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SELECTIVE DIFFERENCES BETWEEN THE TEPHRITID FRUIT FLIES,
DACUS TRYONI AND D. NEOHUMERALIS.

BY J. H. GEE

Thesis for the degree of Doctor of Philosophy,
University of Sydney. April, 1966.

SUMMARY

Dacus tryoni and D. neohumeralis occur sympatrically along the northern and central portions of the east coast of Australia. They are distinguished by a difference in colour of the humeral calli; the calli of D. tryoni are yellow, the calli of D. neohumeralis are brown. When the two species are kept together in population cages, interspecific matings occur. Intermediate forms are produced in the F₁ generation which have both brown and yellow areas of colour on the humeral calli. In the F₂ generation, individuals are found that resemble both species as well as a continuous range of intermediate forms. The range of variation in pattern of colour of hybrid forms was divided into five categories, called colour-forms.

In laboratory populations containing the five colour-forms, the proportion of each colour-form changes with time. The purpose of this study was to analyse some of the selective differences between colour-forms to

account for changes in their relative frequencies in population cages. The approach taken was direct. Selective differences were analysed in population cages when the proportions of colour-forms were changing.

Four population cages were established with F1 hybrids from many interspecific crosses. The colour-form resembling D. tryoni, called grade 5, increased in proportion in all population cages from about 15-20% to about 90-95%. An examination of selective differences revealed that a higher proportion of grade 5 flies mated, and mated mostly with flies of their own colour-form. In addition, grade 5 females had the highest birth rate. The time when these selective differences were found correlates well with the time when the grade 5 flies increased in proportion in population cages.

Further studies of these selective differences showed that in population cages, grade 5 flies mated mostly at dusk, while the remaining colour-forms mated earlier in the day. Therefore, the number of homogamic matings by grade 5 flies was greater than expected. The high birth rate of grade 5 flies is accounted for by the high proportion of these flies that mated. The time of sexual activity of grade 5 flies was confined to a short daily period, while other colour-forms have a much longer duration of daily sexual activity. Because the time of sexual activity of many of the grade 5 flies coincides, there is a greater probability of a male locating a female willing to mate. Hence a higher proportion of grade 5 flies mated.

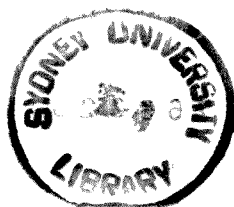
Selective differences between colour-forms in population cages were not influenced by changes in density of either adults or larvae. They were,

however, influenced by changes in the intensity of natural illumination received by the population cages and by changes in proportions of colour-forms in the population cages.

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Section 1.

INTRODUCTION

Dacus tryoni and D. neohumeralis are species of Tephritid fruit flies occurring sympatrically along the northern and central portions of the east coast of Australia. The two species are similar in their morphology but can be distinguished by a difference in colour of the humeral calli. The calli of D. tryoni are bright yellow while on D. neohumeralis they are a dark brown. A review of their distribution and abundance in Australia is given by Birch (1965) and Lewontin and Birch (in press). Gibbs (1965) completed a study of the comparative ecology and sexual isolation of the two species.

Interspecific matings occur when the two species are kept together in the same population cage in the laboratory. Bateman (1958) found that adults of the F1 generation of an interspecific cross were intermediate in pattern of colour, with both brown and yellow on the humeral calli. In addition, he found that the adults of the F2 generation were more variable in pattern of colour than the F1 hybrids. They ranged from completely brown calli (neohumeralis-like) to completely yellow calli (tryoni-like). Gibbs (1965) showed that the fecundity and survival of the F1 hybrids, when kept in the laboratory, were equal to, or greater than that of the parent species.

When laboratory populations are founded with either the two species kept together or with F1 hybrids from an interspecific cross, and kept for a number of generations, the proportion of tryoni-like flies changes relative to that of neohumeralis-like flies. Often, in such populations, one of these forms can become rare or completely eliminated.

Birch (1961) established populations of the two species together in the laboratory at 25°C. These populations were each founded with 500 adults collected from Cairns. Some populations were allowed to increase to a number limited only by the amount of food and space available in the population cages. Under these conditions, neohumeralis-like flies were favoured by selection more than tryoni-like flies.

In other populations kept by Birch (1961), the number of adults was limited to 500 as opposed to about 10,000 in the crowded populations. Tryoni-like flies proved to be superior to neohumeralis-like flies in these uncrowded densities of adults. These results were reached regardless of whether populations were initiated with 20% or 80% *D. tryoni*.

Lewontin and Birch (in press) also kept populations in the laboratory at 25°C. These populations were each founded with 300 F1 hybrids from an interspecific cross of *D. tryoni* and *D. neohumeralis* originating from stocks collected at Brisbane. The populations were provided with a consistent weekly amount of adult and larval food and kept in a limited space. The number of adults

was allowed to increase without restriction. At the end of two years, tryoni-like flies were predominant while neohumeralis-like flies were eliminated; the opposite result to that found in the crowded populations kept by Birch (1961).

A summary of the outcome of selection in populations kept at 25°C by Birch (1961) and Lewontin and Birch (in press) is given in Table 1.01.

The purpose of this study is (1) to analyse selective differences between tryoni-like, neohumeralis-like and intermediate colour-forms and so to account for their different frequencies in population cages, and (2) to account for differences in the outcome of selection in crowded populations cages kept by Birch (1961) and Lewontin and Birch (in press).

TABLE 1.01 Showing a summary of the outcome of selection in population cages kept at 25° C by Birch (1961) and Lewontin and Birch (in press). The outcome of selection in each of the five kinds of populations described was obtained in each of two replicated populations.

	Density of adults per population.	Locality of flies used.	Initial populations.	Colour-form favoured by selection.
Birch (1961)	Uncrowded (500)	Cairns	20% T; 80% N 80% T; 20% N	tryoni-like tryoni-like
	Crowded (Many thousands)	Cairns	20% T; 80% N 80% T; 20% N	neo.-like neo.-like
Lewontin and Birch (in press)	Crowded (Many thousands)	Brisbane	F1 hybrids	tryoni-like

Abbreviations: T= D. tryoni. N = D. neohumeralis.

There are two approaches that could be taken to describe the selective differences between neohumeralis-like and tryoni-like flies. One would be to rear D. tryoni and D. neohumeralis separately and compare their capacities to survive and multiply. However, the results would not be directly applicable to an understanding of the selective differences between tryoni-like and neohumeralis-like flies occurring together with intermediate forms. This is because Levene, Pavlovsky, and Dobzhansky (1954 and 1958) have shown with Drosophila that the capacity for increase of a particular genetic form can be influenced by the number and proportion of other genetic forms coexisting in the same population.

The approach I chose was more direct but one that is not commonly used in such studies. I measured some of the selective differences between neohumeralis-like and tryoni-like flies when they occurred together in population cages while their relative frequencies were changing in response to selection. The main advantage to this approach is that any selective difference found between different forms can be used directly to explain the change in proportion of the different forms in the population concerned.

The limitations of this approach are: (1) analyses of selective differences must not interfere with the births and deaths of the different forms in the population, (2) the change in proportions of forms could occur before there was sufficient time to measure the selective differences.

In this thesis I used the term colour-form to refer to arbitrary divisions or categories of the complete range in variation of pattern of colour of the humeral calli. Only adults of hybrid ancestry were classified by this system. It was not used to refer to either D. tryoni or D. neohumeralis.

Section 2.

PLAN OF EXPERIMENTS.Population cages containing the different colour-forms.

Differences between the results of Birch (1961) and Lewontin and Birch (in press) in the outcome of selection in crowded populations at 25°C could be explained on the basis of (1) the differences in locality from which the founders of the populations originated, or (2) the differences in the way the populations were established and maintained.

I established and maintained populations with adults that originated from Cairns and from Brisbane in a manner similar to that described by Lewontin and Birch (in press). If the tryoni-like forms were superior to the neohumeralis-like forms in the populations originating from Brisbane and the reverse was found in the populations originating from Cairns then (1) would be the most likely explanation. However, if tryoni-like forms were superior independently of locality, then (2) could be the accepted explanation.

Four population cages were established in which changes in the proportions of colour-forms could be recorded and selective differences between colour-forms measured. Two population cages were founded with adults originating from stocks collected at Brisbane; these were called B1 and B2. The remaining two population cages were founded with adults originating from Cairns and were called C1 and C2. The adults used to initiate the population cages were the F1 generation of many interspecific crosses of D. tryoni and D. neohumeralis.

Stocks of both species from Cairns and Brisbane had been kept in the laboratory for two to three generations before the crosses were made. A description of the methods used to collect these stocks and the procedures of keeping them in the laboratory are given in Appendix A.

Each population cage consisted of a rectangular wire frame, 45.5 x 33.0 x 33.0 cm, surrounded by a tightly-fitting nylon cover which could be opened at one end.

The population cages were kept at 25°C ($\pm 1^\circ$), and at 70% relative humidity ($\pm 15\%$). The cages were placed on a shelf against a window. A bank of louvres prevented direct sunlight from falling on the population cages. Additional illumination, from 4:30 am to one half hour before dusk, was provided by two "Consolite 5000" fluorescent tubes located above the population cages.

Procedure of keeping the population cages. Adults in the population cages were provided once each week with a consistent ample amount of water, sucrose, and protein hydrolysate of yeast. Eggs, taken each week from the population cages, were placed on 1,000 ml of medium containing the following ingredients per 100 ml:

Water	94.5 ml
Dried carrot (powdered)	13.5 gm
Dried yeast (Torafood)	4.5 gm
Fungicide (Nipagen)	0.2 gm
Concentrated hydrochloric acid	0.6 ml

Both eggs and larvae were reared outside the population cages.

Eggs were collected from the population cages twice each week; Monday and Wednesday. A hollow dome of apple skin, into which females could oviposit, was placed into each population cage for a daily eight hour period. These apple domes, 4 cm in diameter, were perforated with many pin-pricks and secured around the circumference to a watch glass with melted paraffin wax.

Eggs were removed from the undersurface of the apple dome with a moist brush. They were divided into 10 batches, each of a similar number, and each batch was placed on a square of filter paper. Each square of filter paper was then placed on the surface of 50 ml of medium contained in a plastic cup. Thus the daily collection of eggs from each population cage was placed on 500 ml of medium. Ten containers were used because on several occasions 10 independent samples from each population cage were required to estimate the survival of larvae and the mean weight per pupa.

Each group of 10 cups was kept in a perspex container. The bottoms of these containers were covered with sawdust. Mature larvae leave the medium 8 to 12 days after hatching to pupate in the sawdust. Pupae were sifted from the sawdust 14 to 17 days after hatching, placed in plastic trays, and put into the appropriate population cages. The adults emerged 2 to 5 days later.

Information collected from the population cages.

The density of adults and larvae in the population cages was allowed to increase to the limits that the weekly amount of food and the restricted space would permit. Estimates were made each week of the number of adults in the population cages and the density of larvae. The reason for making these estimates was that I intended to determine if either the density of adults or larvae influenced any of the selective differences that I might find.

To determine the success or superiority of the colour-forms in the population cages, as well as the outcome of selection, estimates were made at regular intervals of the proportions of colour-forms in the population cages.

