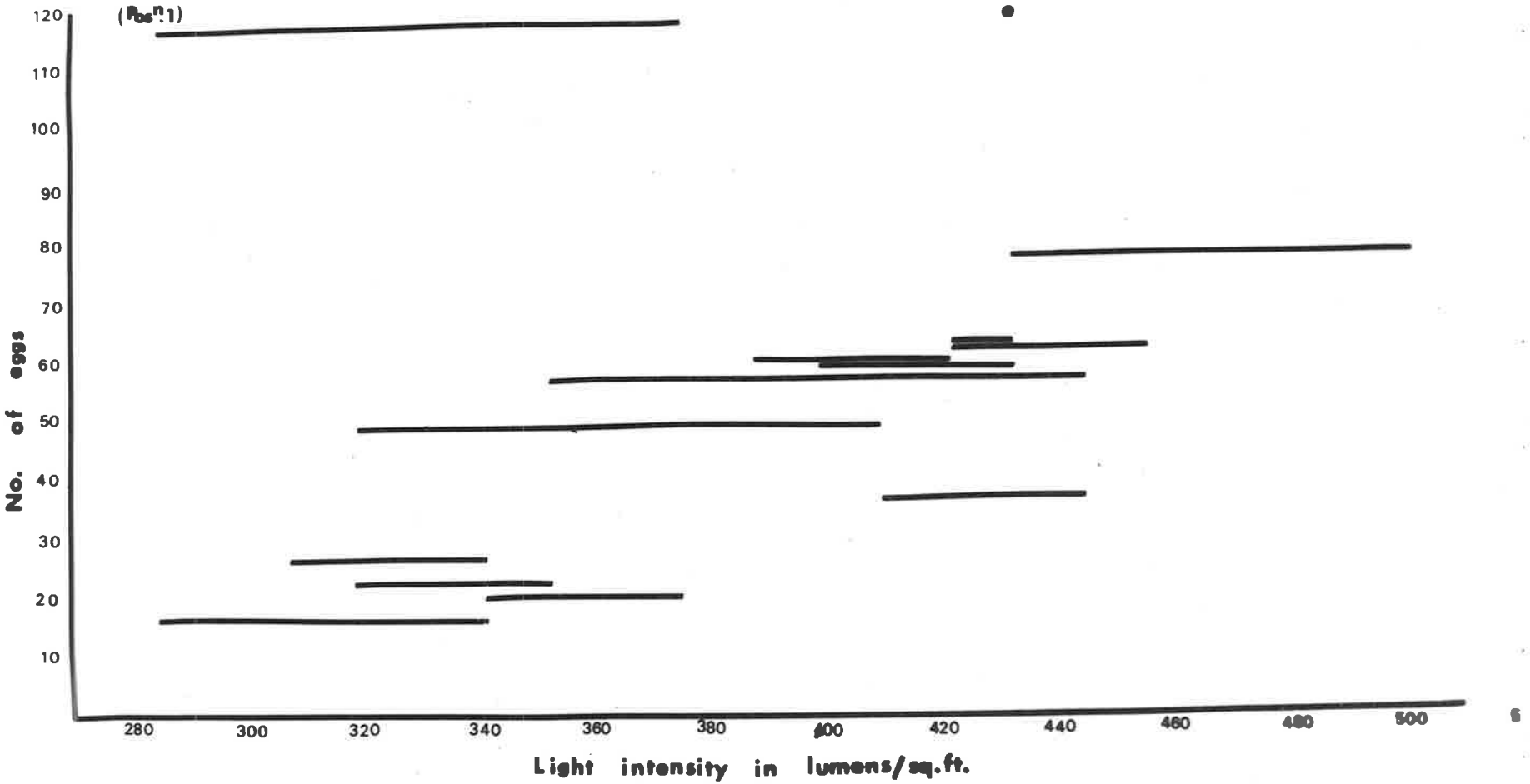

Posn. I
60
50
40
30
20
10
No. of eggs
280
300
320
340
360
380
400
420
440
460
Light intensity in lumen/sq. ft.

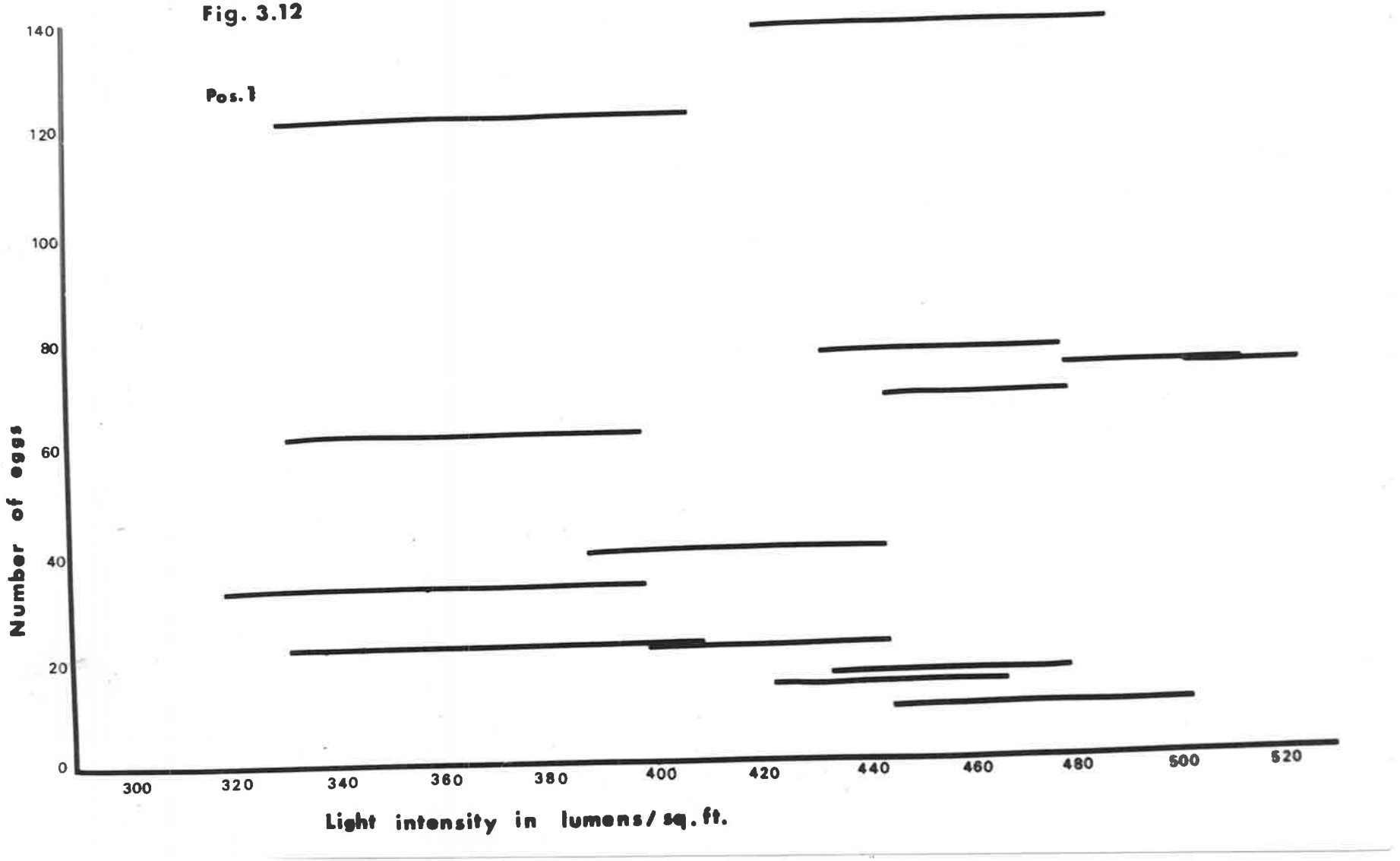
Fig. 3.11


(Pbs^n:1)
120
110
100
90
80
70
60
50
40
30
20
10
No. of eggs
280
300
320
340
360
380
400
420
440
460
480
500
Light intensity in lumens/sq.ft.

Method for Replicate 3:

Because of the significant interference of light intensity differences it was necessary to stratify the positions according to their light intensity so that there would be an equal number of plants from each treatment in each stratum. The light intensities measured at the upper leaves of plants positioned as for Replicates 1 and 2 could not be used, as they differed according to the height of the plants - for instance, position 5 had rank 1 in Replicate 1, when plant C_4 was there, but its rank was only 6 in Replicate 2 when S_2 was there - so the light intensity was measured again at the plant positions but without the plants present. It was measured at about nine inches above the sawdust. (This height was arbitrarily chosen as being about mean plant height). On the basis of these measurements the positions were divided into four strata, with two positions in one stratum and four each in the other three strata. All positions in the same stratum had more or less the same light intensity. One plant from each treatment was then randomly allocated to a position in the stratum with only two positions, and two plants from each treatment to positions in each of the three other strata. They were made as nearly as possible the same height by sinking the pots of tall plants down into the sawdust and heaping the sawdust up under small ones. The light intensities were then measured again, at the upper leaves of the plants, before introducing the butterflies (as shown in Table 3.04).

Fig. 3.12


Pos. 1
140
120
100
80
60
40
20
0
Number of eggs
300
320
340
360
380
400
420
440
460
480
500
520
Light intensity in lumens/sq. ft.

Results and Discussion:

As shown by Figure 3.12 there was no longer a relationship between the light intensity on a plant and the number of eggs it received. Also, equal representation of the two treatments in each of the strata should have removed any remaining masking effect due to the position of a plant. Nevertheless, as shown in Table 3.06, there was no significant difference between the numbers of eggs laid on plants in the two treatments.

TABLE 3.06

Numbers of eggs laid per plant when half of the plants were growing in sulphur-deficient, and half in complete, nutrient solutions. Third replicate.

Plants Numbered	Treatment	
	Complete Nutrients	Sulphur Deficient
1	120	61
2	72	22
3	9	33
4	16	72
5	14	136
6	75	67
7	22	39
Total	328	430

There are two possible explanations why the butterflies did not discriminate between plants in the two treatments:

(1) That there was no significant difference between the levels of mustard oil glucosides produced by plants in the two treatments. As mentioned in Section 3.2.2 (p.137), however,

I did not manage to test thsi analytically. Nor did I have the data to determine theoretically whether there was likely to be a significant difference. To do this it would be necessary to know both:

(a) The relative proportions of sulphur in mustard oil glucosides and in other compounds in brussels sprouts leaves. Niewhof (1969) gave the total sulphur content of brussels sprouts leaves as 143-205 mgms S/100 gms (fresh weight) of leaf, and their mustard-oil glucoside content as 14 mgms per 100 gms (fresh weight). Josefsson (1967) found that the two main mustard-oil glucosides in brussels sprouts leaves were glucoraphanin, which has three atoms of sulphur per molecule, and glucobrassicin, with two atoms of S per molecule. Small quantities of progoitrin and gluconapin (each with 2 s/ molecule) were also present. From these figures it is possible to calculate that probably about 2-3 mgms of the sulphur in 100 gms (fresh weight) of leaves, is incorporated into mustard-oil glucosides, i.e. only between one and two percent of the total sulphur in the sprouts leaves is in the form of mustard oil-glucosides.

(b) The absolute and relative rates of turnover - i.e. of loss from the plant and replacement by uptake from the nutrient solution - of the sulphur in mustard-oil glucosides and in other compo-nds. Although the mustard oil glucosides contain such a small proportion of the total sulphur in the

plant, if their turnover is more rapid than that of other, non-volatile, sulphur compounds, because they are released as vapours, they may nevertheless be significantly affected by a difference in the availability (by a factor of twenty three times) of sulphur in the nutrient solution.

So, as a crude index of whether there was a significant difference between the mustard oil glucoside, levels, I did the following larval feeding test.

David and Gardiner (1966 (b)) showed (in their Table III) that, for most of the mustard-oil glucosides they tested, only a small proportion (less than twenty-five percent) of the Pieris brassicae larvae they were testing fed within the first twenty-four hours when the concentration of mustard-oil glucoside was 0.33×10^{-6}M or even 0.33×10^{-5}M. When it was increased to 0.33×10^{-4}M, however, sixty to eighty percent of the larvae fed within the first day. This concentration of mustard-oil glucosides contains about 3.203 mgm-atoms S/l, if the mustard-oil glucoside has three atoms of sulphur per molecule, and 2.136 mgm-atoms S/l, if it has only two. This is twenty-four to thirty-six times the concentration of sulphur in the "sulphur-deficient" nutrient solution and 1.02 and 1.5 times the concentration in the "complete" nutrient solution. Thus it is possible that Pieris rapae larvae may not feed on the brussels sprouts

plants if the plants' abaility to accumulate an adequate amount of sulphur to manufacture the usual concentrations of mustard-oil glucosides has been significantly reduced by the"sulphur-deficient" treatment.

The eggs had been left on the plants after Replicate 3, so eight days later the stage of development of all larvae that had hatched on plants in the "complete nutrients" treatment was noted and they were transferred to plants in the "sulphur-deficient" treatment, and vice versa. All frass was removed from the plants when the original larvae were removed. Some larvae from plants in the "complete nutrients" treatment were observed feeding on three of the plants in the "sulphur-deficient" treatment, immediately after they were transferred to them. Twenty-four hours later there was frass on or under all plants. Five days later the larvae were all observed again and there appeared to be no substantial difference in their survival or rate of development on plants of the two treatments.

It seems, therefore, that the concentration of mustard-oil glucosides in plants from both treatments were above the feeding threshold of most, if not all, larvae so that such a test could not show whether there was a significant difference between the mustard oil glucosides levels of plants inthe two treatments. But even if larval feeding had been measured more precisely, as David and Gardiner (1966 (b))

measured it, for example, no significant difference may have been detected even if it was there, if, as discussed in the introduction, the larvae have an all-or-nothing feeding response. This leads to the second possible explanation of the butterflies' failure to discriminate between treatments.

(ii) That even though there is a significant difference, the butterflies do not detect and respond to it because they have an all-or-nothing response to mustard-oil glucosides, with a very low threshold.

There are probably much simpler and more reliable indices to the age, stage of development, or nutritional value of a plant, than its level of mustard-oil glucosides. Thus, unless the larvae are actually increasingly stimulated to feed by increasing concentrations (all above their threshold of perception) of mustard-oil glucosides, (and though not disproved, this appears to be unlikely) there would be no selective advantage in, and therefore no selection for, a graded (as versus an all-or-nothing) response by ovipositing females.

3.2.2.3 Conclusions from Both Experiments

If the results from the experiments in 3.2.2.1 and 3.2.2.2, each inconclusive alone, are combined, it is possible to postulate a single hypothesis, consistent with results from both experiments, to explain them.

As discussed in the introduction, insects with an all-or-nothing response to certain attractants or oviposition or feeding

stimulants, may nevertheless discriminate between different concentrations of a volatile attractant if the insects are at a distance from the sources of attractant, because the higher concentration will have a wider sphere of influence in which it will be present at concentrations above the insects' thresholds of perception.

It is postulated that the second explanation in Section 3.2.2.2 (p. 153) is the correct one, and that (in Section 3.2.2.1) the wider sphere of influence of the mustard oil glucosides from the plants in the "complete nutrients" treatment caused significantly more butterflies to be attracted more often into the compartment on that side of the choice chamber (perhaps by a form of odour-induced positive anemotaxis, such as Kennedy and Moorhouse (1969) reported for desert locusts) and so to settle there, when they settled.

As plants in both treatments contained sufficient mustard oil glucosides to exceed the butterflies' threshold at close range, and allow oviposition, and as a butterfly's response could not be increased by a higher concentration of volatile attractant once the threshold was passed, more or less equal numbers of eggs were laid on the leaves (from plants in the "sulphur deficient" treatment) in each side of the choice chamber. The graded response to increasing concentration of mustard oil glucosides that Ma and Schoonhoven (1973) reported for P. brassicae was an electrophysiological response - whether or not a graded response in terms of oviposition by P. brassicae can also be demonstrated remains to be seen. If we assume,

for the reasons discussed at the end of the last section, that the oviposition response of P. rapae (in my experiments) to contact with mustard oil glucosides is, instead, an all-or-nothing response, then this will explain why, when the butterflies had access to the plants, they distributed their eggs independently of the treatments.

3.2.3.1 Experiment to Determine Whether Butterflies Respond to the Differences Between Plants Grown in High Intensity, and Others Grown in Low Intensity, Artificial Light

Method:

Sixteen plants, eight of which had been growing for one to two months under high light intensity and the other eight of which had been growing under low light intensity for two months, were used for the first two replicates of this experiment. (The experiment was repeated with modifications several months later, but as it was modified in the light of the results of the first two replicates, they will be described before the methods for the other four replicates are given).

The same 4 x 4 Latin Square, with two plants from each treatment in each row and each column, was used in both replicates, as it also gave equal representation to both treatments in corner, edge and central positions, therefore counteracting any potential bias due to edge effects. Within the limitation of using the same Latin Square, the plants were randomized among the positions independently for each replicate. The butterflies used for both replicates

(as shown in Table 3.07) were from the first generation of a laboratory reared population.

TABLE 3.07

The numbers of butterflies tested for discrimination between plants grown in high and low intensity light; first two replicates.

Replicate	No. of Butterflies Released				Recollected	
	Females	Males	Time	Date	Time	Date
1	46(2)	43(11)	04.05	23/4	15.10	25/4
2	20(2)	17(5)	01.20	28/4	10.50	30/4

Results and Discussion of First Two Replicates:

As shown by Table 3.08, in each replicate nearly twice as many eggs were laid on plants that had been grown in low light intensity as on those grown in high light intensity, but analysis of variance (Table 3.09) showed that due to the very high residual variance the difference was not significant in either replicate.

The extremely high variances may have partly been caused by using plants that had experienced the high light intensity treatment for different lengths of time and partly because the plants in both treatments had been severely water stressed (by accident) on at least one occasion. Some had been affected more by the water stress than others and so in general the plants were very variable in physical characteristics thus reducing the distinction between treatments. So plants were subjected to the treatments for a longer,

TABLE 3.08

Numbers of eggs laid per plant when half the plants had been grown in high intensity, and the remainder in low intensity, artificial light; first two replicates.