The most important examinations carried out within the population cages were measurements of the selective differences between colour-forms. These were made repeatedly during the early history of the population cages. The reason for repeating these measurements was because the density of adults and larvae and the proportions of colour-forms in the population cages changed considerably, possibly influencing selective differences between colour-forms. Survival of larvae, frequency of homogamic matings by tryoni-like and neohumeralis-like flies, proportions that mate, and rates of birth of the colour-forms were examined to determine if selective differences existed. These measurements were made to account for the change in proportions of colour-forms in the population cages. Time did not permit a complete examination of all

the components of the capacity for increase. Longevity of adults and speed of development were not analysed for selective differences between colour-forms.

Additional experiments. Experiments to determine the influence of changes of particular components of the environment on the selective differences between colour-forms in the population cages were completed using D. tryoni, D. neohumeralis and hybrids from interspecific crosses. These experiments did not involve individuals from the population cages. Details of these experiments are given in the appropriate sections.

Section 3. WEEKLY ESTIMATES OF DENSITY OF FIRST
INSTAR LARVAE AND NUMBER OF ADULTS IN
POPULATION CAGES.

Introduction. This section describes weekly estimates of density of larvae for weeks 1 to 55 and numbers of adults in the population cages from weeks 1 to 31. The reason for making these estimates was because I intended to determine if any of the selective differences between colour-forms were influenced by changes in density of larvae or adults in the history of the population cages.

Materials and methods. The following procedures were carried out weekly on each of the four population cages.

To determine the density of first instar larvae, it was first necessary to estimate the number of eggs laid. This was done after the eggs were removed from the apple dome. Eggs were drawn up into a syringe and then ejected a drop at a time. Knowing that there were about 1,000 eggs per drop and the total number of drops, the total number of eggs was estimated. By determining the percentage hatch of a sample of 200 eggs and multiplying it by the total number of eggs, the number of viable eggs or first instar larvae was estimated.

Eggs were collected twice each week from the population cages and each day's collection was placed on the surface of 500 ml of medium. Differences in the number of eggs between the first and

second collection each week resulted in different densities of larvae. However the number of eggs in the two collections were usually similar (Appendix B). To obtain a weekly measure of the density of larvae, the total number of viable eggs produced each week was divided by the total volume of medium used (1,000 ml).

Each week the number of adults present in each population cage was estimated by adding the number of newly emerged adults to those present and subtracting the number of deaths and escapes.

Numbers of newly emerged adults were determined by first estimating the total number of pupae (the total weight of all pupae was divided by the mean weight per pupa, the latter being obtained by weighing a sample of 200 pupae) and multiplying it by the percentage survival obtained from a sample of 200 pupae.

The number of deaths each week was estimated by dividing the total weight of all dead flies removed from the population cages by the mean weight per dead fly (obtained by weighing a sample of 100 dead flies).

The number of escapes from each population cage was set at 200 per week when there were less than 5,000 adults in the population cage, and 400 per week when this number was exceeded. These numbers were based on several counts. The escapes were mostly dead flies adhering to the food and water containers that were lost when these containers were removed each week and replaced with fresh amounts of food and water.

Weekly estimates of the density of first instar larvae

per ml of medium. During the first 15 weeks, the mean density of first instar larvae per ml of medium did not exceed 3.4 in the Brisbane population cages and 7.5 in the Cairns population cages (Figure 3.01). From week 16 to 23, the mean density of larvae increased and the following maxima were reached in the population cages:

B1	20.3	larvae	per	ml.
B2	15.7	"	"	"
C1	39.4	"	"	"
C2	38.5	"	"	"

Following week 23, the mean density of larvae fluctuated between 3.0 and 39.0 larvae per ml of medium.

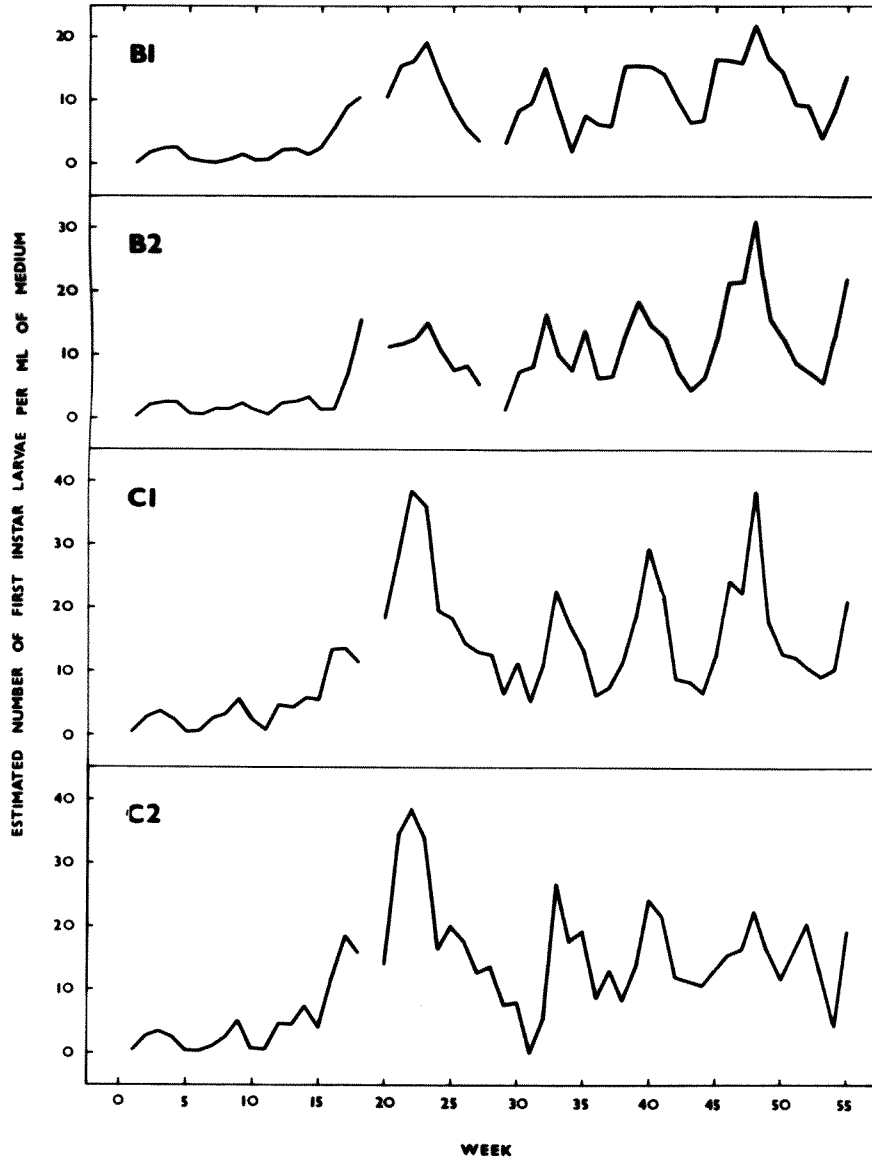
Numbers of adults in the population cages.

In week 30 there was a high mortality of adults in population cage C2. The number surviving was estimated to be about 2,000. By adding the estimated number of newly emerged adults in the following week to this figure I obtained the number of adults in population cage C2 for week 31. The number of adults in C2 was then similar to the numbers in the remaining population cages. The number of adults in each of the remaining population cages could be estimated by visually comparing each population cage to C2.

A second estimate of the number of adults in each population cage in week 31 was made by summing the estimated numbers of newly

FIGURE 3.01

Weekly estimates of the number of first instar larvae per ml. of medium for each population cage; weeks 1 to 55. Eggs were not collected from any of the population cages in week 18, nor from the Brisbane population cages in week 28.



emerged flies from weeks 1 to 31 and then subtracting the estimated deaths and escapes for the same period. The balance was expected to equal the numbers of adults in the population cages in week 31. This was done and the figures are given in Table 3.01.

Table 3.01 gives the two estimates of the number of adults present in each population cage in week 31. It shows that the number of adults estimated by the latter method exceeded the more reliable number estimated by visual observation. The difference between the two estimates was expressed as a percentage of the total number of newly emerged flies for weeks 1 to 31 (Table 3.01). This percentage, calculated for each population cage, varied between 13.5% and 15.4% indicating that a consistent error had been made in the weekly estimates of either the number of newly emerged adults, deaths, or escapes. To allow for this error in the weekly estimates of the numbers of adults in the population cages, the number of newly emerged adults was reduced by this percentage error. The weekly calculations are given in Appendix C.

Figure 3.02 shows the estimated numbers of adults in the four population cages for weeks 1 to 31. All populations increased in number, with some fluctuations, until weeks 28-29, when the following estimated maximum numbers were reached:

B1 - 9,119

B2 - 9,742

C1 -19,610

C2 -11,881

TABLE 3.01 Showing the two estimates of the numbers of adults present in the population cages in week 31.

Population cage	B1	B2	C1	C2
Estimated number of newly emerged adults; weeks 1-31.	72,785	77,422	144,756	142,795
Estimated number of adult deaths; weeks 1-31.	52,039	54,768	100,888	100,032
Estimated number of escapes; weeks 1-31.	6,860	7,060	8,460	8,060
Estimated number in population cage; week 31.	13,886	15,594	35,408	34,703
Estimated number in population cage by visual observation; Week 31.	4,000	4,000	15,000	12,750
Difference between estimates.	9,886	11,594	20,408	21,953
Difference between estimates as a per cent of the estimated number of newly emerged adults; weeks 1-31.	13.5%	15.0%	14.1%	15.4%

FIGURE 3.02

Weekly estimates of the number of adults in each population cage.