Replicate	1		2	
Plants Numbered*	Light Intensity in Which Plants Grew			
	High	Low	High	Low
1	1	3	2	2
2	3	11	1	10
3	4	31	20	9
4	27	23	5	19
5	12	4	13	23
6	1	12	3	10
7	3	30	12	12
8	7	3	6	15
Total	58	117	62	100

* The plants were not paired in any way, e.g. plant number H_1 had no more in common with plant L_1 than with plant L_6.

uniform, period before the next replicates and maintained very carefully to avoid stressing them through either water-stress, or water-logging, etc.

Methods of Replicates 3-6:

Twenty-plants (ten from each treatment, which, by Replicate 3, they had experienced for 11 weeks) were randomized between and

TABLE 3.09

Analysis of variance of the distribution of eggs among plants grown in high and low light intensity

Replicate 1						Replicate 2					
Source of Variation	Sum of Squares	df's	Mean Square	F	P	Source of Variation	Sum of Squares	df's	Mean Square	F	P
Rows	527.7	3	175.9	1.75	N.S.	Rows	257,3	3	85,8	4,01	N,S,
Columns	182.7	3	60.9	< 1	-	Columns	173,3	3	57,8	2,70	N.S,
					0,19						0,07
Treatments	217,6	1	217.6	2.16	N,S.	Treatments	90,3	1	90,3	4,22	N,S.
Residual	805.0	8	100.6			Residual	171,0	8	21,4		
Total	1732.9	15				Total	691,8	15			

within the four strata of a 4 x 5 array, as for the experiments described in Section 2.2.2.2 with each treatment providing half the plants in each stratum. (The light intensities had varied somewhat as plants grew then the lights were raised to compensate, or treatments were adjusted to make them more distinct. The Table in Appendix 3.2 shows the light intensities they had experienced). In Replicate 3 both Alyssum and honey solution were provided as food for the butterflies, which had been pre-conditioned for ten hours beforehand. Before Replicate 4 the plants were re-randomized and the butterflies were again pre-conditioned, this time for twelve hours. Only honey solution was provided for them to feed on during Replicate 4. Too few eggs to be analysed were laid during Replicate 4, so in case the lack of nectar was responsible for the lack of eggs, Alyssum was provided as well as honey solution in Replicate 5, and the plants were not re-randomized. But only one egg was laid in Replicate 5, though the female butterflies had large abdomens and all other conditions seemed normal except that, as shown in Table 3.10, Replicates 3, 4 and 5 each lasted less than twenty-four hours and in each the total time with the lights on did not exceed eighteen hours.

It was tentatively concluded that disruption of a previously unsuspected circadian rhythm of egg-laying was largely responsible for inhibiting the females from laying in Replicates 4 and 5. (The reasons for this conclusion are discussed in Section 4.2.2 after the description of an experiment which showed that the butterflies do

have such a rhythm of oviposition). To remedy this defect Replicate 6 was run for more than twenty-four hours with a ten hour dark period during the natural night. Butterflies were pre-conditioned (for Replicate 6) for fourteen hours and the experimental plants put back under their respective treatment lights for sixteen hours. Plant No. L8 was very sickly and had to be replaced in Replicate 6 by a spare plant from the low light intensity treatment (twelve plants had been subjected to each treatment, so there were two spares per treatment).

Results:

As mentioned in the methods section and as shown by Table 3.11 too few eggs were laid in Replicate 4 for analysis but as in Replicates 3 and 6 they were laid predominantly on plants in the low intensity light treatment. This is the result that was predicted by the results of Replicates 1 and 2, although they were non-significant; in Replicates 3 and 6, however, the differences are significant.

TABLE 3.10

The numbers of butterflies tested for discrimination between plants grown in high and low intensity artificial light: third to sixth replicates

Replicate	No. of Butterflies#		Released		Recollected		Dark Period	
	Females	Males	Time	Date	Time	Date	Time Started*	Time Ended*
3	18(3)	13(1)	03.35	3/11	15.55	3/11	Nil	
4	11(2)	6(2)	15.55	5/11	13.30	6/11	20.30	04.30
5	9(1)	4(1)	17.30	7/11	11.30	8/11	Nil	
6	22(2)	9(2)	15.30	9/11	20.15	10/11	20.00	06.00

* In each case the dark period began on the same date as the butterflies were released among the plants, and ended on the same date as they were recollected.

All butterflies used for these replicates had been caught in the field.

TABLE 3.11

Numbers of eggs laid per plant when half the plants had been grown in high intensity, and the remainder in low intensity, artificial light: Replicates 3, 4 and 6

Replicate	3		4		6	
Plants Numbered	Light Intensity in which Plants Grown					
	High	Low	High	Low	High	Low
1	2	11	0	1	15	32
2	1	6	0	0	5	6
3	2	8	2	0	7	23
4	3	23	0	4	1	50
5	5	19	1	0	0	20
6	0	14	0	2	4	59
7	1	6	0	2	4	15
8	1	17	0	0	2	32*
9	0	5	0	0	1	14
10	0	12	0	1	8	16
Total	15	121	3	10	47	267
s^2	2.50	37.43	Too few eggs laid to be analysed		20.01	282.46
F	14.97				14.115	
P#	<0.02				<0.02	
U	0.5				4.5	
P	<0.001				<0.001	

Because the between plant variance of the plants grown in low light intensity was significantly greater than that for plants grown in high light intensity the results were analysed by the non-parametric Mann-Whitney U test, which does not depend on the variances of the two treatments being homogeneous.

* This is not L8, but the spare plant (from low light intensity) used to replace it.

3.2.3.2 Analysis of the Numbers of Eggs Laid on Plants in Relation to the Total Leaf-area of Each Plant

Method:

Of the eight distributions of eggs analysed, seven were taken from the results of experiments reported earlier, in Chapter 2, as set out in the following table, but the observations which gave rise to the eighth distribution have not been described until this section.

Symbol in Text*	O.P. When Eggs Laid	Section in Which Experiment Described
(a)	Pre-experiment O.P.	2.2.1.2
(b)	O.P. (b)	"
(c)	Replicate 1 with Larvae	2.2.2.1
(d)	First replicate without Larvae	"
(e) & (f)	Replicates 3 & 4 with Larvae	"
(g)	Third replicate without Larvae	"
(h)	Accumulated laying by wild butterflies (uncaged)	3.2.3.2

* The same symbols are used as in Section 3.2.1.2, in which the references to replicates without larvae, which were omitted from the description of the experiment in Section 2.2.2.1, are explained, as is their previous omission.

The eggs in the latter distribution were laid out-of-doors by wild butterflies on young cabbage plants not covered by a cage. Those of distribution (a) were also laid out-of-doors, but by butterflies caged with brussels sprouts plants. The eggs for (b) - (g) were

laid indoors, under artificial lights and in a constant temperature, but also on brussels sprouts plants and in the same sized cage as in (a) (the same that was used for all other experiments and described in Section 1.2.1.1). For (a) - (g), all leaves of the brussels sprouts plants were measured, and the number of eggs laid on a plant was plotted against the total leaf area of that plant. But the eggs laid by the wild butterflies on the cabbages in (h) were counted before a satisfactory method had been devised for measuring leaves so each plant was classified (by subjective assessment) in two ways - into one of six size classes on the basis of:

(i) overall plant size (towards which height and number of leaves would have contributed)

(ii) mean leaf size (i.e. rated according to the proportion of its leaves that were in each of several size classes for leaves judging by eye). As plant size depends on both the size and number of leaves it was possible for a plant to be in a different class by classification (i) from that by classification (ii). Also plants in any particular class by either classification may have had different numbers of leaves, and conversely plants with the same number of leaves may have been in different classes.

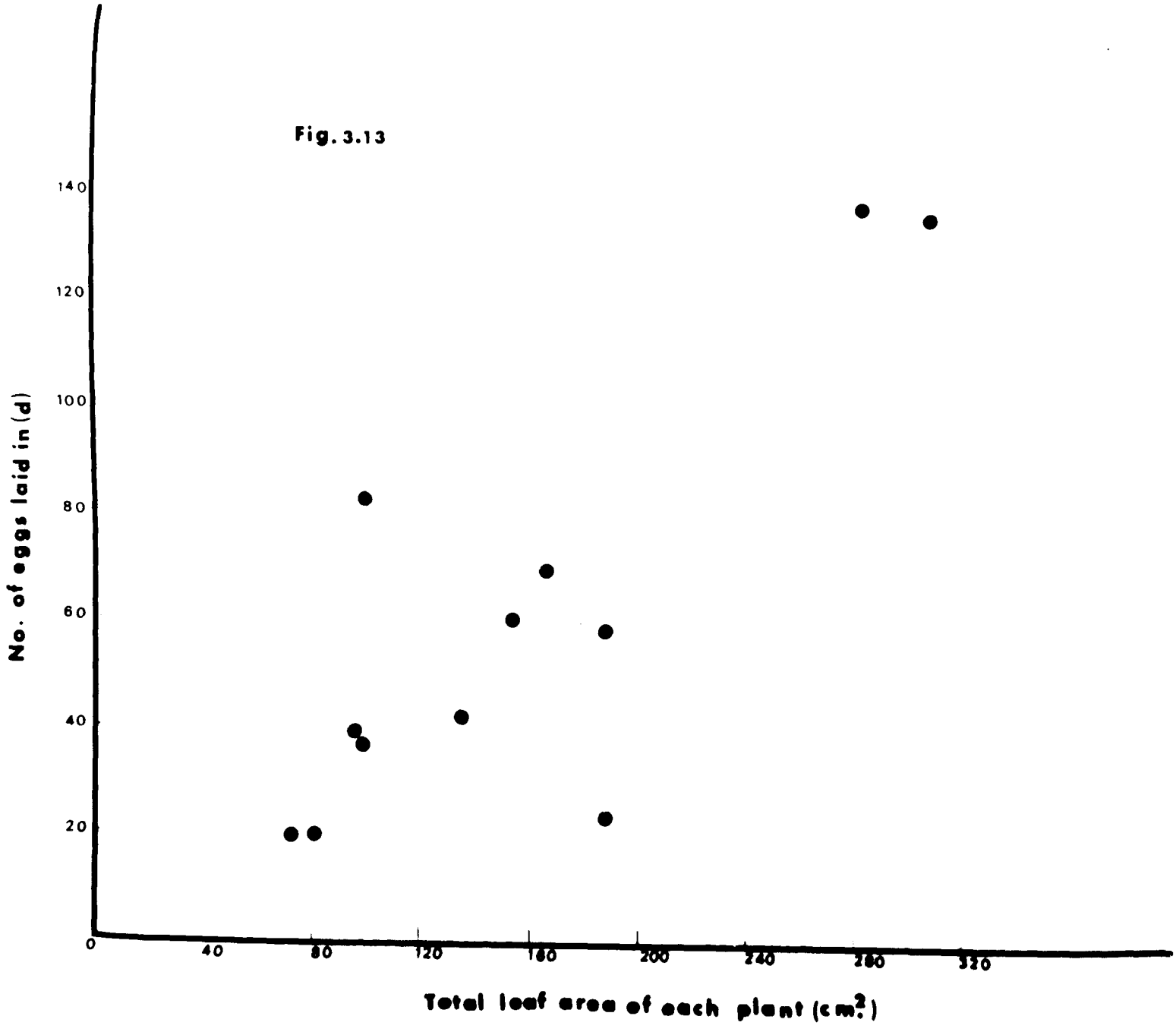
Results and Discussion:

In six of the seven distributions of eggs laid by caged butterflies, including the distribution from (a), when the cage was

out-of-doors in natural daylight and weather conditions, there was no relationship at all between the total leaf area of plants and the number of eggs they received. As shown by Figure 3.13, however, in the other distribution (from (d)) there was a tendency for most of the larger plants to gain more eggs than the smaller ones but there was quite a wide scatter, so that there was no significant regression and the distribution may either have reflected real preferences or have been due to chance (the probability that it was due to chance alone was between 10% and 20%).

The total leaf area of a plant depends on both the number and size of its leaves so that a preference by butterflies for plants with a greater total leaf area could result from responses to either or both of (i) the size of leaves, (ii) the number of leaves, on a plant. As already shown in Figure 3.07, the butterflies did respond significantly to the size of leaves (preferring larger ones), in (d). To test whether the tendency (though non-significant) for larger plants to gain more eggs than smaller ones in (d), could have been influenced by a response to the numbers of leaves on plants, the number of eggs was plotted against the number of leaves for each plant. The values of the same two variables, from (c) and (e), in which there was definitely no relationship between the total leaf area of plants and the number of eggs they received, were also plotted for comparison. There was no relationship between the number of leaves and the number of eggs on a plant in any of the

Fig. 3.13


No. of eggs laid in (d)
140
120
100
80
60
40
20
0
40
80
120
160
200
240
280
320
Total leaf area of each plant (cm.²)

three replicates.

The preference for larger leaves shown by the butterflies in (d) could have been responsible for the tendency for larger plants to gain more eggs in that replicate only if the larger plants do have more large leaves. As shown in Table 3.12, the leaves, already grouped in age-classes, were further grouped into six size-classes, according to the mean area of the leaves in any one age-class. The value of "mean number of eggs per leaf" that was equivalent to the median value of "mean leaf area" for each size-class (from the regression in Figure 3.07) showed the butterflies' relative preference for leaves in that size-class and so was assigned to it as its "coefficient of preference"as shown in Table 3.12.

TABLE 3.12

The "coefficients of preference" of leaves in different size-classes

Size Class	Mean leaf area (cms^2) Min.	Max.	Median Value of Mean Leaf Area	"Coefficient of Preference"
1	1.50	3.49	2.5	0.45
2	3.50	4.49	4.0	1.10
3	4.50	8.49	6.5	2.20
4	8.50	18.49	13.5	5.26
5	18.50	28.49	23.5	9.63
6	28.50	36.50	32.5	13.56

Then the numbers of leaves in each size-class, multiplied by its "coefficient of preference", were summed for each plant to give an

Fig. 3.14

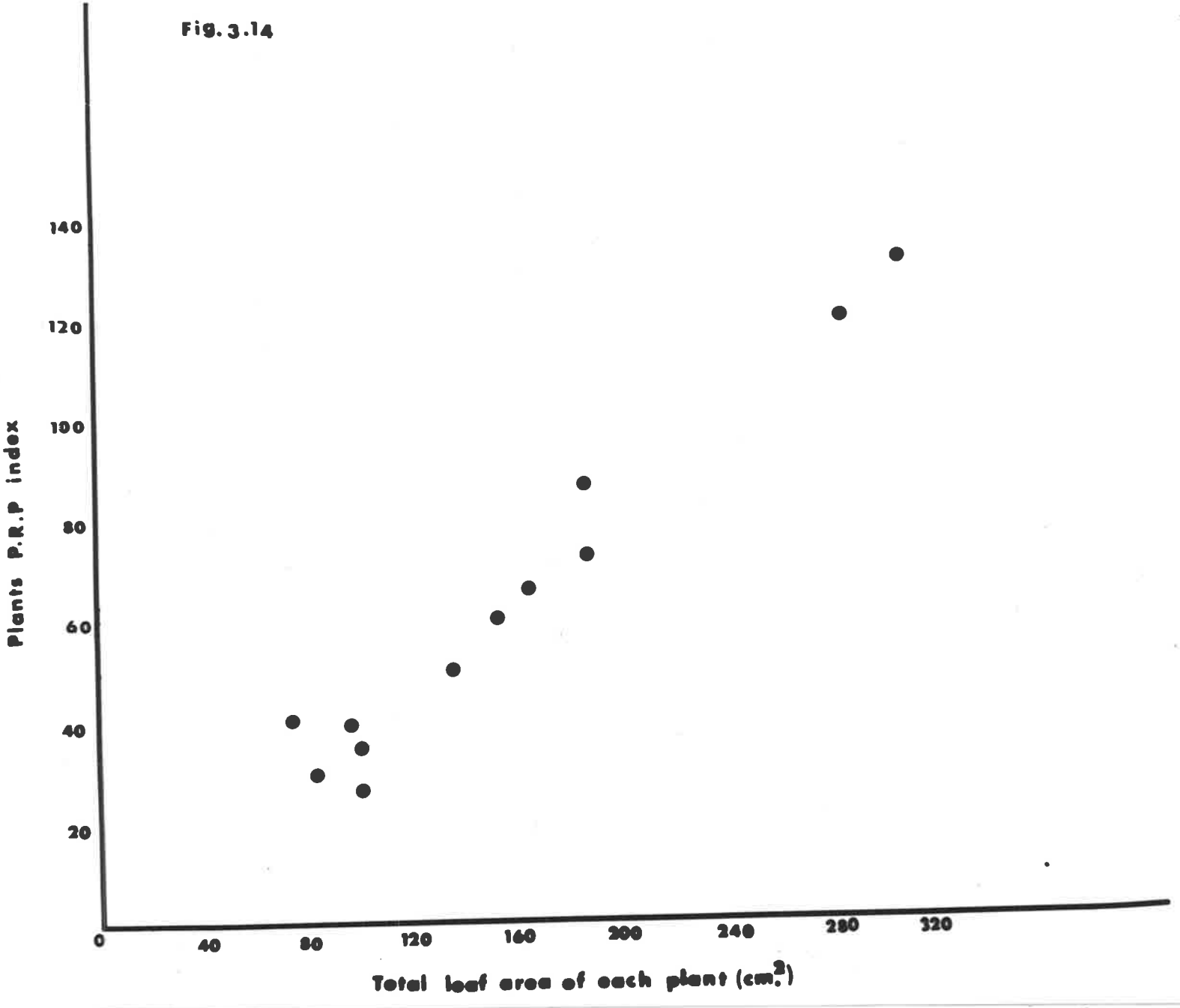
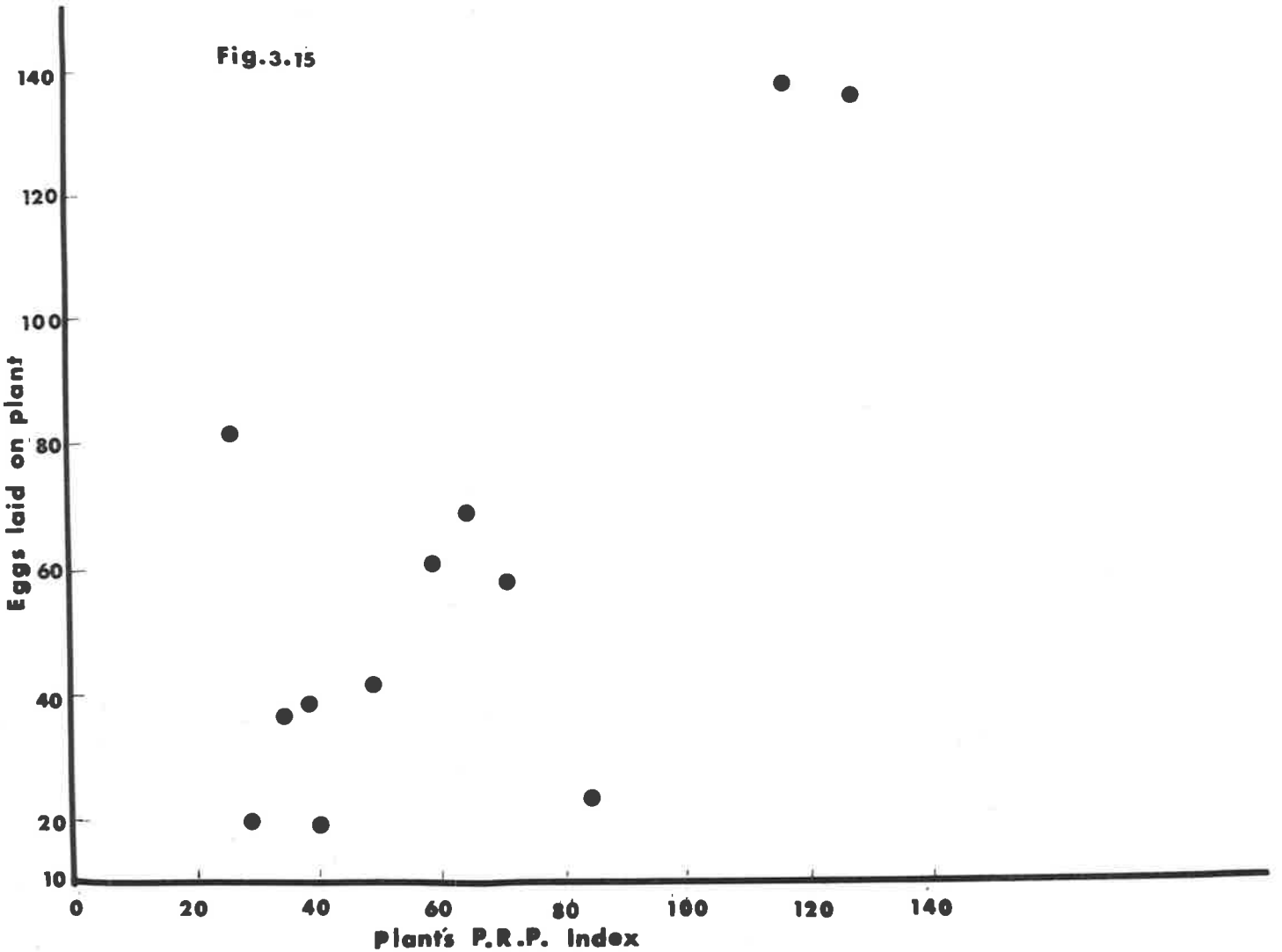