A. Brisbane population cages.

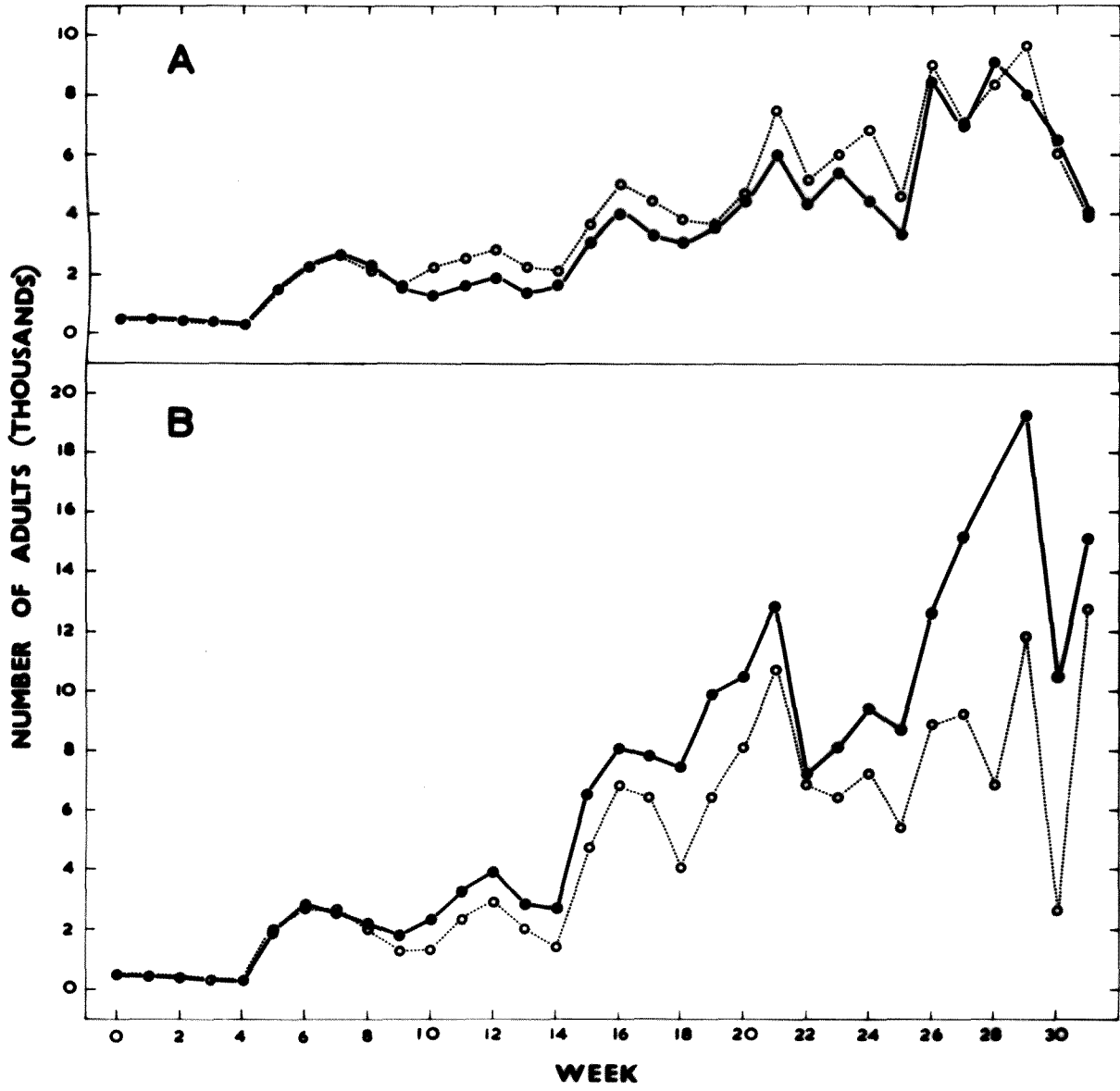
solid circles - B1

hollow circles - B2

B. Cairns population cages.

solid circles - C1

hollow circles - C2



The Cairns population cages contained a greater number of adults than the Brisbane population cages following week 15. An unusually high mortality of adults in week 28 prevented the numbers in population cage C2 from reaching a similar maximum as population cage C1.

Estimates of the numbers of adults in the population cages from weeks 31 to 55 were made but are not given because of the error found in the method. However, I estimated that between weeks 32 to 55 the numbers in the Brisbane population cages fluctuated between 2,000 and 16,000 adults and in the Cairns population cages between 5,000 and 19,000 adults.

Section 4. CHANGES IN PROPORTIONS OF COLOUR-FORMS
IN POPULATION CAGES.

Introduction. This section shows how the range of variation in pattern of colour of the humeral callus was divided into colour-forms and how the proportions of colour-forms changed in each population cage.

The change in proportion of each colour-form was recorded to determine the time in the history of the population cages when selection occurred, as well as the final outcome of selection.

Classification of colour-forms. The range of variation in pattern of colour of the humeral callus was divided into five colour-forms based on a system used by Gibbs (1965) and Lewontin and Birch (in press). The five colour-forms are illustrated in Figure 4.01 A.

Humeral calli of grade 0 flies are completely brown and occasionally contain grey blotches. These adults resemble D. neohumeralis.

Flies called grades 1, 2, and 3, by Gibbs (1965) and Lewontin and Birch (in press) were combined into a single colour-form called grades 1-3. These flies each have a distinct yellow mark on the humeral callus, the area of which does not exceed $\frac{1}{2}$ the total area of the callus.

FIGURE 4.01

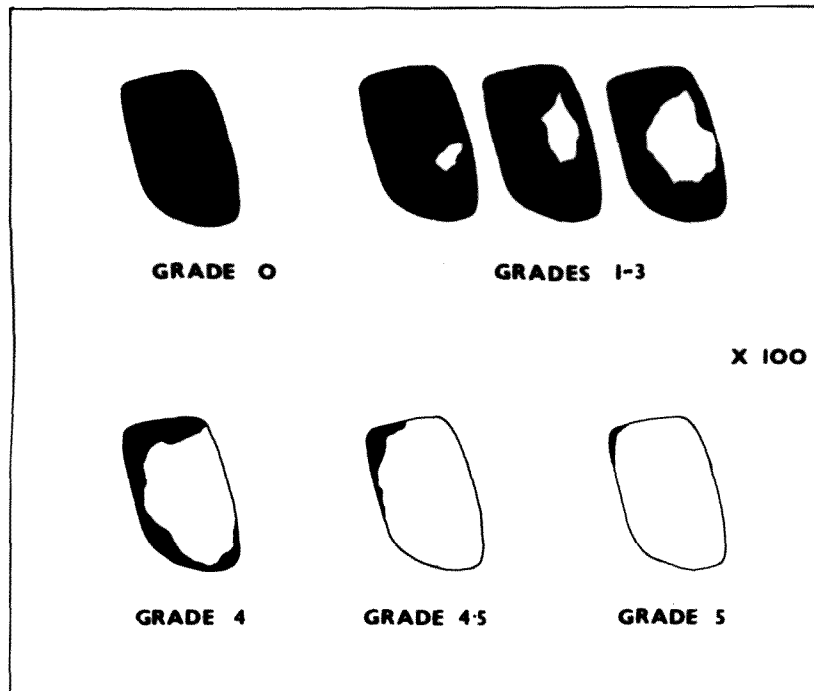
Examples of the pattern of colour on the humeral calli of the five colour-forms.

A. Diagrams of the right humeral callus, dorsal view.

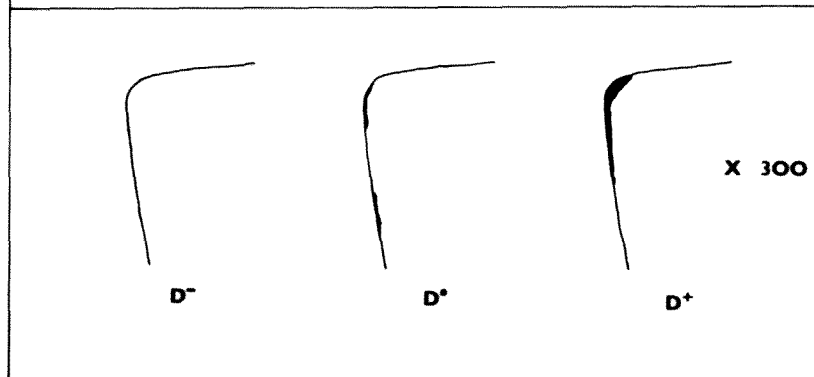
This classification was based on that used and illustrated in detail by Gibbs (1965).

B. Diagrams of the antero-dorsal corner of the right humeral callus showing examples of the three subdivisions of the grade 5 category, based on a classification described by Wolda (personal communication).

A



B



Grade 4 flies have a larger portion of yellow which is between $\frac{1}{2}$ to $\frac{2}{3}$ of the total area of the humeral callus. The area of brown colour usually extends around the full circumference of the callus but occasionally individuals were found with all the brown colour located on the anterior portion of the callus. Gibbs (1965) found that 65.9% of the offspring of 18 interspecific crosses between D. neohumeralis and D. tryoni were of this colour-form.

Grade 4.5 flies have a small portion of brown in the antero-dorsal corner of the humeral callus, less than $\frac{1}{4}$ of the area of the callus.

Flies whose humeral calli are completely yellow or contain only a minute brown mark, usually in the antero-dorsal corner, are classified as grade 5. These adults resemble D. tryoni.

On one occasion a second system of classification was used to divide grade 5 flies into an additional three categories. This system was used by Wolda (personal communication). Figure 4.01B illustrates the three categories. D^- flies have no brown markings on the dorsal side of the humeral callus. D^0 flies have a minute trace of brown along a small portion of the dorsal side of the humeral callus, usually in the antero-dorsal corner. This brown trace is enlarged into a distinct but minute brown mark in the D^+ flies.

Sampling of adults from the population cages. To determine the proportions of colour-forms in the population cages, samples of adults were removed at random, etherized, and then examined under a

binocular microscope. The colour-form and sex were recorded. All adults were returned to their population cages after examination. The first sample was taken in week 7, and subsequent samples were taken every two weeks until week 15, every four weeks until week 59, and every eight weeks until week 99.

When the number of adults in a population cage was estimated to be less than 5,000, a sample of 400 to 600 adults was examined. A sample of 1,000 flies was examined when the numbers of adults in the population cage was estimated to exceed 5,000.

Changes in proportions of colour-forms in population cages.

Rates of change in proportion of each colour-form in the Brisbane population cages are shown in Figure 4.02 and in the Cairns population cages in Figure 4.03.

The proportion of grades 0, 1-3, and 4 decreased in all population cages following week 7. Selection against these forms occurred more rapidly in the Cairns population cages.

Between weeks 7 and 15, the grade 4.5 adults increased in proportion in all population cages. During this same interval the proportion of grade 5 flies in the Brisbane population cages showed little change, while in the Cairns population cages grade 5 flies showed a slight increase in proportion.

After week 15 there was a marked change in the trend of selection in all population cages. Grade 5 flies increased in proportion while

FIGURE 4.02

Changes in the proportion of each colour-form in the
Brisbane population cages; weeks 7 to 99.

solid circles - B1

hollow circles - B2

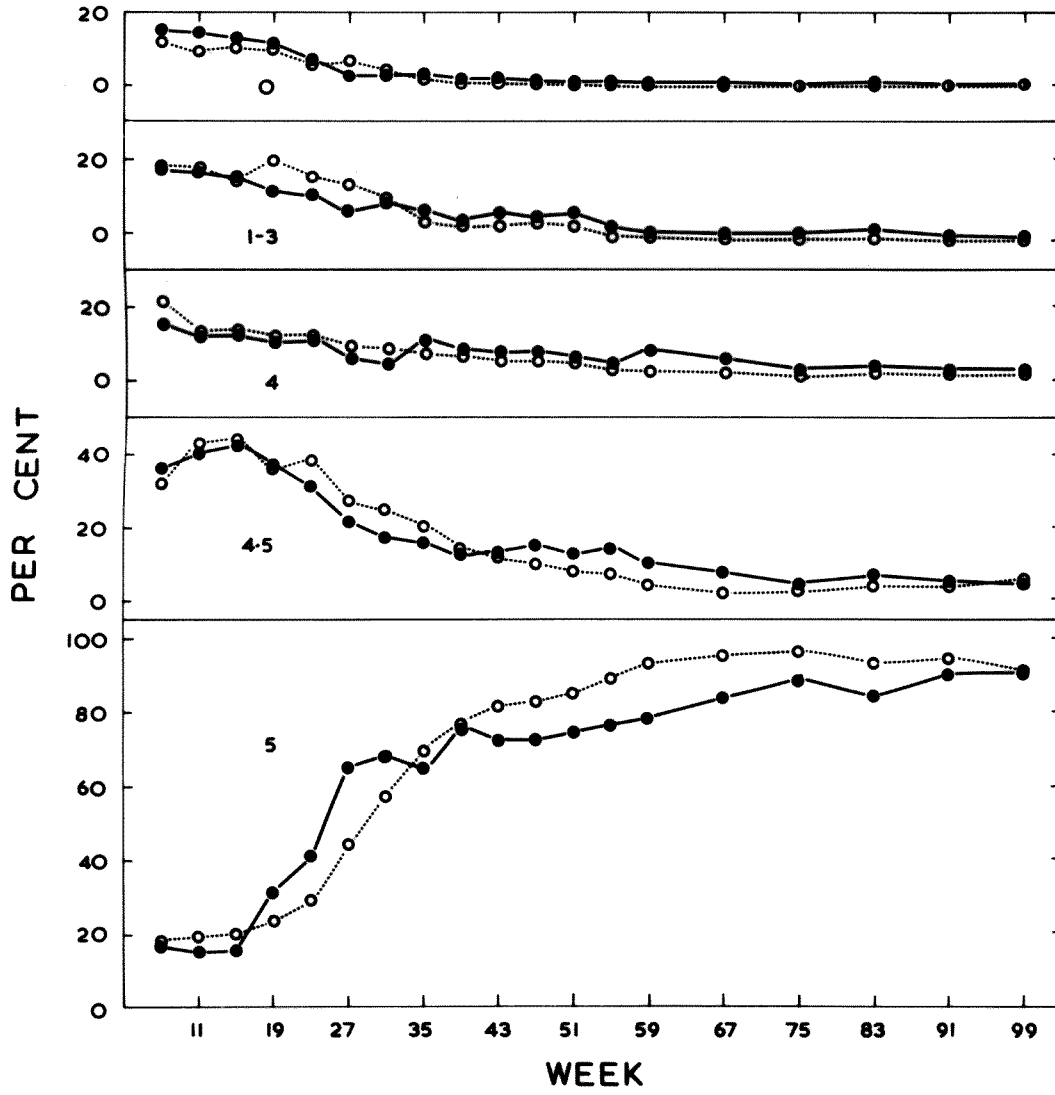
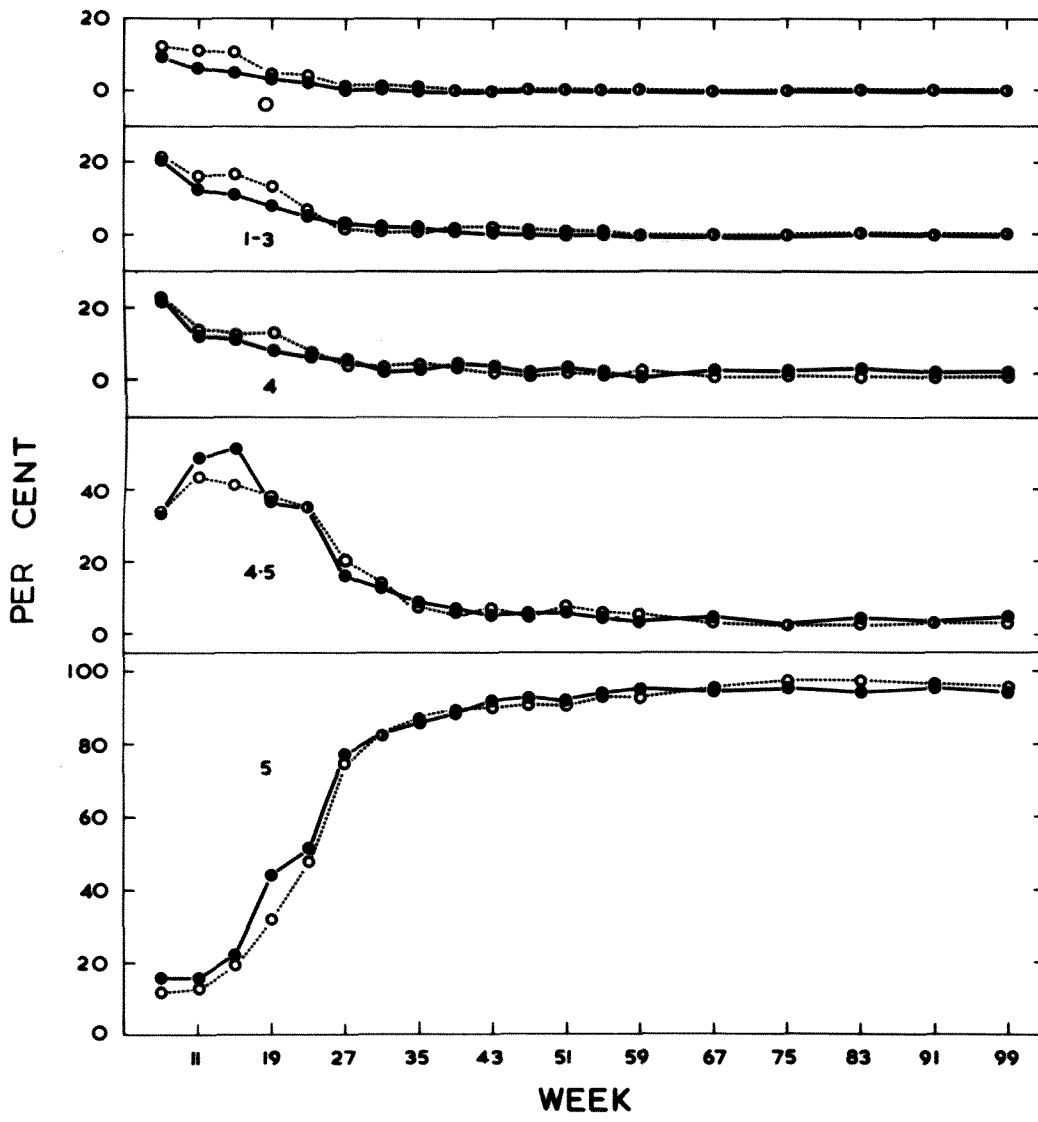


FIGURE 4.03

Changes in the proportion of each colour-form in the Cairns population cages; weeks 7 to 99.

solid circles - C1

hollow circles - C2



the remaining colour-forms decreased. Grade 5 flies increased in proportion in population cages C1 and C2 from an estimated 22.2% and 19.0% to an estimated 76.6% and 73.3% during a 12 week period from weeks 15 to 27. Selection for this colour-form in the Brisbane population cages was not as rapid. Grade 5 flies took twice the time to reach a similar proportion of the population. Increases in proportion from an estimated 16.0% and 20.2% to 75.6% and 75.5% occurred in population cages B1 and B2 over a 24 week period from weeks 15 to 39.

The increase in proportion of grade 5 flies and the decrease in proportion of the remaining colour-forms continued until week 67 in the Cairns population cages and until week 75 in the Brisbane population cages. At these times the proportion of each colour-form became relatively stable and 90 to 95% of the adults in the population cages were classified as grade 5 flies.

Table 4.01 shows a comparison of the proportion of each colour-form in samples taken from the population cages in week 7 and week 99.

Of the grade 5 flies present in the population cages in week 99, the majority had some minute brown markings on the humeral calli. The proportions of the three types of grade 5 flies as classified by Wolda, is shown in Table 4.02.

The number of each colour-form found in the regular sampling of adults from the population cages is given in Appendix D.

TABLE 4.01 Comparison of the proportion of each colour-form in each population cage in week 7 with the proportion found in week 99.

Week	Colour-form	Percentage			
		B1	B2	C1	C2
Week 7	0	14.8	10.8	9.3	12.2
	1-3	17.4	18.2	21.0	21.3
	4	15.5	21.5	21.7	22.5
	4.5	35.7	31.5	32.3	32.5
	5	16.7	18.0	15.5	11.5
Week 99	0	0.1	0.1	0.0	0.1
	1-3	0.6	0.4	0.4	0.2
	4	3.3	1.8	1.5	1.2
	4.5	5.9	6.7	5.0	3.6
	5	90.1	91.0	93.1	94.9

TABLE 4.02 Showing the percentage of each of the three types of grade 5 flies classified by Wolda's system (Wolda, personal communication); from a sample of 150 grade 5 flies from each population cage in week 99.

Population cage	Percentage		
	D ⁻ (no brown)	D ⁰ (brown trace)	D ⁺ (brown mark)
B1	13.3	33.3	53.3
B2	15.3	43.3	41.3
C1	22.0	46.0	32.0
C2	28.0	48.0	24.0

Section 5. SELECTIVE DIFFERENCES BETWEEN COLOUR-FORMS
 IN POPULATION CAGES.

5.0 INTRODUCTION.

I have shown in Section 4 that grade 5 flies increased in proportion from less than 20% to more than 90% of the adults in each of four population cages. The aim of this section is to describe the selective differences between the colour-forms in the population cages and to account for observed changes in their relative frequencies.

Selective differences between colour-forms were examined in survival of larvae, proportions that mate, frequency of homogamic matings, and rates of birth. These examinations were repeated several times during the history of the population cages when the density of adults and larvae was fluctuating and the proportions of colour-forms was changing. This was done because it was possible that some selective differences may have been dependent upon a particular density of adults or larvae, or a particular proportions of colour-forms in the population cage.

5.1 SURVIVAL OF LARVAE.

5.10 Introduction.

A selective mortality of larvae would have a direct influence upon the proportion of colour-forms in the population cages. The object of this section is to determine if there were differences in the survival of larvae between colour-forms in the population cages.

To determine if differential survival of larvae occurred, the following comparison was made. A small proportion of a day's collection of eggs from each population cage was reared at an optimal density for survival of larvae. The remaining eggs were reared in crowded densities in the normal procedure of maintaining the population cages, as outlined in Section 2. It was assumed that there was no selective mortality of larvae in the optimal density. Therefore, any significant difference found on comparison of the ratio of the number of each colour-form from larvae reared in an optimal density with the ratio from a crowded density would be due to differential survival of larvae.

The hypothesis I tested was that differential survival of larvae occurred between colour-forms in the crowded densities of larvae encountered in the population cages.

5.11 Materials and Methods.

An optimal density for rearing larvae was determined in experiments conducted before the population cages were established. These experiments are described in Appendix E.

To examine differences in the frequency of adult colour-forms from larvae reared in optimal and in crowded densities, the following procedure was carried out on a day's collection of eggs from each population cage. The total number of eggs was estimated. Ten samples of either 60 or 80 eggs, depending on the expected hatch, were counted out. Each sample at this optimal density was placed on a square of filter paper and put on the surface of 50 ml of medium in a plastic cup. The remaining eggs, numbering in the thousands, were divided into 10 batches, each of a similar number. Each batch at this crowded density was placed on 50 ml of medium in a plastic cup. Thus, there were 10 cups each containing a known number of eggs at an optimal density and 10 cups containing an estimated number of eggs at a crowded density.