Plants P.R.P index
140
120
100
80
60
40
20
0
40
80
120
160
200
240
280
320
Total leaf area of each plant (cm.²)

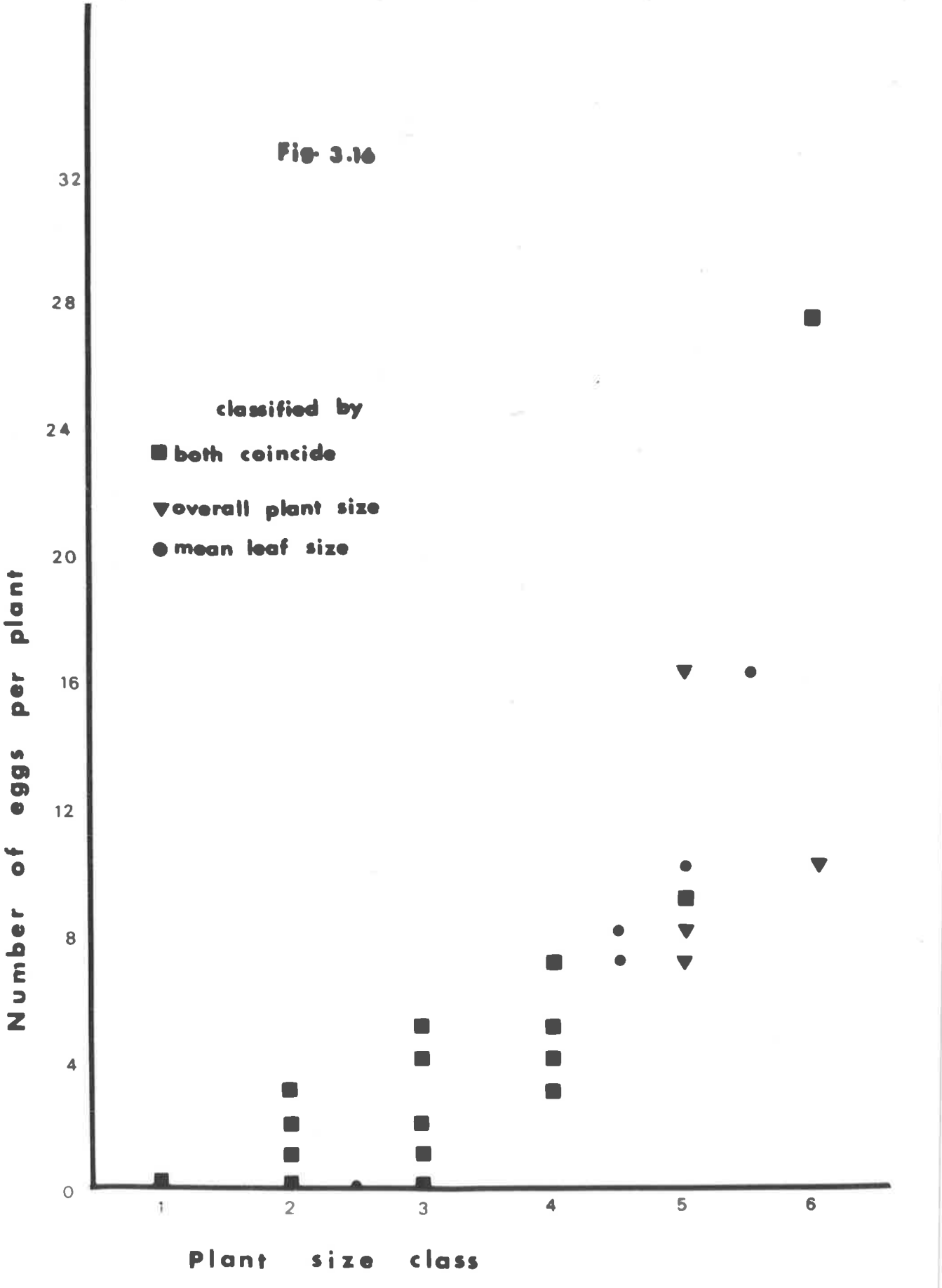
Fig.3.15


140
120
100
80
60
40
20
10
Eggs laid on plant
0
20
40
60
80
100
120
140
Plant's P.R.P. Index

index predicting the butterflies' relative preference for that plant (referred to hereafter as the P.R.P. - "predicted relative preference" - index). Figure 3.14 shows that there was a linear relationship between the P.R.P. index of a plant and its total leaf area, in (d). The relationship between the number of eggs gained by each plant and its P.R.P. index (Figure 3.15) is very similar to that between eggs and total leaf area (Figure 3.13) but (with the exception of the points from the two plants that gave totally atypical results) with generally less scatter, suggesting that the apparent trend in Figure 3.13, is real, and results from the significant preference of the butterflies for larger leaves, in (d) (although, as the two plants that gave atypical results showed, the preference may be overriden). Thus in only one of the seven distributions of eggs laid by caged butterflies was there an apparently real relationship between the total leaf area of plants and the number of eggs they received, and even that relationship was not statistically significant.

As shown in Figure 3.16, however, wild butterflies that were free to visit or fly right away from the uncovered cabbage plants, laid more eggs on plants in the larger size-classes (by either classification), although, unfortunately, only one such distribution was recorded. Nevertheless, as the eggs would have accumulated over a longer time than one epxerimental oviposition period, they were probably laid under a variety of environmental conditions, thus being more representative of the butterflies' behaviour in general, than a

Fig. 3.16


32
28
24
20
16
12
8
4
0
classified by
both coincide
overall plant size
mean leaf size
Number of eggs per plant
1
2
3
4
5
6
Plant size class

single experimental oviposition period would be. Although the heights were not measured of either the plants in the cage, or the uncovered ones exposed to wild butterflies, it could be suggested that the subjective assessment of the relative sizes of the latter plants would have included relative plant height among the factors contrib uting to "size", so that the apparent difference between the behaviour of caged and free butterflies with respect to plant size, could be partly the result of using different methods to estimate plant size. This is unlikely to be so, as plant size was estimated with the aim of determining whether the eggs were distributed randomly over the available leaf area, so that the same attributes were estimated in the uncovered plants as were measured in the covered ones.

As mentioned in Section 3.1.2.3 Gilbert (personal communication 1973) found that wild cabbage butterflies were attracted to any vertical object including an observer, from distances of up to one metre for plants, more for a human observer, and that the strength of the attraction is a function of plant size not merely height. Therefore it seems likely that when the butterflies were confined with the plants in a cage as small as the ones used, plant attributes that the butterflies could perceive only at short range may have been more likely to influence a female's decision of which plant to lay on, whereas when they were free they were attracted from a distance by the large leaves and the larger plants and so flew direct to them. Thus unless the large plants were somehow unsuitable as

oviposition sites, the females would lay eggs on them first, before going to investigate and perhaps lay eggs on other smaller plants whose short range attributes may be more attractive.

3.2.4 Discussion of and Conclusions from all Experiments on, and Analysis of, the Butterflies' Responses to Differences Between Plants

Although individual butterflies differ in the extent to which they respond to different stimuli from the environment, the majority of ovipositing females of Pieris rapae respond to heterogeneity among the host plants available to them by distributing their eggs predominantly on those plants or leaves with certain preferred characteristics, often with the result that the distribution of eggs among plants (or leaves) is much more patchy than random.

In the experiments testing whether butterflies would discriminate between young and old plants (Section 3.2.1.1) and between plants grown in high and low intensity light, (Section 3.2.3.1), they laid more eggs on the young plants, and those grown in low intensity light, respectively, which in each case had softer lighter green leaves that were less stiff and fibrous and rather more fragile than the leaves on the less preferred plants. If the butterflies were choosing plants on which to lay, on the basis of colour alone, they should have preferred the older plants, and those grown in the

higher intensity light, respectively, in the two experiments (assuming their colour preferences are the same as those of Hovanitz and Chang's butterflies mentioned in Section 3.1.2.3). That they did not shows that their response to colour either (i) depends on the other characteristics with which the colour occurs, or (ii) may be overridden by responses to other characteristics that are less obvious, such as surface texture and flexibility of leaves. But, although the leaves of plants in the preferred treatment in the age experiment were similar in colour etc. to those preferred in the light intensity experiment, this may have been just coincidence, with the plants actually being preferred for different reasons in the two experiments.

The plants grown in the lower intensity light also had a much more open growth form than those grown in the high intensity light; i.e. they had longer petioles and internodes so that the leaves were further apart, allowing more light to penetrate between, and be reflected off, them. In the plants grown in the higher intensity light the leaves grew so much closer together that they usually overlapped at least a little, and sometimes little more than the edges of lower leaves were exposed to direct light. The correlation between the bias in the results of the first two replicates of the experiment in Section 3.2.2.2 and the measured light intensities, suggests that the butterflies tend to lay more eggs where the light intensity is high, but the measurements were of the intensity of

vertical light at the tops (or at the level of uppermost larger leaves) of the plants, not of the amount of light penetrating between the leaves. A further experiment (described in Section 4.2.1) to test directly whether the egg-laying behaviour of females alone in the cage was influenced by the intensity of light in the vicinity of the plants when and where they were laying eggs, was done after this experiment, but once again the light intensity between, or reflected off, the leaves, was not measured.

Possibly the butterflies preferred the plants with a more open growth form because light was reflected off more of their leaf surfaces; on the other hand more of the leaves' under surfaces (on which the butterflies prefer to lay their eggs, so long as they are suitably shaped) were accessible in these plants.

Plants of different ages, or growing on different soil types, will sometimes contain different levels of mustard oil glucosides, but the experiments in Sections 3.2.2.1 and 3.2.2.2 showed that (so long as mustard oil glucosides are present) such differences will not usually influence how a female Pieris rapae distributes her eggs - except perhaps when there are well separated localized clumps of plants with different levels of mustard oil glucosides, to which the females are selectively attracted from a distance. Differences in the size of plants, and of their leaves, also seem to be more influential from a distance than when the butterflies are confined close to the plants, as they are in a cage as small as the

ones used throughout this study. Occasionally some butterflies will respond to the size of plants, or rather, the size of leaves (and therefore of plants, if the bigger plants are those with more large leaves) when in a cage. In this case it is the larger leaves (and therefore plants) that are favoured, just as they are by free, wild butterflies, that appear to be attracted by the size of the plants and their leaves from a distance too great for other more subtle stimuli from the plants to reach them. The results in Section 3.2.1.2 and 3.2.3.2 show, however, that in general the pattern in which caged butterflies distribute their eggs among the plants or leaves available to them is not influenced by the size of the plants or leaves.

As well as showing that the preference of the majority of butterflies for certain plant characteristics usually influences, and may sometimes be the strongest factor determining, the pattern in which eggs are distributed by a group of butterflies, the experiments in this chapter have also shown that other factors are usually involved (to varying degrees) in generation of the final distribution - whether they are the preferences of the minority for other plant characteristics, or responses to other components of the environment or to internal stimuli. For instance, although the majority preference for plants that had been grown in low intensity light caused the distribution of eggs to be extremely biased in favour of plants in the low light treatment, the distribution of eggs among plants

within each treatment was also more patchy than random showing that other factors must be involved. (The distribution among plants grown in low intensity light was significantly patchy; the mean number of eggs per plant in the high intensity light treatment was too low to give a significant departure from randomness, with such a small sample size).

The results from the experiment described in Section 2.2.2.3 give an example of the interaction between stimuli from the plants and from their micro-environment. As mentioned in that section some plants were consistently more favourable than others; when the plants were ranked according to the number of eggs they received, for each replicate, there was a significant concordance between their "egg-ranks" in the eight replicates. ($W = 0.550$, $\chi^2_7 = 30.817$, $P < 0.001$; where W is Kendall's coefficient of concordance).

The plants used in that experiment were photographed, so that although the height of plants had not been measured in any of the experiments, their relative heights could be assessed, and the main differences in growth form noted. Although the results were excluded from the analyses in Sections 3.2.1.2 and 3.2.3.2 (because, with only one female laying eggs in each replicate, the numbers of eggs on plants or leaves were too low for meaningful analysis) the leaves of the plants had been measured, just before the start of the experiment.

The plants remained in the same positions throughout all eight replicates and although the intensity of the vertical light shining onto the plants directly from the bank of lights was measured before the experiment and was about the same for each position, the reflected light (from all directions) is not likely to have been equal for the different positions. The positions can therefore be ranked in terms of their probable level of reflected light, according to how near they were to the reflective walls and the bank of lights over the other (rearing) cage. Thus it was possible to rank the plants in order of relative height, total leaf area, mean leaf area, and probable reflected light in that position. These ranks (in conjunction with some description of their growth form) are compared with the "generalised egg rank" of each plant (obtained by ranking the plants according to the sums of their "egg ranks" in the eight replicates - from calculation of the concordance) in Table 3.13.

The results suggest that although confined in a cage, some, at least, of the butterflies were influenced by the size of leaves (and so of plants), preferring to lay their eggs on plants with larger leaves (such as plant number 10) so long as the leaves were well spaced.

It would not be valid to try to fully interpret all the plants' "generalised egg-ranks" in terms of the interactions between the five characteristics of plants and their positions, assessed in Table 3.13, when the assessment and judgement of interaction is so

TABLE 3.13

Comparison of the "generalized egg-ranks" of plants with their ranks (or descriptions) for five characteristics of the plants or their micro-environment. (Data from experiment in Section 2.2.2.3)

Plant No.	Generalized Egg-rank	Rank in Terms of Relative				Comments on Growth Form
		Height	Total Leaf Area	Mean Leaf Area	Probable Reflected Light	
12*	1	3.5	7	6	1	Leaves well-spaced, not flat, and at many different angles
13	2	5	1	1	4	Leaves irregular shaped, not such size range as leaves of 10 but well spaced
10	3	8	2	3	6	Large, round, flat, well-spaced leaves
15	4	6.5	6	7	3	Fairly small, well-spaced leaves
11*	5	1	5	4	7	Leaves rather closer-packed than most others but less than 9 and much less than 14
9	6	2	8	8	5	Leaves small, close together at top of long stem. Not as close as on 14
16	7	6.5	4	5	8	Leaves smaller than on 11 but wider-spaced, round and flat
14*	8	3.5	3	2	2	Leaves very close together all at top of long stem.

* These three plants have 19 leaves each, all other plants have 20 each.

subjective; nevertheless three important points are shown fairly convincingly by this Table.

(i) The result for plant number 12, and to a lesser extent those for plants number 13 and 15, suggest that the intensity of reflected light where a plant is growing may strongly influence the females' response to that plant, but the result for plant 14 shows that reflected light can only be influential if the plant has certain characteristics.