The cups were kept in perspex cages. On the third day after the eggs were laid, the number of unhatched eggs was recorded for each cup containing larvae at the optimal density. By subtracting the number of unhatched eggs from the total number of eggs on the filter paper, the number of first instar larvae in each cup was obtained. Numbers of first instar larvae in each cup at the crowded density were estimated by multiplying the mean percentage hatch of eggs on the cups at the optimal density by the estimated

number of eggs on each cup at the crowded density.

Once the number of first instar larvae had been determined, each cup was placed into a carton with a layer of sawdust on the bottom. The cartons were covered with a gauze lid. Two weeks later, when all the larvae had left the medium to pupate in the sawdust, the cups were removed from the cartons, the sawdust was sifted, and pupae were counted and weighed. All pupae from cups at the optimal density and 50 pupae from each cup at the crowded density were returned to their cartons and covered with sawdust. The remaining pupae from cups at the crowded density were returned to their population cages.

A supply of food and water was placed into each carton. After emergence, adults were kept in cartons until they were classified. Following classification, adults from larvae reared in optimal densities were destroyed, while those from larvae reared in crowded densities were returned to their population cages.

5.12 Comparison of the ratio of the number of each colour-form from larvae reared in an optimal density with the ratio from the crowded densities of population cages.

The ratio of the number of each colour-form from larvae reared at the optimal density was compared with the ratio from a crowded density in a 2 x 5 contingency table. If the total Chi-square from this table were significant, then the two ratios would differ by more than chance and differential mortality between colour-forms

would be indicated in the crowded density.

A limiting feature in the use of contingency tables is that cells with small expected numbers can give unreliable information. To overcome this difficulty, I followed the general rule given by Simpson, Roe, and Lewontin (1960). It states that categories should be lumped when either one cell has an expected number of less than 1 or when more than 20% of the cells have expected numbers of less than 5.

Examinations for selective mortality of larvae between colour-forms in population cages were made during weeks 3, 18, and 22.

Week 3. Table 5.01 shows that in week 3, the density of larvae varied between 2.6 and 3.6 first instar larvae per ml. of medium in the crowded densities. Optimal densities were established at 0.9 first instar larvae per ml of medium. Larvae from the optimal density formed significantly heavier pupae than larvae reared in crowded densities.

Table 5.02 shows that in week 3 in the Brisbane population cages, the ratio of the number of each colour-form from larvae reared in the crowded densities did not differ by more than chance from the ratio of each colour-form from the optimal density. However, for each of the Cairns population cages, the two ratios differed by more than chance ($P < .005$). The percentage of grade 5 flies in the ratio from the crowded density of larvae exceeded the percentage of grade

TABLE 5.01 Mean density of first instar larvae per ml of medium, mean survival of larvae, and mean weight per pupa in both crowded and optimal densities. The figures for survival of larvae and mean weight per pupa in week 3 are the mean of five replicates. The remaining figures are the means of 10 replicates. Differences in the mean survival of larvae and the mean weight per pupa between optimal and crowded densities were compared with the exception of the survival of larvae in week 3. All differences were significant ($P < .05$) except those indicated as ns.

		Density of larvae.	Mean density first instar larvae/ml of med.	Mean per cent survival of larvae.	Mean weight /pupa (mg).
WEEK 3	B1	Crowded	3.0	-	10.5
		Optimal	0.9	77.5	11.7
	B2	Crowded	2.6	-	10.4
		Optimal	0.9	88.2	11.4
	C1	Crowded	3.6	-	9.7
		Optimal	0.9	84.9	12.2
	C2	Crowded	3.5	-	10.7
		Optimal	0.9	81.3	12.0
WEEK 18	B1	Crowded	9.5	60.8	8.4
		Optimal	1.0	79.9	11.5
	B2	Crowded	13.9	62.7	6.5
		Optimal	1.1	85.5	11.4
	C1	Crowded	15.9	68.0	5.6
		Optimal	1.2	82.8	11.2
	C2	Crowded	15.9	64.5	6.1
		Optimal	1.1	88.0	11.5
WEEK 22	B1	Crowded	15.1	77.3	7.1
		Optimal	1.2	76.4 ^{ns}	12.2
	B2	Crowded	11.5	84.6	8.0
		Optimal	1.1	85.3 ^{ns}	12.0
	C1	Crowded	39.7	43.7	4.1
		Optimal	1.3	80.4	12.4
	C2	Crowded	40.3	46.6	4.1
		Optimal	1.3	82.0	12.2

TABLE 5.02 A comparison of the percentage of each colour-form from larvae reared in crowded densities with those from larvae reared in the optimal density. The comparisons are for each population cage on eggs laid during weeks 3, 18, and 22. The total Chi-square values are from contingency tables calculated on the number of each colour-form, as given in Appendix F. Abbreviation: ns = not significant ($P > .05$).

		Density of larvae	Percentage					Total Chi-square.	P
			0	1-3	4	4.5	5		
WEEK 3	B1	Crowded	13.9	18.0	17.5	39.2	11.3	5.69	ns
		Optimal	9.6	18.9	19.4	36.9	15.3		
	B2	Crowded	10.6	22.2	25.0	30.7	11.3	5.84	ns
		Optimal	11.4	22.3	19.2	37.0	10.0		
	C1	Crowded	12.7	24.4	18.1	35.8	8.8	16.21	< .005
		Optimal	11.7	23.4	25.0	37.3	2.5		
	C2	Crowded	10.9	19.7	26.4	32.0	10.9	19.21	< .005
		Optimal	10.2	26.8	27.4	32.8	3.0		
WEEK 18	B1	Crowded	6.2	13.4	7.4	35.5	37.6	3.25	ns
		Optimal	8.2	12.8	9.5	31.5	38.1		
	B2	Crowded	8.4	13.3	10.3	37.5	30.4	12.18	< .025
		Optimal	6.0	16.9	13.1	42.6	21.4		
	C1	Crowded	1.0	5.8	6.3	38.8	48.1	5.65	ns
		Optimal	1.7	5.7	9.2	41.8	41.6		
	C2	Crowded	3.7	5.0	7.4	40.7	43.2	6.96	ns
		Optimal	1.5	6.8	9.9	42.0	39.7		
WEEK 22	B1	Crowded	3.8	5.1	7.1	26.6	57.4	2.82	ns
		Optimal	3.4	6.5	5.5	23.6	60.9		
	B2	Crowded	8.7	13.6	13.2	31.9	32.6	2.13	ns
		Optimal	11.1	14.5	11.0	31.5	31.8		
	C1	Crowded	0.0	1.0	2.7	23.0	73.4	9.54	< .05
		Optimal	0.6	2.3	4.3	18.7	74.0		
	C2	Crowded	1.0	3.7	3.9	25.1	66.2	6.74	ns
		Optimal	1.1	3.2	7.8	25.9	62.1		

5 flies in the ratio from the optimal density in both Cairns population cages. This difference made the greatest contribution to the total Chi-square in the contingency tables for the Cairns population cages.

The contingency tables for each population cage are given in Appendix F.

Week 18. At this time in the population cages, large numbers of viable eggs were produced resulting in crowded densities of between 9.5 and 15.9 first instar larvae per ml of medium. The survival of larvae at these crowded densities was lower than at the optimal density (Table 5.01). In addition, pupae reared from larvae at the optimal density were significantly heavier than those reared from larvae reared in the crowded densities.

Table 5.02 shows that in week 18, in population cage B2, the ratio of the number of each colour-form from larvae reared in the optimal density differed significantly from the ratio of colour-forms from larvae reared in the crowded density. The percentage of grade 5 flies from the crowded density was higher than the percentage of grade 5 flies from the optimal density. This difference made the greatest contribution to the total Chi-square.

In the remaining three population cages, the ratio of the number of each colour-form from larvae reared in the optimal density did not deviate significantly from the ratio of colour-forms from the crowded densities (Table 5.02).

Week 22. Estimated crowded densities of first instar larvae per ml of medium of 11.5 and 15.1 were found in the Brisbane population cages, and 39.7 and 40.3 in the Cairns population cages

(Table 5.01). These latter densities were among the highest recorded in any population cage during weeks 1 to 55. The survival of larvae in the Brisbane population cages was similar in optimal and crowded densities. However, the percentage survival of larvae in the crowded densities of the Cairns population cages was almost half that found in the optimal density. In all population cages, the mean weight per pupa from larvae reared in the optimal density was significantly heavier than that from larvae reared in the crowded densities (Table 5.01).

Table 5.02 shows that in population cage C1, in week 22, the ratio of the number of each colour-form from larvae in the optimal density deviated significantly from the ratio of colour-forms from the crowded density ($P < .05$). There were no significant deviations in the comparisons for the remaining population cages.

In summary, the ratio of the number of each colour-form from larvae reared at an optimal density occasionally deviated significantly from the ratio of colour-forms from crowded densities. Such differences occurred in population cages C1 and C2 in week 3, in B2 in week 18, and in C1 in week 22. Before drawing any conclusions from these results, the survival of larvae from the Cairns population cages in week 3 was re-examined.

5.13 A further examination of survival of larvae from the Cairns population cages during week 3.

It will be recalled that the population cages were founded with an F1 generation of many interspecific crosses between D. neohumeralis and D. tryoni. Thus, the eggs laid in week 3 would be the F2 generation of these interspecific crosses. Bateman (1958) found that 11.7% of the adults in the F2 generation of an interspecific cross of the two species collected at Cairns were grade 5 flies. Similarly, Gibbs (1965) found that 12.0% of the flies in an F2 generation from an interspecific cross were grade 5 flies. The two species in the latter example were collected at Rockhampton. Both workers reared the F2 generation in uncrowded densities as larvae.

Percentages of grade 5 flies similar to those described by Bateman (1958) and Gibbs (1965) were found in the F2 generation in week 3 when larvae were reared in optimal and crowded densities in the Brisbane population cages, and in the crowded densities in the Cairns population cages. But the proportion of grade 5 flies from larvae reared in the optimal densities in the Cairns population cages was less than that in the crowded densities and therefore less than that obtained by Bateman (1958) and Gibbs (1965). Therefore the frequency of grade 5 flies in the Cairns population cages was reduced when the larvae were reared in the optimal density.

There are two explanations to account for the reduced frequency of grade 5 flies. (1) There is a selective mortality in the larval stage against grade 5 flies from Cairns. This is dependent upon an optimal density of larvae and a low proportion of grade 5 flies (10 to 15%) in the population. (2) An experimental error occurred decreasing the frequency of grade 5 flies.

To determine which of the above explanations was most likely to be correct, I repeated the comparison by rearing larvae from an F2 generation of an interspecific cross in optimal and crowded densities. If an experimental error occurred in week 3, it was unlikely to occur again.

Eggs of an F2 generation of an interspecific cross between D. tryoni and D. neohumeralis were obtained. Some were placed on 10 cups of medium at the optimal density while others were placed on an additional 10 cups of medium at a crowded density similar to that found in the Cairns population cages in week 3. The procedure used to rear the larvae to adults was similar to that described in Section 5.11.

The mean density of larvae, survival of larvae, and mean weight per pupa from crowded and optimal densities are given in Table 5.03. These figures are similar to the corresponding data for population cages C1 and C2 in week 3. Pupae, reared from the optimal density as larvae were significantly heavier than those from the crowded density. Table 5.04 shows that the ratio of the number of each colour-form from larvae reared in the two densities did not differ

TABLE 5.03 A comparison of the mean density of first instar larvae per ml of medium, mean survival of larvae, and mean weight per pupa for larvae reared in the optimal density with those reared in crowded densities. The larvae are the F2 generation of an interspecific cross between D. tryoni and D. neohumeralis. Each figure is the mean of 10 replicates.

Density of larvae.	Mean density first instar larvae/ml of medium.	Mean survival of larvae.	Mean weight /pupa (mg).
Crowded	3.4	79.3% ns	11.5 (P<.001)
Optimal	1.1	79.4	12.4

TABLE 5.04 A comparison of the percentage of each colour-form from larvae reared in a crowded density with that from larvae reared in the optimal density. The total Chi-square is from a contingency table given in Appendix G. Abbreviation: ns = not significant ($P > .05$)

Density of larvae	Percentage					Total Chi-square	P
	0	1-3	4	4.5	5		
Crowded	10.7	12.5	18.1	37.4	21.3	3.21	ns
Optimal	10.1	15.4	17.6	38.3	18.6		

from each other by more than chance. This is opposite to the result obtained in week 3 in the Cairns population cages. Therefore the cause of the reduced frequency of grade 5 flies reared from larvae in the optimal density in week 3 in the Cairns population cages was due to an experimental error. I also concluded that the significant differences found in population cages B2 in week 18 and C1 in week 22 were the result of chance variation.

It is possible for both density of larvae and proportion of genotypes to influence the relative viability of coexisting genotypes of larvae. This was found in Drosophila by Lewontin (1955) and Lewontin and Matsou, (1963). The examinations for selective mortality were completed at several combinations of density of larvae and proportion of colour-forms in the population cages. These examinations revealed that there was no differential mortality of larvae. Therefore it is likely that selective mortality of larvae did not occur at any time in the history of the population cages.

5.2 PROPORTION OF ADULTS THAT MATE.

5.20 Introduction.

Colour-forms whose sexual activity and success at locating a mate are greatest, will be at a selective advantage because these forms will make the greatest contribution to the succeeding generation. The object of this section is to determine if such differences existed between colour-forms and if so, to describe the relative proportion of each colour-form that mated.

The hypothesis I tested was that there were differences in the proportion that mated between colour-forms in population cages. The ratio of the number of each colour-form in two samples of adults from the population cages was compared. One sample consisted of copulating flies; the other was taken at random. Both samples were taken at similar times. If the ratio of the number of each colour-form in the two samples did not differ by more than chance, then a similar proportion of each colour-form would have mated at that time. If, however, the two ratios differed by more than chance, then a difference between colour-forms in the proportion of adults that mated would be indicated.

5.21 Materials and methods.

An adequate sample of copulating flies from the population cages was collected by constant sampling over a period of approximately two

weeks. During the daily sampling interval the cages were searched constantly and all flies seen in copula were collected. Each pair was kept in a glass tube until they were identified to sex and colour-form under a binocular microscope. All flies were returned to their population cages following identification.

Flies, sampled at regular intervals from the population cages to estimate the proportions of colour-forms, provided random samples for the above comparison. The method used to collect these flies is described in Section 4.

5.22 The relative proportion of each colour-form that mated in population cages.

Plan of analysis; Samples of copulating flies were collected from population cages during weeks 7-9, 12-13, 16-18, and 25-26. These samples were taken immediately following the regular sampling of the population cages in weeks 7, 11, 15, and 23 when the proportions of colour-forms present were estimated.

The ratio of the number of each colour-form in the two samples from each population cage was compared in a 2 x 5 contingency table. A significant total Chi-square would indicate that the ratio of the number of each colour-form in the two samples deviated from each other by more than chance. Differences in the proportion that mated between colour-forms would be indicated.

The relative proportion of each colour-form that mated was determined from an index calculated by dividing the percentage of each colour-form in the sample of copulating flies by its percentage in the population cage.

Weeks 7-9. Table 5.05 shows that in each population cage, the ratio of the number of each colour-form in the sample of mating flies did not differ significantly from the ratio of the number of each colour-form in the sample taken at random. The contingency tables for each comparison are given in Appendix H.

Because of the similarity of the ratios of the number of each colour-form of the two samples from each population cage, the indices of the relative proportion of each colour-form that mated in weeks 7-9 (Table 5.06) are similar and vary between 0.83 and 1.24.

TABLE 5.05 A comparison of the percentages of colour-forms in the sample of mating flies with the percentages of colour-forms in the random sample of adults. The samples were taken from all population cages during weeks 7-9, and 12-13. The total Chi-square values are from contingency tables, calculated on the number of each colour-form as given in Appendix H.

Abbreviation: ns = not significant ($P > .05$)

	Sample of adults	Percentage					Total Chi-square	P	
		0	1-3	4	4.5	5			
Weeks 7-9	B1	Random	14.8	17.4	15.5	35.7	16.7	.11	ns
		Mating	15.0	17.2	15.8	36.2	15.8		
	B2	Random	10.8	18.3	21.5	31.5	18.0	2.59	ns
		Mating	10.9	16.2	20.4	37.0	15.5		
	C1	Random	9.3	21.2	21.8	32.3	15.5	3.02	ns
		Mating	11.5	22.8	18.0	33.8	14.0		
	C2	Random	12.2	21.3	22.5	32.5	11.5	1.00	ns
		Mating	10.1	21.8	22.1	34.2	11.8		
Weeks 12-13	B1	Random	14.2	17.2	13.2	39.2	16.2	12.41	< .025
		Mating	5.5	18.7	13.3	35.9	26.6		
	B2	Random	9.4	17.6	11.4	42.8	18.8	2.60	ns
		Mating	6.7	18.3	14.4	40.0	20.6		
	C1	Random	6.4	13.4	16.0	48.3	16.0	20.63	< .005
		Mating	1.8	15.9	14.7	37.6	30.0		
	C2	Random	11.2	15.6	17.1	43.4	12.7	6.96	ns
		Mating	14.1	22.8	16.3	34.8	12.0		

TABLE 5.06 Indices of mating showing the relative proportion of each colour-form that mated. The indices were calculated by dividing the percentage of each colour-form in the sample of copulating flies by its percentage in the population cage.

		Population Cage	Indices of mating of each colour-form				
			0	1-3	4	4.5	5
Weeks 7-9	B1	1.01	.99	1.02	1.01	.95	
	B2	1.01	.89	.95	1.17	.86	
	C1	1.24	1.08	.83	1.05	.90	
	C2	.83	1.02	.98	1.05	1.03	
Weeks 12-13	B1	.39	1.09	1.01	.92	1.64	
	B2	.71	1.04	1.26	.93	1.10	
	C1	.36	1.19	.92	.78	1.88	
	C2	1.26	1.46	.95	.80	.94	
Weeks 16-18	B1	.63	1.17	1.03	.78	1.66	
	B2	1.01	1.14	1.13	.75	1.37	
	C1	.76	.34	.55	.70	2.28	
	C2	.51	.47	.69	.83	2.31	
Weeks 25-26	B1	.60	.84	1.02	.44	1.53	
	B2	.85	.79	.69	.63	1.75	
	C1	.12	.50	.85	.52	1.41	
	C2	.24	.66	.55	.40	1.64	

Weeks 12-13. Table 5.05 shows that in population cages B2 and C2 the ratio of the number of each colour-form in the sample of copulating flies did not differ significantly from the ratio of the number of each colour-form in the random sample of adults. But significant differences were found in population cages B1 and C1. In these population cages, the percentage of grade 5 in the sample of mating flies was greater than their percentage in the population cage.

In population cages B1 and C1 grade 5 flies had the highest indices of mating (1.64 in B1, and 1.88 in C1) while the grade 0 flies had the lowest (0.39 in B1, and 0.36 in C1), as shown in Table 5.06. The indices of mating for all colour-forms in population cages B2 and C2 were similar and varied between 0.71 and 1.46.

Weeks 16-18 and 25-26. A significant deviation of the ratio of the number of each colour-form in the sample of mating flies from the ratio of the number of each colour-form in the sample of adults taken at random was found for each population cage. Table 5.07 shows that in each comparison, the percentage of grade 5 in the sample of mating flies was greater than their percentage in the population cage.

The indices of mating for grade 5 flies were the highest of all colour-forms, varying between 1.37 to 2.31 (Table 5.06). Grade 0 and 4.5 flies usually had the lowest indices of mating, varying between 0.12 and 1.01.

TABLE 5.07 A comparison of the percentage of each colour-form in the sample of mating flies with the percentage of each colour-form in the random sample of adults. The samples were taken from all population cages during weeks 16-18 and 25-26. The total Chi-square values are from contingency tables, calculated on the number of each colour-form as given in Appendix H.

Weeks	Sample of adults	Percentage					Total Chi-square	P
		0	1-3	4	4.5	5		
Weeks 16-18	B1 Random Mating	12.6	16.4	12.6	42.0	16.4	18.48	< .005
		8.0	19.2	13.0	32.6	27.2		
	B2 Random Mating	10.0	14.2	11.4	44.4	20.2	11.17	< .025
		10.1	16.2	12.9	33.1	27.7		
	C1 Random Mating	5.4	11.0	11.0	50.4	22.2	72.17	< .005
		4.1	3.7	6.1	35.4	50.7		
	C2 Random Mating	10.2	17.0	13.4	40.4	19.0	64.83	< .005
		5.2	8.0	9.3	33.6	43.8		
Weeks 25-26	B1 Random Mating	7.2	10.5	10.1	31.2	41.0	61.30	< .005
		4.3	8.8	10.3	13.7	62.7		
	B2 Random Mating	5.9	14.5	12.1	38.3	29.2	67.63	< .005
		5.0	11.4	8.3	24.1	51.1		
	C1 Random Mating	1.7	5.4	6.0	34.5	52.4	60.51	< .005
		0.2	2.7	5.1	17.8	74.1		
	C2 Random Mating	4.1	7.4	7.4	34.2	46.7	110.34	< .005
		1.0	4.9	4.1	13.6	76.4		

In summary, differences in the proportion that mated between colour-forms were indicated in population cages B1 and C1 in weeks 12-13 and in all four population cages in weeks 16-18 and 25-26. In each instance, the grade 5 flies had the highest index of mating. Therefore a higher proportion of grade 5 flies mated than any of the remaining colour-forms in these population cages at the above times.

5.3 BIRTH RATES.

5.30 Introduction.

Those colour-forms whose females possess the highest birth rates will be at a selective advantage. The aim of this section is to test the hypothesis that there are differences in birth rates between colour-forms.

Birth rates are defined as the average number of viable eggs produced per female per unit of time. The birth rates of each colour-form were determined by removing samples of females from the population cages and measuring the number of viable eggs they were able to produce over a period of time. The assumption was made that the relative birth rates of the colour-forms remain unchanged upon removal from the population cage.

Information, relating to birth rates, on the total number of eggs laid per female of each colour-form and the survival of eggs laid by females of each colour-form were also examined.