(ii) In spite of consistently high ranks for all four ranked characteristics, plant number 14 ranked lowest in "generalized egg-rank", i.e. it was least preferred by the butterflies, showing that the lack of suitable stimuli (probably mainly visual, but also, to a lesser extent, proprioceptive, and perhaps even tactile stimuli) related to the growth form of a plant, may inhibit oviposition on that plant, even if it is favourable in all other respects.

(iii) Although the data do not provide any decisive evidence of whether or not the height of a plant has an influence on its favourableness for oviposition by caged butterflies they do at least show, however, that if it has, the influence cannot be very strong, compared with the influence of other characteristics. The results for plants number 13, 15 and especially number 10, show that butterflies confined in a cage with a group of plants will not be

inhibited from laying eggs on an otherwise favourable plant just because it is distinctly shorter than other available plants.

CHAPTER 4

RESPONSES TO STIMULI FROM THE MICRO-ENVIRONMENT OF THE HOST PLANTS

4.1 Introduction

In several of the experiments described so far, the butterflies' responses to specific treatments have been limited, biased, or overshadowed by other responses, apparently to the quality of the micro-environment of individual plants. The two most convincing examples were the apparent influence, on the number of eggs laid, of:

(i) the direction of the sun and wind on the roof (in the pilot experiment described in 2.2.1.1.), and

(ii) differences in the intensity of vertical radiation under the bank of fluorescent lights used for indoor experiments (especially in the first two replicates of the experiment described in Section 3.2.2.2). (The latter experiment is referred to hereafter as "the nuticulture experiment", as the plants were cultured in nutrient solutions).

The butterflies' response to any particular plant also appeared to be influenced by the micro-environment of the position it occupied in the array (especially in Section 2.2.1.2). This influence led to the hypothesis that such stimuli from the environment contributed significantly to the distribution of eggs observed in that experiment.

When plants remained in the same positions in the array,

throughout a series of replicates (see Section 2.2.1.3) the numbers of eggs they received varied less from replicate to replicate than they did when the plants were moved between replicates. This further supports the hypothesis that the position occupied by a plant influenced the butterflies' response to that plant.

Gilbert (personal communication 1973) has evidence that, like the English Pierids that Baker (1968) studied, free, wild, Australian Pieris rapae tend to fly predominantly at a particular angle to the sun's azimuth. It might be suggested that some positions in my experimental arrays were more favourable simply because the butterflies' directional tendencies made them fly predominantly in the vicinity of those positions. But when they were in the experimental cage, with relatively non-directional (except vertical) artificial light, individual butterflies did not show any tendency to orient most of their flight in a particular direction. Thus differences in the degree to which different positions enhance or detract from the favourableness of the plants occupying them, must have resulted from differences in the quality of the micro-environment in those positions.

There are many reports in the literature of the micro-environment associated with individual plants or parts of plants influencing the pattern in which insects distribute their eggs. But most such reports simply cite circumstantial evidence (like that above) rather than testing responses to specific stimuli that the micro-environment may provide. A number of different components of the micro-environment

may provide such stimuli. For instance, as Richards (1940) pointed out, for those females that do not feed on the plant on which they lay their eggs, the proximity and distribution of food sources in surrounding areas may influence the flight path taken by them over smaller areas in which the larval host plants are growing. This in turn would influence the distribution of their eggs among the larval host plants. Of course for Pieris rapae and any other insects with a tendency to fly predominantly in one particular direction, a female's directional tendencies will limit or modify the influence of the distribution of food on their flight paths but are not likely to remove the influence altogether. Greater proximity of flowers for the butterflies to feed from may have contributed to the higher density of eggs laid in the outer rows of the cabbage patch reported by Kobayashi (1957).

The avoidance of direct sunlight and wind by ovipositing females of the psyllid, Cardiaspina densitexta, and the fruit fly, Dacus tryoni, restrict most oviposition to the under surfaces of leaves and fruit respectively (White 1970, and Pritchard 1969, respectively). Nevertheless it is not a specific egg-laying response, as males and immature females show the same response. Female Cactoblastis cactorium tend to restrict their egg-sticks to the underside of second, third and lower segments of a prickly pear plant and to avoid laying on terminal growth. Although this behaviour protects most egg-sticks from direct sunlight, it is not a response to conditions of

the micro-environment, but rather, to the plant. The females oviposit mainly in the dusk or dark, between 6.30 and 8.30 p.m. (Dodd, 1940). He says that in daylight the moths are immobile and not easily disturbed, but at night they fly, so apparently they do not ovipost before sunset. Similarly the preference of female C. densitexta, for leaves on the north rather than the south side of the tree, or any other leaves that are more frequently exposed to the sun is a preference for leaves in a particular condition (localized water stress), rather than a response to stimuli from the micro-climate. The females are not exposed, being on the under surface of the leaves.

Even when the use of artificial substrates for oviposition removes the risk of confusing responses to stimuli from the micro-climate with others to stimuli from the plant, results may be ambiguous if stimuli from the micro-climate are not tested individually with controlled experiments.

Thus Hovanitz and Chang's method of testing whether the position and movement of the sun and the direction of the wind influenced the distribution of eggs in a small cage, did not allow for possible variation in the strength of the wind. Nor, apparently, did they consider whether the butterflies' directional response to the sun might have been affected by the small size of the cage.

The butterflies' responses can only be tested meaningfully by

testing one component at a time e.g. testing the effect of wind in non-directional artificial light, or of the sun, in still air. Kobayashi (1966) tested the responses of individual female Pieris rapae crucivora to directional light without interference from wind, so that his results are more meaningful. He found that they laid more eggs in the direction of the sunlight, but that he could reverse the butterfly's preference by covering the south and west walls of the cage with black vinyl. These findings could be taken as evidence that the directional tendency of P. rapae crucivora is to fly towards the sun, as it is for P. rapae in England at the end of summer. But Kobayashi's experiment was done in June, so P. rapae crucivora in Japan is not behaving in quite the same way as P. rapae in England.

On the other hand, Kobayashi used only two females, on two occasions each, and it is possible that they may not have shown the same directional preference as the majority. (Gilbert (personal communication 1973), observing free, wild P. rapae, found that although the majority of flights were at an angle of approximately 260° to the sun's azimuth, the preferred direction for any one flight could be towards any point of the compass). But this may not have been a serious defect, as Kobayashi restricted all subsequent experiments to cloudy days. He also covered all four walls of the cage with frosted vinyl. The light intensity would thus have been much more uniform and any directional stimulus may also have been reduced.

Nevertheless, the spectral composition of daylight differs from

that of the light given by the fluorescent tubes in my experiments, the butterflies he used were from a different sub-species, and he did not measure the intensity of light in his experiments. Thus Kobayashi's demonstration of the influence of sunlight on the distribution of eggs by P. rapae crucivora neither supports, nor even tests, the hypothesis that Pieris rapae responds to differences in the intensity of artificial light. So I did the following experiment.

4.2.1 Experiment to Test the Egg-laying Response of Single Females to a Gradient in Light Intensity

Method:

Eight brussels sprouts plants were set up (as described in Section 1.2.3) in the controlled temperature room in which larvae and adults were later reared. Instead of being placed directly under the bank of lights, the cage and lights were each displaced along their long axis till the east end of the bank of lights was over the centre of the cage. This meant that the lights were nearer the (white) west wall of the room, increasing reflection from it, and the plants nearer the east wall. Thus there was a gradient in vertical and near vertical light intensity; high to low, from west to east. The plants were randomized among positions before the first, third and fifth replicates. Once they were in position the vertical light intensity was measured at the upper leaves of all plants. The horizontal light intensity facing west (the light side of plants), and east (the shaded side), was also measured for all plants, at about the height of the middle leaves.

The butterflies used for the first two replicates had been reared in the laboratory (they were probably about the third consecutive generation reared in the laboratory). As discussed in Section 1.2.1.2, the whole population of laboratory reared butterflies was so unhealthy by this time that it took seventeen attempts (comprising ten to twelve different females altogether) to find two females that would actively fly around laying enough eggs to get a worthwhile record of their behaviour in (a) and (b). Before the third replicate about five female and two to three male butterflies were caught in the field, but they were put to feed and preconditioned in the cage with the laboratory reared butterflies. Thus the origin of the butterflies used in replicates (c) and (e) (the same female was used for these two replicates), and (d), is uncertain. Probably they were some of those caught in the field as only five to six attempts were required to obtain three records of behaviour this time.

Results and Discussion

There was no consistency between results from different females and no relationship between the light intensity measured at a plant and the number of eggs a female laid on it. Even the results of (c) and (e) were not consistent. Although the same female was used for these two replicates, the plants were in different positions. Neither the same positions, nor the same plants were consistently favoured.

Thus the results seem to completely contradict the evidence from the nutriculture experiment (Section 3.2.2) that the butterflies were laying more eggs on plants with a higher vertical light intensity at the upper surfaces of their upper leaves - why?

There were three main differences between the nutriculture experiment and this one: (i) In this experiment the plants were growing in soil (in pots), whereas those in the nutriculture experiment were growing in two different nutrient solutions, one with a normal and the other with a very low sulphur content. This difference, however, seems unlikely to have influenced the butterflies responsiveness to light intensity. (ii) In the nutriculture experiment a group of butterflies, some of which were gravid females, were present all the time, whereas in this experiment there was only one butterfly present per replicate (and for the first three replicates they were apparently not very fit and active). (iii) The nutriculture experiment was done in a dimmed or dark laboratory (according to whether it was day or night time, respectively), where even the nearest reflective surfaces were covered with dark cloths to reduce the level of reflected light, so that most of the light on and around the plants was vertical radiation. In the controlled temperature room where this experiment was done, however, there was a generally very high level of reflected light due to the white walls, as well as probably some light from the other bank of lights (over the rearing cage, as described in Section 1.2.3). As a result, a plant in a position in which the intensity of light, in one or more

of the directions in which it was measured, was low, may have had leaves (and not necessarily its uppermost leaves) so positioned that they could catch horizontal or diagonal reflected light from a direction in which light intensity was not measured, and might have been high. Such a plant might therefore have been at least as attractive to an ovipositing female as a plant for which the intensity of vertical light at the upper surface of the upper leaves (where a butterfly is more likely to just settle temporarily, than to lay), was high.

Thus although this method of measuring light intensity may be adequate when almost all the light is direct vertical radiation from above, it probably does not give an adequate picture of the variety of light intensities associated with the plants when the level of reflected light is high. In the field, spatial differences in light intensity are generally not constant throughout the day. Either they are very temporary, (for example, differences caused by patches of cloud shadow) or else they are caused by the reflected light, or shadows, from trees, buildings, other plants, etc. Although such reflected light or shadow is temporary in that it lights or shades different plants at different times of day, it is relatively permanent in that the same plants are affected at about the same times of day every day (except when the sky is so overcast that the light is very diffuse and uniform, with no shadows at all). As reflected light contributes so much to spatial differences in light intensity in the field, the hypothesis that such differences are

likely to influence the pattern in which a female Pieris rapae distributes her eggs, cannot be effectively tested with only the simple method for measuring light intensity that was used for this, and earlier, experiments.

An alternative to a method relying directly on measurement of light intensity, that may nevertheless test the hypothesis even if in a rather crude and simple way, would be to place a bowl-shaped structure (open upwards) around the stem and lower leaves of each plant. All bowls should be the same colour outside, but bowls on half the plants should be lined with smooth aluminium foil (for maximum reflectivity) while those on the remaining plants should be lined with a dull grey or green material (green may be better if the aluminium foil is reflecting the green of the plants) of about the same depth of colour as the foil appears when in position, but with a rough, non-reflective surface. Then both individual females (whose behaviour could also be observed), and groups of females, should be tested to determine whether they lay more of their eggs on plants in the treatment with more reflected light. Due to a shortage of time and animals I was unable to do this experiment.

4.2.2 Experiment Testing Whether Pieres: rapae has a Circadian Rhythm of Egg-laying

Introduction:

Anomalous results obtained when the duration of ovi-position periods was reduced in the experiment described in Section

3.2.3.1 suggested that the probability of female P. rapae laying eggs may not be equal throughout the daylight hours, but that they may lay most of their eggs during a shorter preferred period, related to the time of day. Because of the potential influence of such behaviour in determining which of a set of conditions of the micro-environment will influence the butterflies' distribution of eggs, the experiment testing whether the butterflies do lay predominantly at a preferred time of day is described in this chapter.

Method:

The twenty plants used for this experiment were divided into five categories such that the differences in appearance etc. between the four plants in the same category were relatively much less than differences between plants in different categories. The four plants from each category were then individually randomized between two groups such that each group of ten plants comprised two randomly chosen representatives of each category. There were four oviposition periods (each of approximately 3 hours duration) per replicate. The two groups of plants were used for alternate O.P.s, both within and between the two replicates as shown in Table 4.01. After each O.P. the butterflies were recollected and the plants removed and replaced by plants of the other group before the butterflies were returned to the cage for the next O.P. As shown in Table 4.02 the interval between O.P.s was generally between 15 and 30 mins, during which time the butterflies remained in the light and at the same temperature as during O.P.s.

The plants were randomized among the ten alternate positions of a 4 x 5 array; i.e. in four rows, two of three plants per row, alternating with two of two plants per row, in the experimental set-up described in Section 1.2.2, with pots of flowering Alyssum or honey solution occupying the alternate positions in the array. During the second and subsequent O.P.s the eggs were counted and removed from those plants used in the previous O.P. so that the plants were ready for the following O.P.

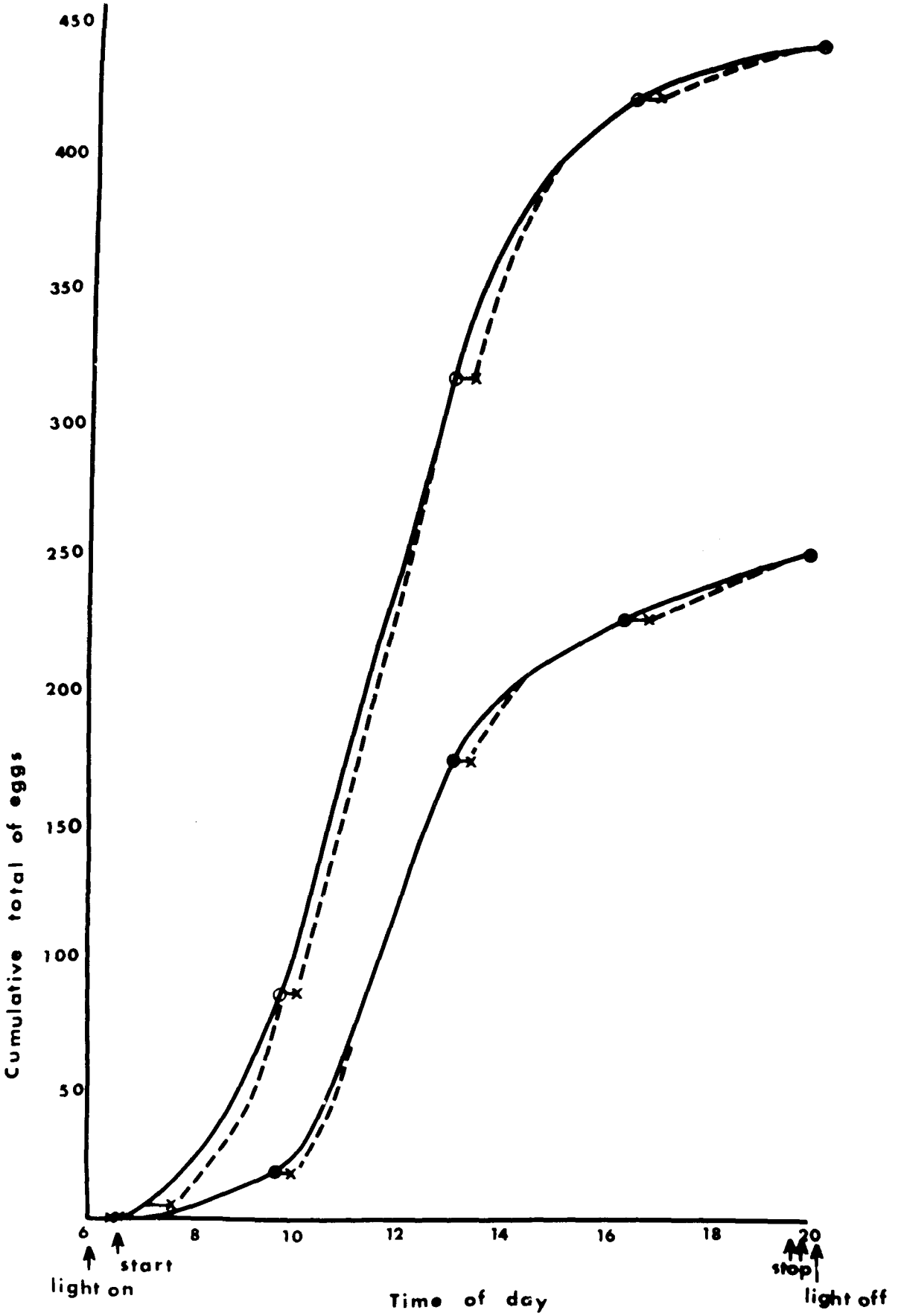
TABLE 4.01

Plants used to test whether Pieris rapae have a circadian rhythm of egg laying

Replicate & Date	O.P.	Group of Plants	Replicate & Date	O.P.	Group of Plants
1	(a)	I	2	(a)	II
	(b)	II		(b)	I
30/11	(c)	I	1/12	(c)	II
	(d)	II		(d)	I

All butterflies used for this experiment had been reared in the laboratory. Thirty-six females and twenty-eight males that had been used in previous experiments, and fifty-one newly reared butterflies were stored at 10°C, 14 hrs. light/10 hrs. dark (light between 06.00 hrs. and 20.00 hrs.), until they were needed for the experiment. The day before the experiment they were conditioned for eight hours (at ∿ 21.5°C) and then returned to 10°C until next morning, when fifty females and ten males were used for the experiment.