5.31 Materials and methods.

Females were collected from the population cages by placing an unperforated apple dome into each population cage. When these apple domes were covered with females attempting to oviposit, the apple domes

were quickly removed and females shaken off into holding cages. This procedure was repeated until a sufficient number of females had been collected. These females were later identified, without anesthetic, under a binocular microscope and placed into containers according to colour-form.

Birth rates of females from the population cages were determined in weeks 8, 10, 19, and 28. In weeks 8 and 10, females were kept in clear plastic cups with a nylon sleeve over the opening. There were five to ten females per cup. For subsequent tests in weeks 19 and 28, females were held in groups of five in a glass tube with a perforated cap over the opening. Each container was provided with sugar and water. Due to the difficulty of managing a large number of containers, females could not be held individually in separate containers. Information on the percentage of females of each colour-form that were inseminated could not be obtained.

Two days were required to collect, identify, and place the females in containers. On the third day, a small perforated apple dome was placed into each container. Apple domes were provided for five hours for one day in weeks 8 and 10 and for five hours for each of two consecutive days in weeks 19 and 28. Once eggs were collected females were returned to their population cages.

Apple domes were removed from the containers at the end of each five hour period. Using a small moist brush, eggs were removed from the underside of the apple dome, transferred to a square of

moist filter paper, and kept in covered petri dishes at 25°C.

The total number of eggs produced per container was counted and following a 72 hour period after laying, the number unhatched was counted and subtracted from the total number of eggs to give the number of viable eggs per container. The number of females alive at the end of egg collecting was divided into the total number of eggs and the number of viable eggs to give the total number of eggs per female and the number of viable eggs per female.

5.32 Birth rates of females from the population cages.

Weeks 8 and 10. Mature adults in the population cages were scarce in weeks 8 and 10. As a result, samples collected at these two times were combined and analysed as one.

The total number of eggs per female, the number of viable eggs per female, and the percentage of eggs that hatched were analysed in a 2 (localities) x 5 (colour-forms) factorial analysis of variance, with two replicates. Each replicate was composed of females from one of the two population cages from each locality.

The results of these analyses showed that there were no significant differences in survival of eggs between colour-forms or localities. An average of 45.2% of the eggs laid by Brisbane females hatched while 60.2% of the eggs laid by Cairns females

hatched. (Table 5.08). The total number of eggs per female and the number of viable eggs per female did not differ between colour-forms, but there was a significant difference between localities. Females from Brisbane produced an average of 9.4 eggs per female of which an average of 4.5 were viable. These figures were lower than those produced by Cairns females who laid an average of 13.6 eggs per female of which 8.1 were viable (Table 5.08). The tables of analyses of variance are given in Appendix I.

Week 19. Females, removed from the population cages in week 19, were provided with apple domes for a five hour period on each of two consecutive days. The total number of eggs per female, the number of viable eggs per female, and the survival of eggs were examined in an analysis of variance similar to that completed on the data collected in weeks 8 and 10. The data are given in Table 5.09.

There were no significant differences between colour-forms or localities in the survival of eggs.

TABLE 5.08 Mean percentage survival of eggs, mean total number of eggs per female, and mean number of viable eggs per female of each colour-form from Brisbane and Cairns population cages in weeks 8 and 10. Each figure is the mean of two replicates. Each replicate is composed of females from one of the two population cages from each locality. The tables of analysis of variance are given in Appendix I.

Locality	Colour-form	Number of females/replicate		Mean total number of eggs/female	Mean number of viable eggs/female	Mean percentage survival of eggs.
		R1	R2			
Brisbane	0	16	16	7.8	4.0	51.3
	1-3	18	20	8.3	3.3	32.3
	4	20	20	7.9	2.0	26.2
	4.5	20	20	11.2	7.8	70.4
	5	20	20	11.9	5.6	46.9
				Mean	9.4	4.5
Cairns	0	16	18	7.7	5.8	70.0
	1-3	20	16	13.1	6.2	46.8
	4	20	20	15.2	8.5	57.5
	4.5	20	20	14.9	9.6	64.5
	5	20	20	16.9	10.6	62.4
				Mean	13.6	8.1

TABLE 5.09. Mean percentage survival of eggs, mean total number of eggs per female, and mean number of viable eggs per female of each colour-form from Brisbane and Cairns population cages in week 19. Each figure is the mean of two replicates. Each replicate is composed of females from one of the two population cages from each locality. The tables of analysis of variance are given in Appendix I.

Locality	Colour-form	Number of females/ replicate		Mean total number of eggs/female	Mean number of viable eggs/female	Mean percentage survival of eggs.
		R1	R2			
Brisbane	0	25	25	17.7	10.8	65.9
	1-3	"	"	18.5	11.6	61.8
	4	"	"	24.7	16.0	61.5
	4.5	"	"	24.0	14.4	59.8
	5	"	"	26.0	20.1	77.6
Cairns	0	"	"	9.6	4.5	45.9
	1-3	"	"	17.2	12.7	73.2
	4	"	"	19.7	13.3	67.0
	4.5	"	"	31.9	21.9	68.6
	5	"	"	32.4	23.2	72.2

An average of 65.4% of the eggs hatched. Of the analyses on the total number of eggs per female and the number of viable eggs per female, the difference between localities was not significant, nor was the interaction between locality and colour-form. But differences between colour-forms were significant ($P < .025$). On the basis of these results, differences between colour-forms in the total number of eggs per female and the number of viable eggs per female were tested for significance using Duncan's New Multiple Range Test.

Table 5.10 shows that the number of viable eggs per female and the total number of eggs per female laid by grades 4.5 and 5 flies were significantly greater than those laid by grade 0 flies ($P < .05$).

Week 28. This examination of birth rates was carried out on females from the Brisbane population cages only. At this time the grade 5 flies in the Cairns population cages were estimated to be 80% of the adults and it was unlikely that a sufficient number of grades 0 to 4 could be collected to give an adequate test for differences between colour-forms. The frequency of grade 5 forms in the Brisbane population cages increased at a slower rate and more grades 0 to 4 forms were available. However, numbers of these colour-forms were restricted so grades 0 and 1-3 were combined into one category. Grades 4.5 and 5 each composed a separate category. Grade 4 females were omitted from the analysis as there were too few to make up one

TABLE 5.10 Mean total number of eggs per female and mean number of viable eggs per female produced by each colour-form, (mean of Brisbane and Cairns females), from data collected in week 19. Figures indicated by an asterisk (*) are significantly greater than those indicated by the arrow ($P < .05$).

Colour-form	Total number of eggs per female	Number of viable eggs per female.
0	13.7 ←	7.8 ←
1-3	17.9	12.3
4	22.2	14.7
4.5	28.0 *	18.2 *
5	29.2 *	21.7 *

category, and I did not want to combine this colour-form with any of the others. Thus the survival of eggs, total number of eggs per female, and number of viable eggs per female were each analysed in a single factor analysis of variance with two replicates. There were three levels of colour-forms in each analysis: grades 0-3, grade 4.5 and grade 5.

These analyses showed that there were no significant differences between colour-forms in survival of eggs. An average of 68.6% of the eggs hatched. Significant differences were found between colour-forms in the total number of eggs per female, and the number of viable eggs per female. Differences between all colour-forms were tested for significance using Duncan's New Multiple Range Test.

Table 5.11 shows that the total number of eggs per female and the number of viable eggs per female laid by the grade 5 females is significantly greater than those laid by grades 0-3 and 4.5.

In summary, all examinations (weeks 8 and 10, 19, and 28) showed that grades 4.5 and 5 females had the highest birth rates. Differences between colour-forms in weeks 8 and 10 were not significant. But in week 19, significant differences were found between grade 0 and grades 4.5 and 5 females from both localities. In week 28 when only Brisbane females were tested, grade 5 females had a significantly higher birth rate than grades 0-3 and 4.5 females. Grade 4 females were not tested at this time.

TABLE 5.11. Mean percentage survival of eggs, mean total number of eggs per female, and mean number of viable eggs per female from Brisbane population cages only, collected in week 28. Each figure is the mean of two replicates and each replicate is composed of females from one of the two population cages. The tables of analysis of variance are given in Appendix I. Differences between means were tested for significance by using Duncan's New Multiple Range Test. Figures indicated by an asterisk (*) are significantly greater than those indicated by the arrow ($P < .05$).

Colour-form	Number of females/replicate		Mean total number of eggs/female	Mean number of viable eggs/female.	Mean percentage survival of eggs.
	R1	R2			
0-3	75	75	6.1	4.4	70.3
4.5	"	"	10.5*	6.3	61.4
5	"	"	14.7*	10.9*	74.1

5.34 The relationship between the proportion of each colour-form that mated and the birth rates of the colour-forms.

The proportion of females inseminated is one of the factors influencing the birth rate of a group of females. It is not surprising to find then, that differences in rates of birth between colour-forms are to some extent correlated with the differences in proportion that mate between colour-forms.

In weeks 7-9 a similar proportion of all colour-forms mated. In weeks 8 and 10, there were no differences in the rates of birth between colour-forms. But in weeks 16-18, the proportion of grade 5 flies that mated was much greater than that of grade 0 flies. Grade 5 females in week 19 produced more viable eggs per female than did the grade 0 females. A similar result was found in the Brisbane population cages when the proportion of each colour-form that mated in weeks 25-26 was compared with the rates of birth measured in week 28.

Such a correlation was not found with grade 4.5 flies. In week 19, the grade 4.5 females possessed a high birth rate similar to grade 5 flies. At this time in the population cages, the proportion of grade 4.5 flies that mated was less than half that of the grade 5 flies that mated. A similar result was found in the Brisbane population cages in weeks 25-28.

Barton Browne (1956) found that uninseminated females of D. tryoni produced almost no eggs. If this occurs among the five

colour-forms nearly all the eggs produced are from inseminated females. An examination of the percentage of eggs that hatched from each colour-form showed that there were no differences between colour-forms. Thus a higher survival of eggs from grade 4.5 females did not account for their high birth rate in the presence of a low proportion that mated.

In conclusion, the differential proportion of colour-forms mating has a great influence on the birth rates of each colour-form. But grade 4.5 flies possess a selective advantage of having a high birth rate which compensates for their disadvantage of mating in a low proportion.

Section 5.4 TIME OF MATING OF COLOUR-FORMS AND
FREQUENCY OF HOMOGENIC MATINGS IN
SAMPLES OF COPULATING FLIES FROM
POPULATION CAGES.

5.40 Introduction.

The object of this section is twofold. The first is to describe differences in time of mating during the day between colour-forms in the population cages. Second, and more important, is to assess the effect of such differences on the number of homogamic matings by each colour-form. Differences in time of mating between colour-forms will result in a non-random choice of mates and will influence the frequency of all combinations of matings.

Gibbs (1965) has shown that a great majority of the offspring from homogamic matings by grades 0 and 5 flies resemble the parents. Heterogamic matings by grades 0 and 5 and matings involving grades 1-3, 4, and 4.5 flies produce a large proportion of intermediate colour-forms.