Fig. 4.01


450
400
350
300
250
200
150
100
50
Cumulative total of eggs
6
8
10
12
14
16
18
20
light on
start
stop
light off
Time of day

Results and Discussion:

As shown by Table 4.02, on both days the butterflies laid more of their eggs in the second (mid-morning till early afternoon) O.P. than in all the other three O.P.s put together. Figure 4.01 shows that on the first day of the experiment when the butterflies probably had more mature eggs in their ovaries, the favoured period extended from about 08.30 hrs. till about 14.30 hrs, but the following day, when they probably carried fewer mature eggs, the favoured period did not start until about 10.00 hrs, but nevertheless lasted till about 13.30 or 14.00 hrs.

TABLE 4.02

Evidence of a circadian rhythm in the egg-laying behaviour of Pieris rapae

Replicate	O.P.		(a)		(b)	(c)	(d)
1	Duration of O.P.	Start	06.35	07.36*	10.00	13.15	16.50
		Finish	07.06*	09.42	12.54	16.18	19.54
	Total eggs laid		86		229	103	22
2	Duration of O.P.	Start	06.30		10.00	13.15	16.42
		Finish	09.40		13.00	16.12	19.45
	Total eggs laid		17		156	53	24

*At 07.06 hrs. the lights went off due to a mistaken setting of the time clock. The mistake was found and the lights switched on again by 07.36 hrs.

The evidence that the butterflies do have a circadian rhythm of oviposition helps to explain the apparently anomalous results of the experiment testing whether butterflies would discriminate between plants grown in different light intensities described in Section

3.2.3.1 (hereafter referred to as experiment L.I.3). As already mentioned, until and including all but the last replicate of that experiment, the butterflies had been subjected to unnatural and inconsistent light and dark regimes, so that (as tentatively concluded, before the existence of a circadian rhythm was confirmed) their circadian rhythm may have been completely upset in some cases; in others it would have become independent of natural daylight hours, in some cases being in phase and in others out of phase with the natural day. But the existence and importance of a circadian rhythm was not suspected until experiment L.I.3, probably because in earlier experiments the testing period always spanned at least (usually more than) twenty-four hours. Sometimes the experiments were run in continuous light, but, when the lights were not on continuously, by chance they must have been on at a time that overlapped at least to some extent with the peak period of oviposition of at least some of the butterflies in the group.

All the butterflies used for replicates 3 to 6 of the experiment L.I.3 had been caught in the field only a day or two before replicate 3; those used for replicates 3 to 5 had also been pre-conditioned in the daytime so that at the start of replicate 3 their circadian rhythm was probably still in phase with the natural day. But before replicate 4, the butterflies were preconditioned at night then stored at 10°C, in the dark, during the time of day when peak oviposition usually occurs, until the start of replicate 4, by which time their circadian rhythm might have started a phase-shift to be

fitted to the new light regime. For any butterflies that still retained the tendency for a circadian rhythm with a peak period of oviposition more or less in phase with natural daylight, replicate 4 would probably have reinforced the rhythm; on the other hand the reversal to a light period in the daytime again, in replicate 4, would probably have further confused and weakened the circadian rhythm in those butterflies in which it had already begun to shift phase. Although replicate 5 was run in continuous light, it only lasted for eighteen (not twenty-four hours), and only the very last hour or two could have been in phase with the natural peak oviposition period. (There may not have been any overlap at all). Nevertheless if all butterflies had been in optimum reproductive condition some eggs would be expected; as the same field-caught butterflies had been used for all three replicates (3, 4 and 5) however, as well as being active and exposed to high temperatures during two pre-conditioning periods, ageing may have reduced their ability to lay eggs, thus accentuating the effect of a disrupted circadian rhythm. (Although their abdomens appear large as if full of eggs, old butterflies sometimes seem unable to lay them). More than two thirds of the butterflies used for replicate 6 had not been used before; they were stored before use in the same 14 hours light/10 hours dark photoperiod that was used for replicate 6, and laid many more eggs than were laid by almost as many females in replicate 3.

All subsequent experiments were timed to take advantage of the peak period of laying as much as possible.

CHAPTER 5

STATISTICAL ANALYSIS OF OBSERVED DISTRIBUTIONS OF EGGS

5.1 Introduction

In the experiments described and discussed in Chapters 2, 3 and 4, controlled variation of the environment in which insects were ovipositing was used to test whether various environmental stimuli influence the pattern in which the females distribute their eggs. Manipulation of the phsyiological condition of the females, to determine whether internal stimuli also influence the distribution pattern, is more difficult. But for phytophagous insects that lay their eggs on the larval host plant (Nishijima's categories (i) and (iii) of Section 3.11) an alternative method is possible. Statistical analysis of the distribution of eggs among the available units of host plant, and of the behaviour that generates it, can provide information on which characteristics of the distribution arise as a response to physiological stimuli, and which as a response to environmental stimuli. Theoretically it would even be possible (in some cases) to estimate the relative contributions of responses to internal and external stimuli. In practice, however, insufficient data has been collected in the appropriate way and the appropriate statistical methods have not been developed fully enough.

The distribution pattern of many insects' eggs does not differ

significantly from a negative binomial distribution; other contagious distributions (e.g. the Neyman type-A distribution) are also relatively common. Random or more uniform distributions of eggs are rarer. But determining whether a distribution is contagious, random, or more uniform, gives relatively little information on the behaviour that generated it, as any particular distribution may have arisen through several alternative forms of behaviour. Nevertheless Monro (1967) used just such analysis, together with measurements of how consumption of resources varied with population density and egg distribution, as the basis of hypotheses about the oviposition behaviour of Dacus tryoni and Cactoblastis cactorum. Consequently his hypotheses depended as much on teleological assumptions as on information from the analyses. Subsequent work (by Pritchard, 1969, on Dacus, and by Monro himself, cited by Birch 1971, on Cactoblastis) has shown, however, that in each case there is little or no evidence for the sort of behaviour that Monro proposed was most important. Instead, alternative behaviour which he considered less likely, appears to be responsible for the distributions.

As already mentioned in Section 2.1.2.2, Harcourt (1961) studied a wild population of Pieris rapae in Canada. He found that at densities higher than about two eggs per plant the frequency distribution was significantly more patchy than random but did not differ significantly from a negative binomial distribution. When the mean density was less than two eggs per plant the distribution did not differ significantly either from random or from a negative binomial

distribution. The relative degree of aggregation (as indicated by the parameter k from the negative binomial distribution) was more or less independent of the mean number of eggs per plant, but if he used quadrants of cabbage plants as sampling units, instead of half or whole plants, the estimate of k was different. This was a reflection of heterogeneity in the spatial distribution on any one plant; on windy days the females oviposited predominantly on the lee quadrant of the plants. Harcourt was mainly concerned more with achieving an adequate statistical description of the population to enable transformation of data for analysis of variance. As he was not studying oviposition behaviour he did not analyse the distribution any further.

On the other hand, Kobayashi, studying the Japanese subspecies Pieris rapae crucivora, has not only analysed the distribution of eggs by wild butterflies ovipositing in cabbage fields, he has also watched individual butterflies ovipositing in a large net cage, recording the number of visits to individual plants and the number of eggs laid at each visit. In his earlier studies, Kobayashi (1957, 1960) found that generally both the spatial distribution and frequency distribution (among plants) of eggs laid by wild butterflies in the cabbage fields was patchy, apart from in two or three consecutive censuses, for which the frequency distribution did not differ significantly from random, in spite of the normal heterogeneity of the environment. Kobayashi considered the latter distributions were

evidence that the patchy distribution usually found is not simply a response to heterogeneity of the environment.

The contagious distributions that Kobayashi found in the field were best fitted by a variety of theoretical distributions; the most recent were fitted best by the negative binomial distribution, which was also the predominant form of distribution when butterflies were laying eggs in his "net house". (That is, if the eggs laid on the central and peripheral plants were analysed separately. The butterflies invariably laid more eggs on the peripheral plants than the central ones, so in 1965 Kobayashi analysed only the results from the central plants). He tested six different densities of butterflies and found that for those distributions that did not differ significantly from a negative binomial (which was the majority), a common value of k could be fitted to distributions of eggs laid by the same number of females, even if the density of eggs differed. There was a tendency for these values of k to increase, (or 1/k, the measure of aggregation, to decrease) as the density of ovipositing females increased from 1 to 32 females in the cage (which was 0.03 → 1.0 females/m^3). When there were 32 females in the cage, the distribution, though contagious, was less skew than a negative binomial. There was only one test at this maximum density, while the other two distributions that did not fit the negative binomial each had another replicate which did fit it at the same density of females. Nevertheless Kobayashi took the former as evidence that as the degree of aggregation falls with increasing density of ovipositing females, so

the distribution also changes from negative binomial to a less skew form of distribution. According to this hypothesis the variety of forms of distribution that Kobayashi found in the field were the result of differences in the density of females ovipositing at any one time and place. But as shown in Section 5.2.1, even at densities up to about 30-40 butterflies/m^3, South Australian P. rapae distributed their eggs among the plants (in a small cage) in a pattern that did not differ significantly from a negative binomial distribution. Thus unless the Japanese sub-species differs from the South Australian one in this respect, the hypothesis above is insufficient to explain the variety of distribution patterns found in the field.

In the net-house experiments mentioned above, Kobayashi (1965) had counted only the number of eggs per plant, and even that on only the central plants. In subsequent experiments (one each with 1, 2, 4 and 8 females in the cage), however, he also recorded the number of visits to each plant. He found that the frequency distribution did not differ significantly from a Poisson (Kobayashi 1966). For the first three experiments (not the one with eight females present) he also also recorded the number of eggs laid at each visit. But, in each case, the only visits which he recorded were those at which one or more eggs were laid. As before, he found that most of the frequency distributions of eggs among plants fitted a negative binomial distribution. As he had found that the distribution of eggs among visits did not differ significantly from a logarithmic distribution,

he concluded that the negative binomial was generated by the model of randomly distributed colonies (Anscombe 1950).

In his earlier net-house experiments, as there was no consistent correlation between the number of eggs and the number of leaves on a plant, Kobayashi (1965) dismissed the possibility that differences between plants had any influence on the distribution of eggs. He concluded that "the contagious distribution at high parental density reflects the innate oviposition habit of the species..." Certainly the good fit between the distributions Kobayashi recorded and theoretical ones seems to be evidence that in the relatively uniform environmental conditions of Kobayashi's net-house, responses to internal stimuli were predominant in the females' oviposition behaviour.

But for three of the six egg-counts for which Kobayashi counted the number of eggs laid per visit, there was a significant correlation between the number of eggs per visit and the number of visits per plant, i.e. more eggs were laid per visit to plants that received more visits. This is surely evidence that Kobayashi's butterflies were not altogether indifferent to differences between plants, even in the net-house.

The experiments reported in Chapters 2, 3 and 4 showed that although certain characteristics of the host plants or micro-weather were often the strongest factors influenceing how female

P. rapae distributed their eggs, the distributions could rarely be accurately predicted simply in terms of the females' response to these main stimuli. Thus responses to internal stimuli, such as the behaviour Kobayashi reported appears to be, would have been contributing significantly to the observed distributions. So the nine sets of observations reported in Section 5.2.2 were recorded, to gain preliminary information on what it was possible, and what useful, to measure, test or specifically record.

Then the same methods (of observing and recording the behaviour of individual butterflies) were used when testing whether females (alone in the cage) would respond to larvae on a plant (Section 2.2.2.3) or to a gradient in light intensity (Section 4.2.1). The results are recorded in 5.2.3, and analyzed in terms of total eggs per visit etc.

But analysing the distribution of eggs by observing the number of eggs per visit and visits per plant is, of course, not practical when more than a very few butterflies are ovipositing. Even then probably about one observer per butterfly is needed to achieve an accurate record.

But Iwao and Kuno (1968, 1970) developed a statistical method by which the distribution of eggs laid by any number of females can be analysed to give some information about the distributions of eggs per visit and visits per plant. Iwao (1968) showed that for a number of different types of theoretical spatial distributions there is a

linear relationship between the mean crowding, $\overset{*}{m}$ (Lloyd, 1967), and mean density, m, with a slope β and an intercept α that are characteristic of the distribution being studied. Iwao (1968) and Iwao and Kuno (1971) analysed records (from the literature) of the spatial distribution of a number of biological populations in terms of the relationship between mean density and mean crowding. They concluded that among species which distribute themselves (or their eggs, etc.) contagiously, three main types of distribution are found. They can be distinguished by the combination of values of α and β:

(i) $\alpha > 0$, $\beta \simeq 1$. The individuals occur in groups or colonies (which are the basic components (b.c.s) of the population) whose mean size remains constant over a wide range of mean densities. (This is indicated by $\alpha > 0$). The basic components (groups) are distributed at random among units of the habitat (this is indicated by $\beta \simeq 1$).

Iwao and Kuno gave Kobayashi's (1965) field data and his (1966) results from the net-house observations, as examples of this type of distribution. The basic components were visits, distributed at random among the plants (habitat units), and with a mean number of eggs/visit (individuals/b.c.) that was greater than one and relatively stable over a range of densities of eggs.

(ii) $\alpha \simeq 0$, $\beta > 1$. Individuals occur singly (i.e.the basic components of the population are individuals not groups) but

are contagiously distributed (as shown by $\beta > 1$) with a tendancy for aggregation that is independent of mean density, such as in negative binomial distributions with a common k. (But Kobayashi's data, above, show that distributions described by (i) can also take the form of a negative binomial, but generated differently through compounding of the logarithmic and Poisson distributions). Iwao and Kuno considered this type (ii) distribution less common than the other two, and suggested that the former may have resulted from the animals response to heterogeneity in their habitat. The analysis in Section 5.3.1 (Figures 5.04 and 5.05 show that in most of my experiments with, or observations on, female P. rapae ovipositing alone in the cage, the eggs were distributed according to this model. It is not so clear whether the distributions in the experiments with groups of females (shown in Figures 5.01 and 5.02) fall into this category or the third one. The evidence is discussed in Section 5.3.1.

(iii) $\alpha > 0, \beta > 1$. Iwao and Kuno considered this the most common form of distribution among arthropods and probably also among many other animals and even plants. The basic component of the population is a group (shown by $\alpha > 0$) and though the distributions (of individuals among units) are often negative binomial they do not have a common k. Either the mean number of individuals per group varies or

the groups themselves are distributed contagiously.

Another measure of the distribution pattern of populations is Morisita's (1959) index of dispersion I_δ. As a single measure of dispersion the information it gives about a distribution is very limited - a little more than given by the variance-to-mean ratio, or k of the negative binomial distribution but less than the $\overset{*}{m}$-m regression analysis. But more recently Morisita (1964, 1971) has developed his theory further so that I_Δ (the dispersion index for the whole population rather than just a sample) can be broken down into partial dispersion indices which show the relationship of the total population to the dispersions within each of its components. For example he determined the interrelationship of inter- and intra-sample dispersions, and how the total dispersion of a population may arise from the inter- and intra-group dispersions.

He showed (1971) that by means of partial dispersion indices for the various behavioural components that generate a distribution it is theoretically possible to calculate the slope and intercept of the $\overset{*}{m}$-m regression line for that distribution. Although he does not give any means of calculating the errors involved, the value of β calculated in Section 5.3.2 by Morisita's formulae, agrees quite well with the slope of the $\overset{*}{m}$-m regression line, for the distributions of eggs laid by nine different females ovipositing alone in the cage, as shown in Figure 5.05. The data was not collected in the appropriate way to enable the calculation of α. Basically β depends on

the dispersion of females' visits among plants, while α depends on both the dispersion of visits among plants and the dispersion of eggs among visits.

But analysis of the distribution of an insect's eggs among units of the host plant into the components of eggs/visit and visits per plant does not necessarily completely separate responses to internal (physiological) and external (environmental) stimuli. The ovipositing female may receive stimuli from some plants that make her stay longer and lay more eggs at an average visit to those plants compared with other plants, i.e. the distribution and even mean number of eggs per visit may be influenced by stimuli from the plants as well as internal, physiological ones. Similarly the physiological state of a female may influence the distribution of her visits among plants. A female that is very active may fly around all the plants (thus exposing herself more or less equally to stimuli from all of them) relatively frequently, and so distribute her visits among them according to the differences between the plants. On the other hand a female that is relatively inactive may settle again on the same plant each time she flutters off a leaf, for many settles, rather than fly further to another plant. Then again she may leave that plant but just fly straight to an adjacent one, not exposing herself to stimuli from any of the other available plants. In these cases the butterfly's physiological condition is limiting the number of plants she can choose between for any one visit. Such limited choice

may especially affect the distribution of total visits (and perhaps also eggs) among plants in a relatively short period of time such as an experimental oviposition period.

If, instead of analysing the dispersions or distributions of eggs per visit and visits per plant, we look at the dispersions of eggs per settle and settles per plant, however, it is more possible to separate responses to external and internal stimuli.

In theory it should be possible to split these two dispersions into their components, as Morisita did for the dispersion of visits among plants, but the statistical relation between these components has not been determined. Nevertheless it appears that each of these dispersions has one component that measures the response to internal stimuli only, and one that measures the response to external stimuli. Thus the dispersion of settles among plants can be split into the mean intra-plant, inter-time unit dispersion of settles per plant, which will depend only on internal stimuli, and the interplant dispersion of the mean number of settles per plant which will depend on differences between the plants. Similarly the dispersion of eggs per settle can be theoretically split into a mean intra-leaf surface dispersion of eggs among settles, due to internal variability in the females tendency to lay eggs, and an inter-leaf surface dispersion of the mean number of eggs per settle, which will depend on differences in attractiveness of the leaf surfaces.

But in practice it would be necessary also to analyse settles on under and upper surfaces of leaves separately as it is already known that most settles on under surfaces appear to be associated either with attempts to oviposit or at least with testing the leaf's suitableness as an oviposition substrate, whereas many settles on upper surfaces seem to have no association with attempted oviposition.

5.2 Observations and Analyses

5.2.1 Testing Whether the Distributions of Eggs Among Plants Differ Significantly from a Negative Binomial Distribution

Anscome's T statistic was used for this test, but as it requires a large sample only those distributions for which the sample size (number of plants) was equal to or greater than twenty, could be used. Kobayashi (1966) rejects the null hypothesis that his distribution does not differ significantly from a negative binomial if T is greater than its standard error. As a deviation as great as 1.96 standard errors is possible, before the probability is less than five percent, the latter criterion will be used for rejecting or retaining the null hypothesis, rather than Kobayashi's. In all the above experiments except 2.2.1.2 (a) and (b) the volume of the cage was approximately 1 cubic metre, thus the density of butterflies was much greater than in Kobayashi's (1965 or 1966) observations (his cage was about 32 cu.m.), but the distributions still did not differ significantly from negative binomial.

TABLE 5.01

Negative Binomial Distribution of Eggs by Female P. Rapae in a Laboratory Cage

Experiment in Section	T	SD_T	T/SD_T	Probability
Experiments with no treatments effects:				
2.2.1.2 (a)	-281.78	380.47	0.741	>0.05
" (b)	172.31	202.82	0.850	"
3.1.2.3 (p.124)	-525.00	492.89	1.065	"
(a)	-2639.29	6395.97	0.413	"
2.2.1.3 (b)	39.63	35.95	1.107	"
(c)	-283.04	1003.46	0.282	"
*(d)	-138.49	722.73	0.192	
*(e)	-174.08	462.87	0.376	"
2.2.2.2	-868.57	1154.93	0.752	"
Experiments with treatment effects:				
3.2.3.1 (3)	-382.08	554.03	0.690	"
(6)	-3725.84	8169.57	0.456	"

*These two replicates were done several weeks after (a)→(c) so that the leaves had changed. Consequently the results of these two replicates could not be analysed in Section 2.2.1.3.

Although it is probably due to chance, it is a strange coincidence that the 3 most aggregated distributions (those with the highest values of 1/k) occurred when there was one plant per female - at both higher and lower densities of butterflies the values of 1/k decrease. It would be interesting to determine whether there is any significance in this result.

TABLE 5.02

Degree of Aggregation of the Distribution of Eggs among Plants in Relation to the Number of Butterflies Present

Experiment in Section	Rep	No. of Females	Total Butterflies	1/k	Plants per Female
2.2.1.2	(a)	30*(2)	46 (8)	0.335	0.800
"	(b)	36(24)	55(40)	0.354	0.667
3.1.2.3	(p 124)	26 (8)	34(11)	0.463	0.769
	(a)	18 (3)	40(13)	1.149	1.111
2.2.1.3	(b)	15 (2)	27 (4)	0.178	1.333
	(c)	13 (2)	23 (4)	0.482	1.539
	(d)	33(13)	44(15)	0.397	0.601
	(e)	34(19)	43(25)	0.602	0.741
2.2.2.2		25 (1)	25 (1)	0.273	0.800
3.2.3.1	(3)	18 (3)	31 (4)	0.951	1.111
	(6)	22 (2)	31 (4)	1.094	0.909

* Numbers of butterflies that died during each experiment given in parenthesis.

5.2.2 Analysis of Five Preliminary Records of Oviposition Behaviour

Method:

The method was as described in Section 1.2.3. Six plants were used for the observations on the first butterfly, five plants for another eight sets of observations, but, as explained below, only five of them could be analysed. Six butterflies were used for

the nine sets of observations.

Results and Analysis:

Because of the small number of plants used, no really rigorous test is available to determine whether distributions among plants of eggs, or of total visits, or visits at which eggs were laid, were distributed randomly or not, in each oviposition period. If it is borne in mind however that the tests are not rigorous, and if a number of different tests are done and compared, some tentative conclusions may be drawn, for those distributions with a mean of more than 5 eggs per plant, or per visit. The five such analyses that are possible are shown in Table 5.03.

On the other hand the distribution of eggs per visit can be analysed rigorously for five of the nine records. It was possible to calculate expected random frequencies for all but one of the nine records, but for three of these the expected frequencies were too low to allow analysis. The five plants used for each of these records (and the six for the first one) were selected from a total of ten - on several occasions the same combination of plants was used, for instance, the five plants that were used on 31/1 were also used on 25/1, 3/2 and 4/2. As Table 5.03 shows, the distributions of both eggs per plant and visits per plant (both total visits and those at which eggs were laid) were probably random on 31/1 and 4/2. Although the mean numbers of eggs and visits on 25/1 and 3/2 were

TABLE 5.03

Tentative Analysis (not Rigorous) of the Distributions of Eggs and Visits Among Plants by Single Females

Date / Female		23/12 Lb,Lb	30/1 R,O	31/1 R,O	2/2 R,Dg	4/2 O,B
Distribution of eggs among plants	$\bar{x}$	6.50	9.00	9.80	18.00	8.00
	χ^2	18.692	12.222	4.980	48.556	5.750
	dfs	5	4	4	4	4
	$s^2/\bar{x}$	3.74	3.06	1.25	12.14	1.44
	$\sim$ P	<0.01	<0.02	>0.05	<0.001	>0.05
Tentative Conclusion		Patchy	Patchy	Random	Patchy	Random
Distribution of V_os/ plant (V_o = Total Visits)	$\bar{x}$	8.17	9.20	13.40	12.80	11.80
	χ^2	6.224	8.130	7.403	41.156	1.763
	dfs	5	4	4	4	4
	$s^2/\bar{x}$	1.25	2.03	1.85	10.29	0.44
	$\sim$ P	>0.05	>0.05	>0.05	<0.001	>0.05
Tentative Conclusion		Random	Random	Random	Patchy	Random
Distribution of V_1s/ plant (V_1 = Visits at which > 1 egg laid)	$\bar{x}$	-	8.00	9.20	12.40	6.6 or 6.8
	χ^2	-	10.500	5.739	39.129	5.03 or 4.53
	dfs	-	4	4	4	4
	$s^2/\bar{x}$	-	2.63	1.44	9.78	1.26 or 1.13
	$\sim$ P	-	<0.05	>0.05	<0.001	both >0.05
Tentative Conclusion		-	Patchy	Random	Patchy	Random

too low to analyse them, the data suggested that the plants were visited and eggs laid among them, at random on these dates, as well. Figure 5.03 shows that when analysed by Iwao and Kuno's m^*-m regression method the distributions do indeed appear to be random. The

fact that the only four random distributions all occurred on the same group of plants, although different females were ovipositing, seems to be further evidence that in most other cases heterogeneity between the plants contributes significantly to heterogeneity in the distribution of eggs. Nevertheless, only one of the three patchy distributions of eggs in Table 5.03 appeared to have arisen as a result of a patchy distribution of total visits among plants. But this did not mean that the butterflies were behaving as Kobayashi (1966) reported for P. rapae crucivora; instead a patchy distribution of visits at which eggs were laid suggests the possibility of oviposition in response to stimuli from the plant once the female has visited and settled on it.

TABLE 5.04

Analysis of Frequency Distribution of Eggs Among all Vists (i.e.V_os)

Date n		23/12 49	30/1 46	31/1 67	2/2 64	4/2 59
Result of test against expected frequency	$\bar{x}$	0.796	0.978	0.761	1.406	0.678
	χ^2	0.981	28.359	20.720	37.076	2.863 or 3.787
	dfs	1	1	1	2	1
	P	>0.05	<0.001	<0.001	<0.001	>0.05
	Comment	Random	More Uniform	More Uniform	More Uniform	Random

n = total visits in O.P.

5.2.3 Behaviour Records from Experiments (Already Reported)

Testing Specific Responses

Fifteen further records of the behaviour of females

ovipositing alone in the cage were collected: two from preliminary experiments (done in the same constant temperature room as the experiments with groups of butterflies) testing the methods; number of larvae etc., for the experiment in Section 2.2.2.3; five from the experiment in Section 4.2.1; and the eight replicates of the experiment in Section 2.2.2.3. Six plants were used in the preliminary tests, and eight in each of the other two experiments so that, as the mean number of eggs per plant was less than five in all but four sets of observations, the distributions among plants of eggs, total visits, and visits at which eggs were laid, were not tested for each set of observations to determine whether they differed significantly (and in which direction) from random.

Instead the distributions of eggs laid, and of visits to plants, in all eight replicates of the experiment in Section 2.2.2.3 were summed, so that the means were high enough for valid analysis of the resulting total distribution. This method was considered to be valid as on the whole the butterflies were all behaving the same way, as shown by a significant concordance between their preferences for plants (Table 5.05). Female No. 2 appeared to behave aberrantly on 15/9 but normally on 18/9, so two sets of concordances were calculated, one omitting, the other including, both records from female 2.

TABLE 5.05

Concordance between the choice of plants by butterflies in the Experiment in Section 2.2.2.3

Distributions of			W*	χ^2	dfs	P
Eggs per plant	Female 2	Included	0.550	30.817	7	<0.001
		Excluded	0.694	29.131	"	<0.001
V_os per plant	Female 2	Included	0.697	39.043	"	<0.001
		Excluded	0.386	16.220	"	<0.05
V_1s per plant	Female 2	Included	0.491	27.474	"	<0.001
		Excluded	0.584	24.536	"	<0.001

*W = Kendall's coefficient of concordance (Siegel 1956). As the table shows there was a closer concordance between the distributions of total visits (v_os) when the data for female No. 2 were included but it made little difference in the case of eggs or visits at which eggs were laid (v_1s) so the results from all eight replicates were summed for the analysis shown in Table 5.06.

TABLE 5.06

Summed Distributions for the Experiment in Section 2.2.2.3

Distributions of	$\bar{x}$	χ^2	dfs	$s^2/\bar{x}$	P
Eggs among plants	39.875	133.037	7	19.01	<0.001
*V_os " "	36.625	68.966	"	9.85	<0.001
*V_1s " "	28.000	78.929	"	11.28	<0.001

* V_os = Total visits, V_1s = visits at which 1 or more eggs were laid.

Thus, on the whole the distributions among plants of eggs, total

vists and visits at which eggs were laid, were all significantly more patchy than random. The distributions of eggs were analysed both among V_os (for the experiment in Section 4.2.1 as well) in Table 5.07, and among V_1s (Section 2.2.2.3 experiment only).

The expected frequencies were calculated for logarithmic distributions with the same mean as the mean number of eggs per visit at which eggs were laid. Because the χ^2 test requires each class to have an expected frequency of five or greater, it was not possible to test whether the observed distribution differed significantly from the expected ones except for replicate 5. Nevertheless as shown in Table 5.08 there was a marked similarity between the observed and expected frequencies in four of the eight distributions, and a large difference between observed and expected frequencies occurred in only one replicate.

TABLE 5.07

Distribution of Eggs Among Total Visits

Experiment	Rep.	$\bar{x}$	χ^2	dfs	P	Distribution
In Section 2.2.2.3	1	0.732	13.217	1	<0.001	More Uniform
	2	5.143	Not analysable, appears very patchy			
	3	0.727	" "			" more uniform
	4	0.944	14.717	1	<0.001	More uniform
	5	1.875	6.634	2	<0.05	" "
	6	1.200	4.966	1	<0.05	" "
	7	0.685	21.673	2	<0.001	" "
	8	1.000	19.197	2	<0.001	" "
In Section 4.2.1	1	1.260	4.509	2	>0.05	Random
	2	0.344	Not analysable, appears to be patchy			
	3	0.516	0.131	1	>0.05	Random
	4	0.778	(5.078 or (2.416	1	<0.05 or >0.05	More Uniform or Random
	5	0.660	10.829	1	<0.001	More Uniform

TABLE 5.08

Comparison of Logarithmic Distribution Frequencies with Observed Frequencies of Eggs per V_1

No. of Eggs per Visit (x)	Rep. 2 $\bar{x}$ = 6.000		Rep. 5 2.000		Rep. 4 1.172		Rep. 6 1.429		Rep. 8 1.267	
	E*	O	E	O	E	O	E	O	E	O or —
1	1.95	1	17.08	16	24.93	25	15.27	15	48.17	46 47
2	0.92	1	6.11	5	3.33	3	3.75	4	8.82	13 12
3		0		6	0.59	1	1.23	1	3.01	0 0
4		1	6.81	1	0.15	-	0.75	1		1 0
5	<2.75	0		0		-		-		- 1
6		1		2		-		-		- -
7-8		0		-						
9		1	χ_1^2= 1.89							
10-13		0								
14 +		1	P > 0.05							

Eggs/visit	Rep. 1		Rep. 3		Rep. 7	
x	E	O	E	O	E	O
1	26.19	26	12.31	12	35.01	35
2	1.81	2	1.69	2	0.99	1
3 +		-		-		-

*E = expected logarithmic frequency, O = observed frequency.

Thus it appears that in this respect my butterflies were behaving similarly to those Kobayashi studied. But it is unfortunate that he did not record visits at which eggs were not laid, as analysis by Iwao and Kuno's m^*-m regression method (in the next section) suggests that there is, after all, a real difference in behaviour between Kobayashi's butterflies and those I used. This suggests that "visits at which eggs are laid" are not necessarily

any more biologically meaningful as units, than are "total visits". As mentioned in the introduction, "settles" seem to be more standard for butterflies in different physiological states and so are probably better units than either sort of visits.

5.3.1 Analysis by Iwao and Kuno's m^*-m Regression Method

All experiments with groups of butterflies were divided among two classes as follows:

Experiment in Section	No. of Distributions
(i) Experiments or observations with no significant treatment effect (Figure 5.01)	
2.2.1.2	2 (both O.P.s (a) and (b)
3.1.2.3	1 (Comparison between plants grown inside and outside)
2.2.1.3	5 (Include the two extra reps. mentioned above)
4.2.2	8 (4 per replicate, 2 replicates).
2.2.2.1	8 (Includes the three extra without larvae mentioned in 3.2.1.2 and 1 other with larvae but too few eggs).
2.2.2.2	1
(ii) Experiments with Specific Treatment Effects (Figure 5.02)	
2.2.1.1	2 (O.P. (b) of ea. of (a) and (b)
3.2.1.1	1
3.2.2.2	3 (From reps. 1, 2 and 3)
3.2.3.1	4 (reps. 1, 2, 3 and 6).

Thus there were twenty-five records of distributions for (i) and ten for (ii). As mentioned in 5.2.2 above, four of the distributions from the nine preliminary observations on single females appeared

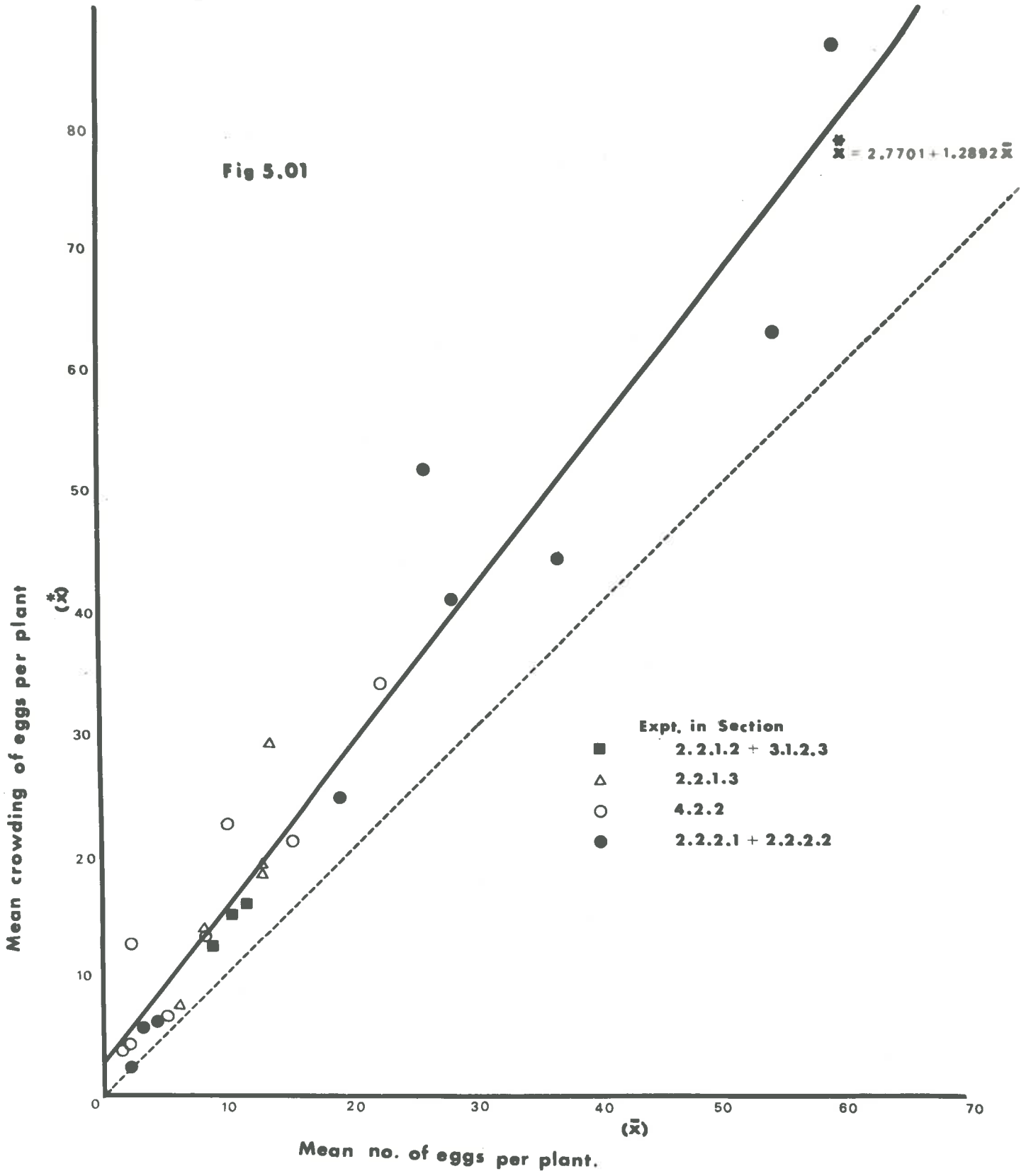
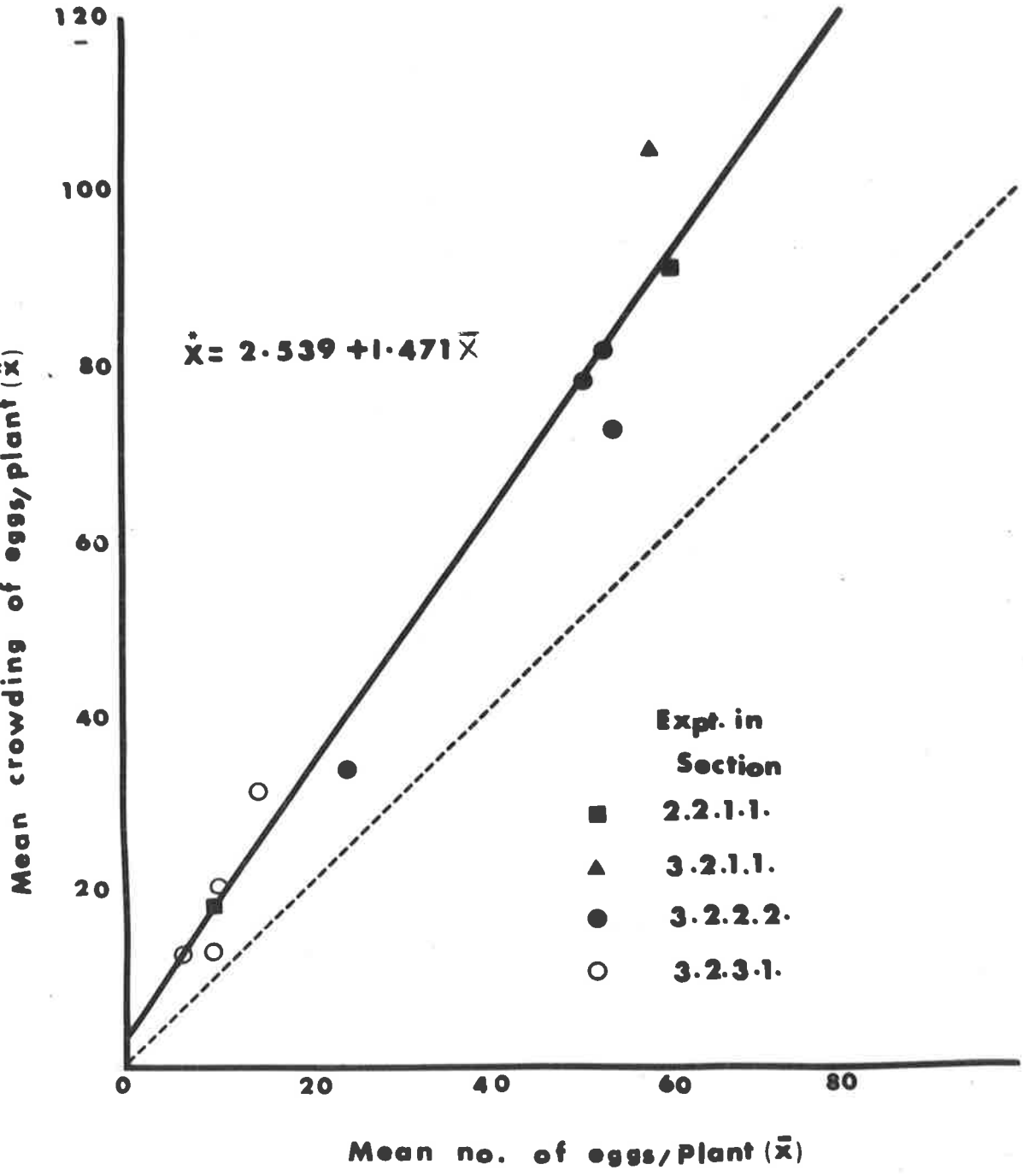


Fig 5.01
$\bar{x}^* = 2.7701 + 1.2892\bar{x}$
Expt. in Section
2.2.1.2 + 3.1.2.3
2.2.1.3
4.2.2
2.2.2.1 + 2.2.2.2
Mean crowding of eggs per plant ($\bar{x}^*$)
Mean no. of eggs per plant. ($\bar{x}$)
0
10
20
30
40
50
60
70
80

Fig. 5.02


120
100
80
60
40
20
0
20
40
60
80
$\dot{X} = 2.539 + 1.471 \bar{X}$
Mean crowding of eggs/plant ($\dot{x}$)
Mean no. of eggs/Plant ($\bar{x}$)
Expt. in Section
2.2.1.1.
3.2.1.1.
3.2.2.2.
3.2.3.1.

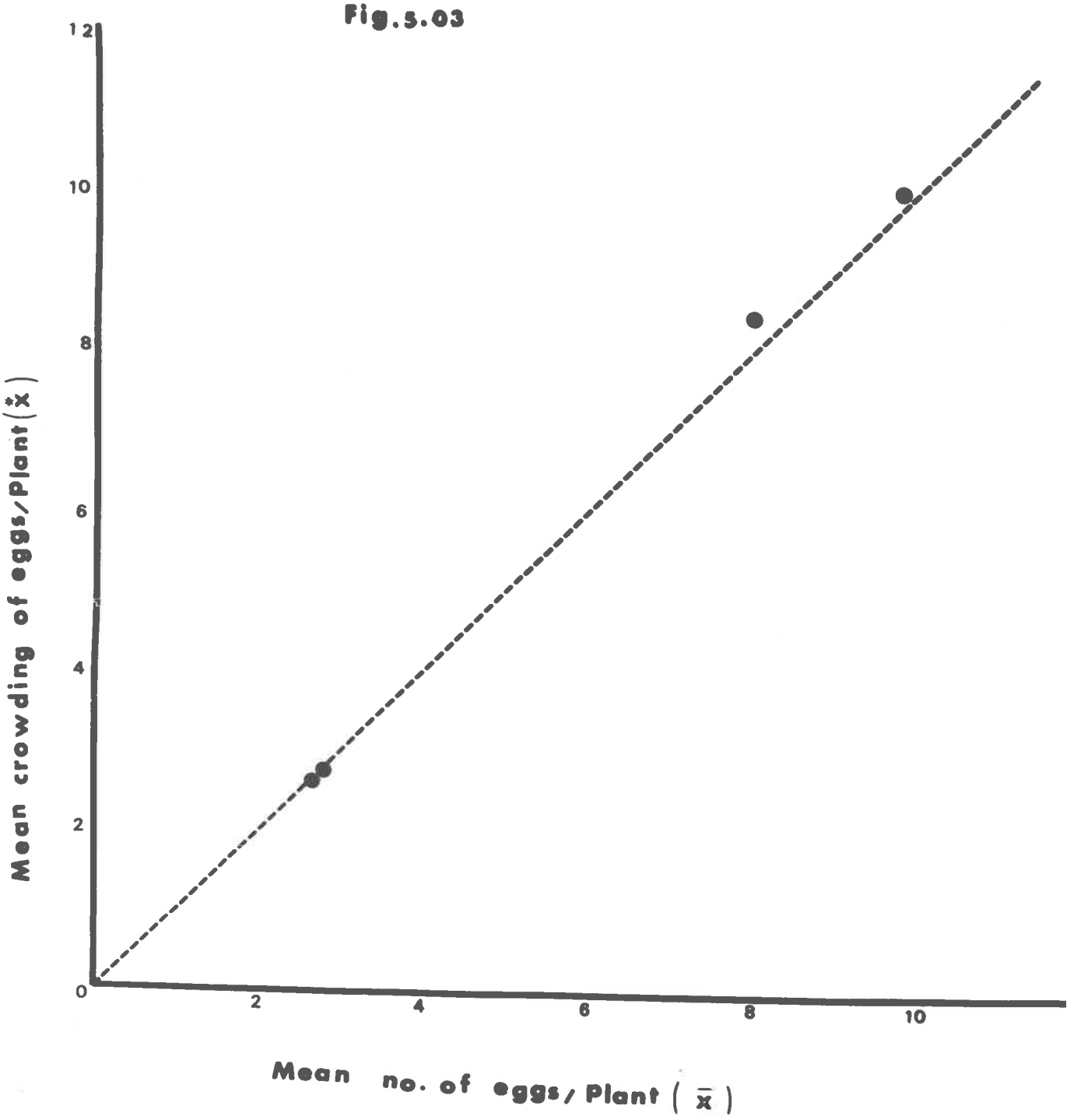
to be random; they were the only ones out of all distributions of eggs laid by single females that eappeared to be so, so they were analysed separately.

Although there was not a significant or consistent response to the light gradient in the experiment in Section 4.2.1 the light may have been influencing some of the butterflies. Even if it was not, the butterflies in that experiment were not behaving very normally, so the distributions from Section 4.2.1 are omitted from the analysis. There were therefore nineteen distributions of eggs laid by single females, as follows:

Experiment in Section	No. of Distributions
(iii) Apparently random distributions (Fig. 5.03)	
5.2.2	4 (on 25/1, 31/1, 3/2 and 4/2).
(iv) Apparently non-random distributions (Fig. 5.04)	
5.2.2	5 (on 23/12, 14/1, 18/1, 30/1 and 2/2).
5.2.3	2 (Preliminary to 2.2.2.3)
2.2.2.3	8

The mean crowding of each distribution was plotted against its mean density for each of the classes, in the figure indicated in the table. The value of $\overset{*}{x}=\overline{x}$, which is the expected relationship for a random distribution, is shown on each graph by a broken line. The calculated regression equations are also shown on the appropriate graph, but the 95% confidence limits of the estimates a & b, of α and β respectively (which are also necessary for interpretation

Fig.5.03


12
10
8
6
4
2
0
2
4
6
8
10
Mean crowding of eggs/Plant (x*)
Mean no. of eggs/ Plant (x̄)

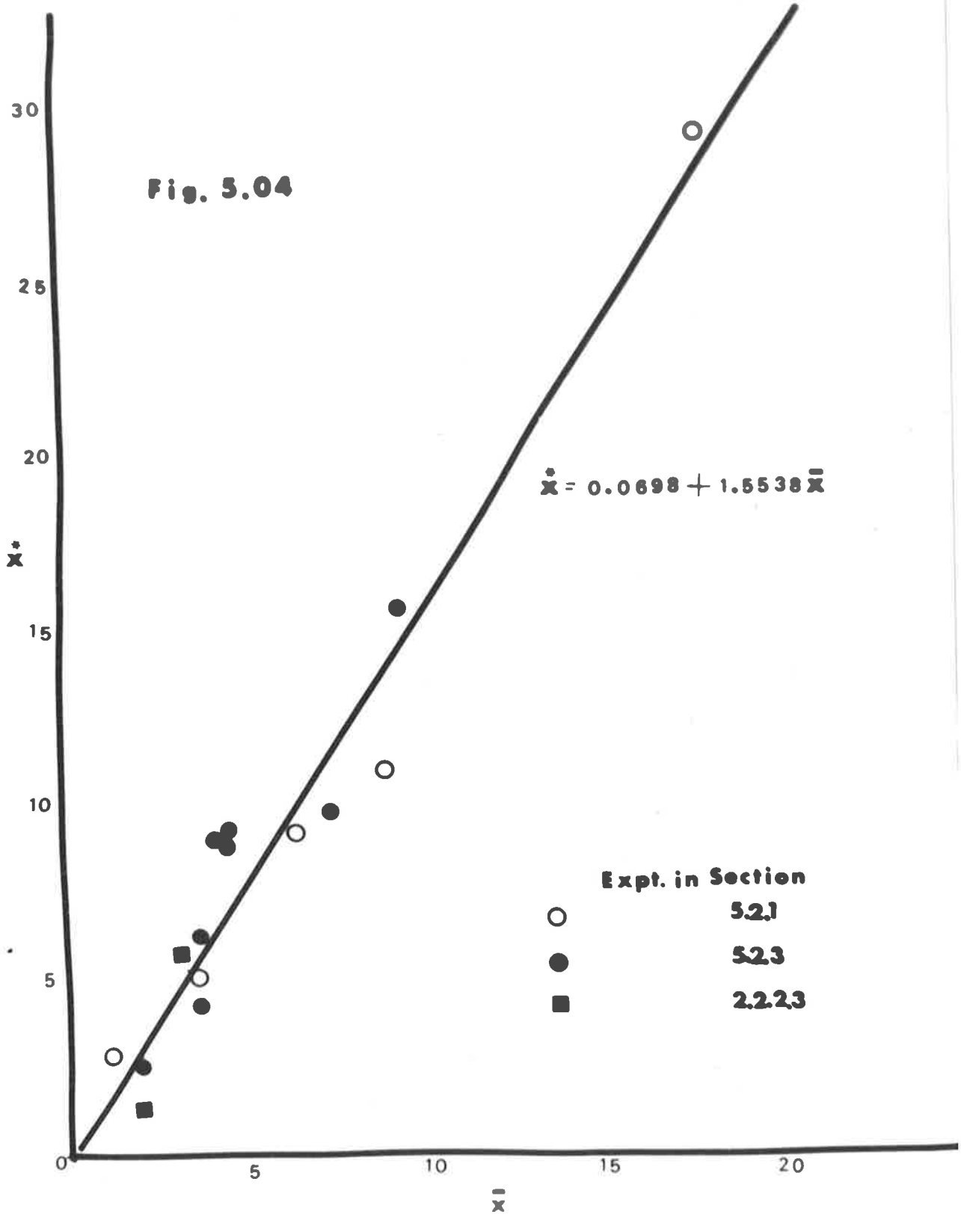


Fig. 5.04
30
25
20
15
10
5
0
5
10
15
20
$\dot{\bar{x}}$
$\bar{x}$
$\dot{\bar{x}} = 0.0698 + 1.5538\bar{x}$
Expt. in Section
5.2.1
5.2.3
2.2.2.3

of the graphs) are shown below.

TABLE 5.09

Confidence Limits of Estimates of the Slope and Intercept in m^*-m Regressions

Class	a	95% Conf. Lims.	b	95% Conf. Lims.
(i)	2.770	-0.563 → 6.103	1.289	0.433 → 2.145
(ii)	2.539	-6.536 →11.714	1.471	-0.298 → 3.239
(iv)	0.070	-1.502 → 1.642	1.554	1.326 → 1.782

Conclusions From m^*-m Regression Analysis

(a) Experiments with single butterflies.

Figure 5.03 further supports the hypothesis that the females distributed their eggs at random among these five plants, on each of the four occasions they were used together.

As mentioned in the introduction, although the distribution of eggs/visit (by butterflies in the 2.2.2.3 experiment) did not appear to differ significantly from a logarithmic distribution, when only those visits at which eggs were laid were counted, Figure 5.04 shows that my butterflies were not behaving as Kobayashi's did. The confidence limits for b are so narrow that there can be no doubt that the butterflies were not visiting these plants at random. Although the confidence limits for a are wider, there seems little doubt that eggs are being laid

independently, as in Iwao and Kuno's model (ii). Thus it is perhaps more realistic to look at the distribution of eggs among total visits.

(b) Experiments with groups of butterflies.

The values of a and b in both samples of distributions of eggs laid by groups of butterflies suggest, however, that when in company, the females may distribute their eggs according to Iwao and Kuno's third model instead. Although the experiments in Section 2.2.3 failed to show significant interactions between adults, the apparent change in distribution pattern shown by comparing Figures 5.01 and 5.02 with 5.04 and 5.05, suggests they may occur. Perhaps females congregate (subjective observations suggest that they certainly congregate to rest - occasionally but not always they may also do so for oviposition) so that the basic component of the population would be a group of eggs, but a group with a changing mean. On the other hand the confidence limits of both a and b are very wide for both samples of distributions by groups of females, so that, in practice their behaviour may not differ significantly from that of females laying alone. But it could even differ in some other direction without these data really showing the difference.

5.3.2 Analysis by Morisita's Indices of Dispersion

Morisita (1971) (Section III.2.iii(2)) expressed the relationship between the mean density and mean crowding of eggs laid by

flying insects theoretically, in terms of the dispersion and mean number of eggs per visit, and the dispersion and mean number of visits to plants. For the particular case of a series of distributions of eggs each laid by a single female, he calculated that the slope β of the $\overset{*}{m}$-m regression line would be given by the product of two dispersion indices - $I_{\Delta Q}$ (the dispersion among plants of the mean number of visits per female, per unit time, to the i^{th} plant) and $I_{\Delta qz}$ (the mean dispersion among females, of visits per unit time, to the same plant). As the oviposition behaviour of females had been timed in the experiment in Section 2.2.2.3 it was possible to calculate these dispersion indices (shown in Table 5.10). (As the duration of "sits" (q.v. Section 2.2.3) has no influence on the distribution of eggs the units of time used were units of FLP time). The same plants must be available to all females (i.e. in all replicates that are to be compared - or among which the inter-female dispersion is to be measured) so that not all the fifteen distributions from Figure 5.04 could be used. Instead the eight records from the experiment in Section 2.2.2.3 and the oviposition record from the undyed female laying eggs in the presence of males only, in Section 2.2.3 were used. The latter experement was done on the same plants only a matter of days after the former.

Figure 5.05 shows the observed regression of mean crowding on mean density for these distributions (only counting those eggs laid within the timed FLP record).

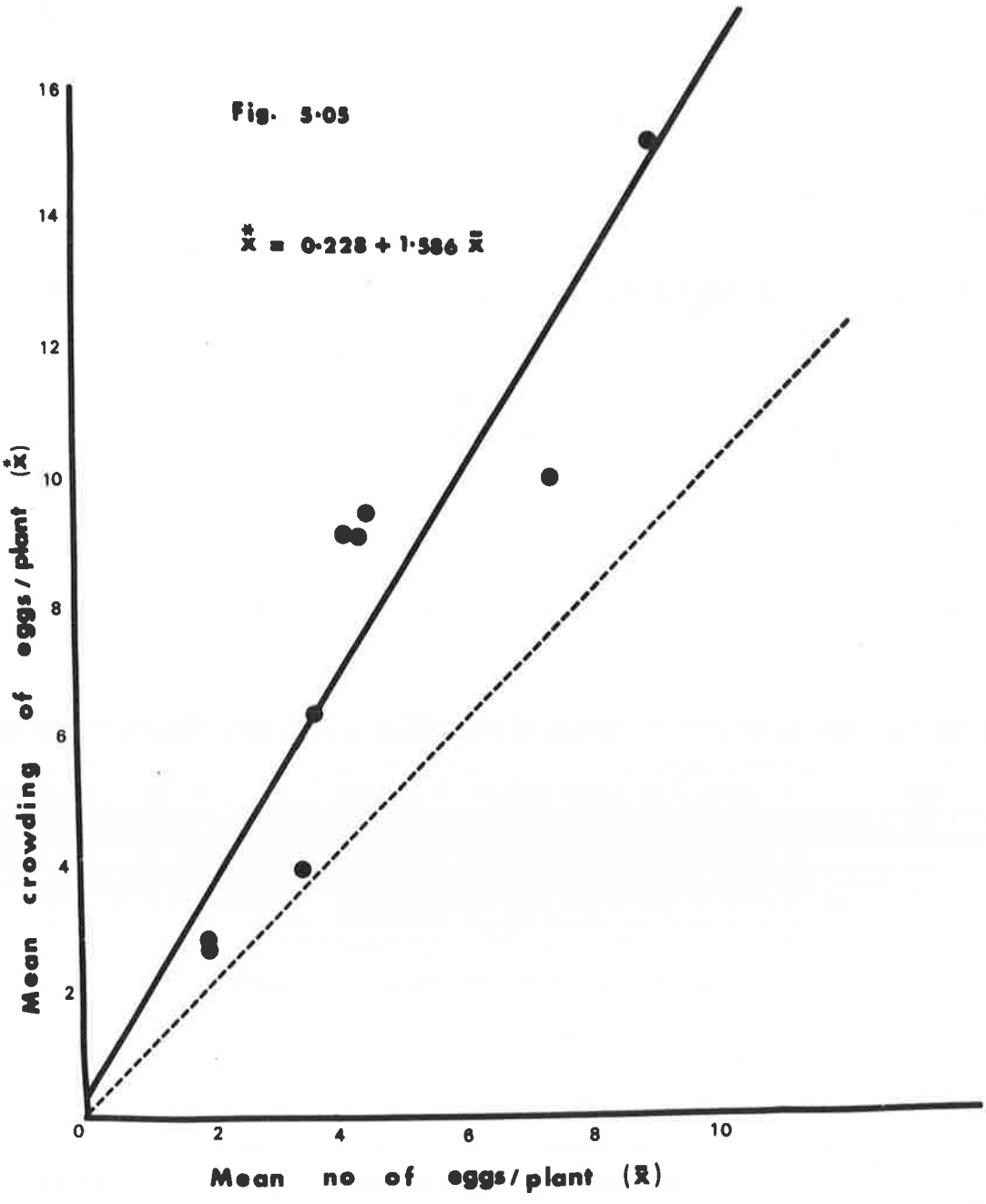


Fig. 5·05
$\overset{*}{x} = 0.228 + 1.586\,\bar{x}$
Mean crowding of eggs / plant ($\overset{*}{x}$)
Mean no of eggs / plant ($\bar{x}$)
0
2
4
6
8
10
12
14
16

TABLE 5.10

Comparison of the Empirical and Theoretical Estimates of β*

$I_{\Delta qz}$	$I_{\Delta Q}$	$\hat{\beta}$	b	95% Confidence Limits
1.436	1.165	1.674	1.586	$\pm$ 0.600

* $\hat{\beta}$ = theoretical estimate of β.

b = empirical estimate of β.

The agreement between the two estimates is surprisingly close, suggesting that in this case total visits, from which $I_{\Delta qz}$ and $I_{\Delta Q}$ were calculated, are more relevant than only those visits at which eggs were laid.

As the theoretical estimate of β lies above the empirical one, it seems most unlikely that β is really less than or equal to one, although the lower of the 95% confidence limits is 0.986. As before, therefore, we can conclude that visits are distributed contagiously, both among plants and through time. When settles instead of visits were used to calculate $I_{\Delta qz}$ and $I_{\Delta Q}$, the estimate of β was higher still (1.715), but the relative contributions of $I_{\Delta qz}$ and $I_{\Delta Q}$ to $\hat{\beta}$ had changed. The contribution of $I_{\Delta Q}$ had increased, and that of $I_{\Delta qz}$ had decreased, probably because of the change in the record of Female No. 2 laying on 15/9. She visited plants almost equally, whereas the number of settles per plant showed plant heterogeneity, better. Nevertheless the differences between females in

their level of activity and their preferences for certain oviposition sites (measured by $I_{\Delta qz} = 1.353$) was still greater than the overall discrimination of differences between plants (measured by $I_{\Delta Q} = 1.267$).

Conclusions to Analysis by Morisita's Indices

Calculation of $\hat{\alpha}$ involves calculating dispersion indices from a large number of independent time units. As most of my observations were made in contiguous time units it was not possible to calculate a theoretical estimate of α with my data. Thus more data need to be collected in appropriate ways before Morisita's theoretical analyses can be adequately tested. At the same time the analyses need to be developed further, both to give some estimate of the errors involved, and also to show the interrelationships between other dispersions, such as the components of the dispersion of eggs among settles, and those of settles among plants.

5.4 Conclusions About Statistical Analyses of Egg-distributions

Although far from complete, the analyses and discussion above show (much more convincingly than the experiments in Chapters 2, 3 and 4 did) that the butterflies' oviposition behaviour was being influenced by internal as well as external stimuli. Thus both a direct experimental study of behaviour and a less direct statistical

analysis of distributions are necessary to achieve a better understanding of the oviposition behaviour of phytophagous insects. The statistical approach, being a more recent development, seems to require more work before it can fulfill its potential of measuring the relative contributions of internal and external stimuli to a female's behaviour, in any particular set of conditions.

APPENDIX I

DETERMINATION OF THE RELATIONSHIP BETWEEN THE AREA AND VOLUME OF INDIVIDUAL LEAVES OF BRUSSELS SPROUTS PLANTS

The individual areas of thirty leaves were measured (either directly or by means of a paper shape carefully cut to fit the leaf) on an electronic planimeter. [This method could not be used for measurement of leaf area in experiments as it required detachment of the leaves for direct measurement, or slow and impractical making of paper leaf images. Nevertheless measurement of a few test leaves by electronic planimetry gave a standard against which the accuracy of other method of estimating leaf area could be assessed.

Of other methods tested, measurement by the sliding circle device described in Section 1.2.1.3 gave the most efficient combination of speed, convenience and accuracy]. The areas of fifteen of the thirty leaves were also measured with the sliding circle. The volumes of all thirty leaves were estimated by immersion - the mean of the estimates from three to five immersions was used. Satisfactory estimates were possible for twenty-two of the leaves, eleven from those leaves measured both ways and eleven from those measured only by electronic planimetry.

Plotting leaf volume against area showed that there was a curvilinear relationship, so the data were transformed to logarithms which gave a linear relationship, so that the regression equations

could be calculated. The equations were

(a) for area measured by electronic planimeter:

log volume = - 1.634 + 1.176 log area

(b) for area measured by sliding circle:

log volume = -2.047 + 1.424 log area.

APPENDIX 2.1

JUSTIFICATION FOR TESTING HYPOTHESES (a) AND (d) OF TABLE 2.10 BY THE SAME EXPERIMENT

If the plants remain in the same positions throughout a series of O.P.s, in each of which the same butterflies are allowed to lay eggs on them, then it is unlikely that the same plants would consistently receive more eggs unless the butterflies were responding to differences either between plants themselves or between local micro-weather conditions of the different positions in the array. If the butterflies do respond preferentially to some plants, then the favourableness of any i^{th} plant as a site for oviposition (where i can take values $1 \rightarrow t$, t being the total number of plants used in the experiment) will be a function of components from the plant itself (p_i) and its micro-environment (m_i). If the butterflies respond to eggs, there will also be a component from any eggs present (e_{ij}), where j can take either of two values, a or b, depending on whether the butterflies' responses to eggs is density dependent or not.

The three components, p_i, m_i and e_{ij} together constitute the potential of the i^{th} plant as an oviposition site. One further component contributes to change the potential to the actual frequency with which the i^{th} plant is used for oviposition (i.e. the number of eggs that plant received) - the remainder, r_i. This is the chance component of how frequently the plant or leaf is visited, of whether or not the butterfly lays an egg or more on it, and if so, of how

many she lays, and how often. Thus the number of eggs a plant receives can be expressed symbolically, as a function of these four components, as follows:

$$n_i = f[p_i, m_i, (e_{ij})] + r_i$$

where j = either a or b, and e_{ij} is put in parenthesis as it would be omitted if the butterflies do not respond to eggs, or if there are no eggs present. Probably the simplest model of the relationship between the components would be additive, i.e.,

$$n_i = p_i + m_i\ (+e_{ij}) + r_i \qquad \ldots(1)$$

Then in the experimental method described above for testing level (a) of Table 2.10, p_i and m_i would retain the same values throughout a series of O.P.s. Thus unless there was a response to eggs the differences between the number of eggs laid on the i^{th} plant in different O.P.s, would depend only on the values of r_i in those O.P.s. The smaller the total number of eggs laid on all plants in a given O.P., the larger the contribution of each plant's r_i to its n_i in that O.P. The contribution of r_i may also be relatively large if p_i and m_i tend to cancel, rather than reinforce each other. Thus if the butterflies do not respond to eggs, and if, in each of several O.P.s, the plants are ranked according to the number of eggs they receive, then if there is a significant correlation between the plants' ranks in the first and second of any three consecutive O.P.s there will not necessarily also be a significant correlation

between their ranks in the second and third O.P.s.

On the other hand, if the butterflies do respond to eggs, and if the eggs laid in one O.P. are left on the plants for the following one, the difference between the numbers of eggs laid on the i^{th} plant in these two O.P.s depends on both r_i and e_{ij}, and especially on whether j = a (i.e. the response is density dependent) or b (the response independent of density). As discussed in the main text (p.50), a density dependent response would almost certainly reflect the relative numbers of eggs on the different plants, whereas a response to eggs that is independent of density is not likely to do so.

For the k^{th} replicate, therefore,

$$e_{iak} = x(n_{i(k-1)}) = x(p_{i(k-1)} + m_{i(k-1)} + r_{i(k-1)}) \quad \ldots(2)$$

[in general, but as mentioned above, in the particular experimental method where plants remain in the same positions throughout, p_i and m_i are constants $\therefore e_{iak} = x(p_i + m_i + r_{i(k-1)})$] but $e_{ibk} \neq x(n_{i(k-1)})$ (where x is a constant). Thus if the butterflies have a density-independent response to eggs, it would be possible (just as it is if they do not respond to eggs at all) for there to be a significant rank correlation between the first two of three consecutive O.P.'s, when eggs were removed from the plants after the first O.P. before the second, and yet no significant correlation between the ranks of plants in the second and third O.P.s, although eggs laid in the second O.P. were left on the plants throughout the third O.P.

If they have a density dependent response to eggs, however, the probability that there could be a significant correlation between the plants' ranks in the first and second O.P.s (when no eggs present) yet not between the second and third O.P.'s (when eggs from the second remained for the third) is extremely low. If we call the first, second and third O.P.s (k-2), (k-1), and k, respectively, then from equation (1):

$$n_{i(k-2)} = (p_i+m_i) + r_{i(k-2)}$$

$$n_{i(k-1)} = (p_i+m_i) + r_{i(k-1)}$$

and $$n_{ik} = (p_i + m_i) + e_{iak} + r_{ik} .$$

Substituting for e_{iak} from equation (2) we get:

$$n_{ik} = \underbrace{(x+1)\ (p_i+m_i)}_{\text{constant component}} + \underbrace{x(r_{i(k-1)}) + r_{ik}}_{\text{variable component}} \quad \ldots(3)$$

It can be seen that the effect of a density dependent response to eggs is to reinforce the contribution of the constant component, and to make the variable component in O.P. k more similar to the variable component in O.P. (k-1). If there is a significant correlation between the ranks of plants in O.P.s (k-2) and (k-1), then for most plants the absolute value of the constant term in the equation for each plant will be greater than that of the variable term for each O.P.; that is, for most plants:

$$|p_i + m_i| > |r_{i(k-2)}| \text{ and } |p_i + m_i| > |r_{i(k-1)}| .$$

Then unless the total number of eggs laid on all plants in O.P. k is very much less than the total number laid in O.P. (k-1), the values of $|r_{ik}|$ are not likely to be very much greater than the values of $|r_{i(k-1)}|$; they would probably be about the same order of size so that for most of the plants $|r_{ik}|$ will also be less than $|p_i + m_i|$. Thus, as r_{ik} is the only component of the total response to any one plant in O.P. k (symbolized by equation (3)) that tends to make the plant's rank in O.P. k different from its rank in O.P. (k-1), if there is a significant correlation between the ranks of plants in O.P.s (k-2) and (k-1), there will almost certainly be a significant correlatinn between their ranks in O.P.s (k-1) and k.

APPENDIX 2.2

TABLE 1

Numbers of larvae used per plant in the experiment reported in Section 2.2.2.1

Plant No.	No. of unit larvae*	Estimates of density (unit larvae/cc)#		cm^2 per unit larvae
		Method 1	Method 2	
		REPLICATE 1		
1	5.16	0.54	0.45	55.51
3	4.30	0.92	0.69	44.12
4	3.44	0.84	0.59	48.44
6	1.72	1.02	0.68	43.08
7	2.58	0.97	0.59	52.85
12	3.50	0.89	0.64	44.13
		REPLICATE 2		
2	1.72	0.80	0.58	48.14
5	2.58	1.18	0.79	37.40
8	2.58	1.56	0.87	39.03
9	6.02	0.61	0.50	51.84
10	2.58	1.34	0.81	38.69
11	3.87	0.89	0.60	48.81
		REPLICATE 3		
2	3.52	1.54	1.06	27.22
3	7.54	2.17	1.53	18.72
4	4.28	1.16	0.80	36.32
8'	5.28	1.25	0.94	28.71
9	11.56	1.36	1.10	23.41
11	5.28	1.64	1.10	27.00
		REPLICATE 4		
1'	5.73	1.29	0.90	31.75
5	3.52	2.03	1.21	26.57
6	3.63	2.65	1.68	18.18
7	4.02	1.60	0.95	33.67
10	4.28	1.70	1.08	28.18
12'	6.46	1.80	1.25	22.98

* See pp.17+18 (Section 1.2.1.4) for definition of unit larvae

\# See pp.11+12 (Section 1.2.1.3) for discussion of methods used to estimate plant volume and hence density of larvae.

m.c.c. = 3.24 unit larvae per cc.

TABLE 2

Numbers of larvae used per plant in the experiment reported in Section 2.2.2.3

Plant No		Total leaf area (cm^2) as at 11/9	Volume Estimates (cc) as at 11/9# Method 1	Method 2	No. of unit* larvae added Rep 1 14/9	Rep 2 15/9	Rep 5 18/9	Rep 6 20/9
GROUP I	9	215.60	7.6	5.3	7.24	8.50	10.00	8.80
	11	239.69	9.7	7.2	8.00	9.50	12.00	10.56
	12	223.57	8.1	5.9	7.74	8.50	11.00	9.80
	13	285.71	10.6	8.0	8.74	9.00	14.00	12.32
					Rep 3 16/9	Rep 4 17/9	Rep 7 23/9	Rep 8 24/9
GROUP II	10	263.41	8.2	6.5	9.50	10.00	9.58	10.34
	14	261.21	9.6	7.2	9.50	10.50	11.10	10.60
	15	229.29	8.3	6.0	8.00	10.00	8.04	8.34
	16	250.16	9.6	7.1	9.00	10.50	9.34	9.32

* See pp.17+18(Section 1.2.1.4) for definition of unit larvae.

See pp.11+12(Section 1.2.1.3) for discussion of methods used to estimate plant volume.

m.c.c. = 3.24 unit larvae per cc.

APPENDIX 3.1

METHOD OF GROWING BRUSSELS SPROUTS PLANTS HYDROPONICALLY FOR THE EXPERIMENT IN SECTION 3.2.2

Each plant was grown in an individual container (~ 50-70 cc capacity) of nutrient solution. The container was wrapped in aluminium foil to prevent the entry of light and consequent growth of algae, fungi, etc. It was covered with a waxed hardboard disc, which also served to support the plant, whose stem passed through a hole in the centre of the disc. Cotton wool inside a split piece of plastic tubing was packed around the stem where it passed through the disc to prevent the entry of light via the hole. The cotton wool was changed regularly to avoid growth of fungi.

The solution was continuously aerated with a gentle stream of compressed air via a plastic tube (1mm diam) that passed through the disc to the bottom of the containers - if possible almost under the roots of the plant. The solutions in all containers were topped up every two to three days and completely renewed every two to three weeks or more frequently if the plants did not seem healthy. The solutions used were Hoagland's complete nutrient solution and a sulphur-deficient equivalent in which the only sulphur came from the $FeSO_4$ in the FeEDTA solution.

The solutions (minus the FeEDTA) were prepared in bulk every two to three weeks. The FeEDTA was prepared at least once a week

and stored in a dark bottle at 5°C. It was not mixed with the rest of the Hoaglands until immediately before the plants' solutions were replenished.

APPENDIX 3.2

LIGHT INTENSITIES IN WHICH PLANTS HAD GROWN IN PREPARATION FOR THE EXPERIMENT IN SECTION 3.2.3.1

For Replicates	High Light Treatment		Low Light Treatment	
	Lumens/sq.ft.	Approximate Duration	Lumens/sq.ft.	Approximate Duration
(a) and (b)	275→425	1 week	60→139	2 months
	∿ 225	1 week		
	∿520→780	1 month		
	∿910→936	Few days only		
	∿455→585	2 weeks		
(c) →(f)	390→585	3 weeks	60→125	3 weeks
	455→936	3 weeks	179→253	3 weeks
	260→780 (range)	5 days	100→220 (range)	
	455→650 (most)		100→125 (short ones)	∿5 weeks
			138→193 (tall ones)	
			grew 206→275	
	390→780	$3\frac{1}{2}$ weeks	so changed to	
	455→780	4→5 days	138→206	4→5 days

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