Consider the following examples in Table 5.12 using data from crosses by Gibbs (1965). Each of the three examples shows the expected number of each colour-form produced by (1) homogamic matings of two different pairs of colour-forms and (2) heterogamic matings between the same four flies. The total number of each colour-form expected in the offspring of homogamic and heterogamic matings can be compared. It is assumed that 100 offspring will result from each mating.

TABLE 5.12 Showing how different matings can influence the frequency of colour-forms in the succeeding generation. It is assumed that 100 offspring result from each mating. Data from Gibbs (1965).

		Expected frequency of colour-forms among the offspring.				
		0	1-3	4	4.5	5
EXAMPLE 1						
Homogamic matings:						
0 x 0	- - - - -	97.5	2.5	0.0	0.0	0.0
4 x 4	- - - - -	20.6	12.9	25.8	28.7	12.0
Totals		118.1	15.4	25.8	28.7	12.0
Heterogamic matings:						
0 x 4	- - - - -	51.4	29.4	18.9	0.0	0.0
4 x 0	- - - - -	51.4	29.4	18.9	0.0	0.0
Totals		102.8	58.8	37.8	0.0	0.0
EXAMPLE 2.						
Homogamic matings:						
5 x 5	- - - - -	0.0	0.0	0.0	17.6	82.4
4 x 4	- - - - -	20.6	12.9	25.8	28.7	12.0
Totals		20.6	12.9	25.8	46.3	94.4
Heterogamic matings:						
5 x 4	- - - - -	0.0	1.3	18.0	42.8	37.7
4 x 5	- - - - -	0.0	1.3	18.0	42.8	37.7
Totals		0.0	2.6	36.0	85.6	75.4
EXAMPLE 3.						
Homogamic matings:						
5 x 5	- - - - -	0.0	0.0	0.0	17.6	82.4
0 x 0	- - - - -	97.5	2.5	0.0	0.0	0.0
Totals		97.5	2.5	0.0	17.6	82.4
Heterogamic matings:						
5 x 0	- - - - -	0.0	13.9	65.9	20.0	0.1
0 x 5	- - - - -	0.0	13.9	65.9	20.0	0.1
Totals		0.0	27.8	131.8	40.0	0.2

Table 5.12 shows how the frequency of some combinations of matings can influence the proportions of colour-forms in the succeeding generation. Any mechanism increasing the number of homogamic matings by grades 0 and 5 will increase the frequency of these colour-forms in the following generation.

An increased frequency of homogamic matings by grade 0 or 5 flies is not a selective difference in that it is a component of the capacity for increase. But its presence can influence the rate of selection in the event that grades 0 or 5 possess other selective advantages. For this reason, I have included it in this section.

Gibbs (1965) has shown that D. tryoni mate at dusk from 6.00 - 6:30 pm and that D. neohumeralis mate during the day between 8:30 am and 2:00 pm. He also found that F1 hybrids from an interspecific cross between the above two species mated between 3:30 - 6.00 pm. It is likely that grade 0 flies mate at a time similar to D. neohumeralis and that grade 5 flies mate at a time similar to D. tryoni. The remaining grades, 1-3, 4, and 4.5 may mate at times intermediate to that of grade 0 and 5.

Thus it was likely that the colour-forms mated at different times during the day in population cages. If this were so, frequencies of some combinations of matings would be affected and the rate of selection influenced.

5.41 Differences in time of mating between colour-forms in population cages.

Plan of analysis. Data on time of mating during the day of the colour-forms was available from samples of copulating flies collected from the population cages during weeks 7-9, 12-13, 16-18

and 25-26. However, before the data was analysed, I combined the samples of copulating flies taken from the population cages in weeks 12-13 with those collected in weeks 16-18. The samples taken at this time in the history of the population cages were similar because they both represented the system of mating in the population cages when the grade 5 flies were starting to increase in proportion. In addition, samples of copulating flies from population cages B1 and B2 were combined as were those from population cages C1 and C2. Differences in proportions of colour-forms between the two replicated population cages from each locality were slight when each sample was taken. These differences would have little or no effect on either the frequency of homogamic matings or the time of mating of the colour-forms. Thus there were six samples of copulating flies available for analysis; one sample from each locality collected during weeks 7-9, 12-18, and 25-26.

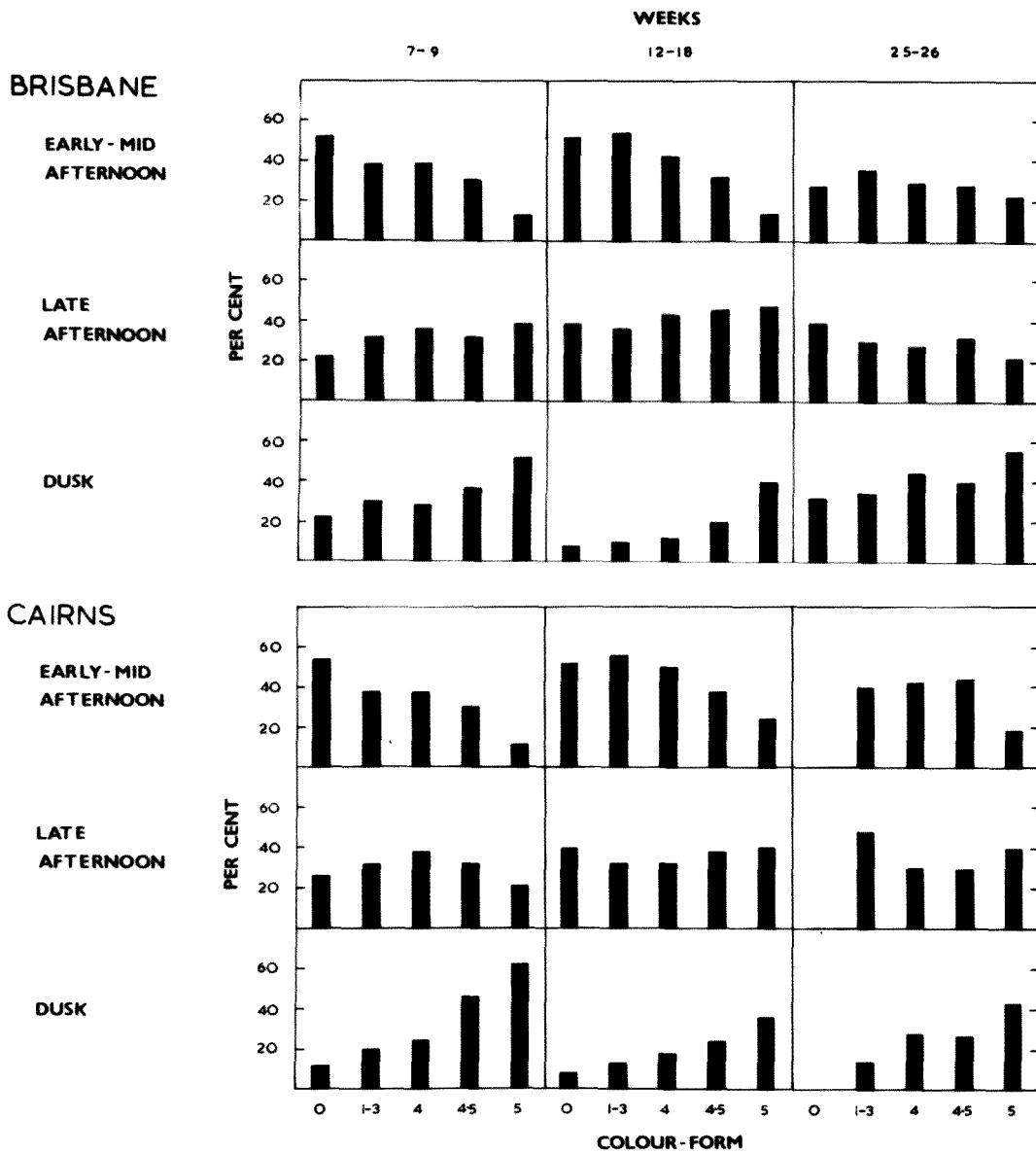
Information on the time of mating of the colour-forms was obtained by dividing the daily period of sampling into three intervals. These were early to mid afternoon, late afternoon, and dusk. The interval in which each pair was found copulating was recorded. The percentage of each colour-form mating in each interval was then calculated.

Results. Figure 5.01 shows in weeks 7-9, that over 50% of the grade 0 flies in both Brisbane and Cairns population cages mated during early to mid afternoon. Grades 1-3, 4, and 4.5 from both localities each mated with a similar percentage in all three intervals. But over 50% of the grade 5 flies from both localities copulated at dusk. Samples collected from the Brisbane and Cairns population cages in weeks 12-18 showed that over 75% of grades 0, 1-3, 4, and 4.5 each mated from early to late afternoon. Over 75% of the grade 5 flies from both localities mated from late afternoon to dusk. In weeks 25-26 however, the sample of mating flies from the Brisbane population cages showed that there was little difference between colour-forms in their time of mating. Over 60% of all colour-forms mated from late afternoon to dusk. At this time in the Cairns population cages over 70% of grades, 0, 1-3, 4, and 4.5 each mated from early to late afternoon, while 71.6% of the grade 5 flies mated from late afternoon to dusk. The data is given in Appendix J.

Thus differences in the time of mating between colour-forms were found in the population cages. These differences could influence the frequency of homogamic matings by the colour-forms, particularly grade 5 flies.

FIGURE 5.01

Showing the percentage of each colour-form that mated in three daily periods. The information is from samples of copulating flies taken from the Brisbane and Cairns population cages during weeks 7-9, 12-18, and 25-26. There was not a sufficient number of grade 0 flies in the Cairns sample, taken in weeks 25-26, to give a meaningful result. The smallest number that percentages were calculated from was 30 flies.



5.42 Frequency of all combinations of mates in samples of copulating flies from the population cages.

Plan of analysis. The observed frequency of all combinations of mates in the samples of copulating flies was compared to the expected frequency to determine if the observed number of any combination deviated from the expected. Expected frequencies were calculated on the assumption that all flies captured in the samples mated completely at random. The observed and expected frequencies were compared in a R x C contingency table. As mentioned earlier, the two combinations of particular interest were the number of homogamic matings by grades 0 and 5 flies.

The hypothesis I set out to test was that the frequencies of some of the observed combinations of mates deviated from the expected.

Each of the six samples of copulating flies from the population cages described in Section 5.41 were analysed (one sample for each locality collected during weeks 7-9, 12-18, and 25-26).

Weeks 7-9. A significant departure of the observed frequency of some combinations of mates from the expected frequency was found in the Brisbane and Cairns samples. The total Chi-square was 39.90 in the Brisbane contingency table and 37.23 in the Cairns contingency table. (Tables 5.13 and 5.14). Both are significant ($P < .005$).

TABLE 5.13 Contingency table for Brisbane flies showing the observed and expected numbers of pairs for all possible combinations of mates. The data is from samples of copulating flies collected from population cages B1 and B2 during weeks 7-9. The total Chi-square is 39.90 with 16 degrees of freedom ($P < .005$).

MALE COLOUR- FORMS		FEMALE COLOUR-FORMS					TOTAL
		0	1-3	4	4.5	5	
0	Observed	8	7	2	13	5	35
	Expected	5.4	5.8	7.1	11.7	4.9	
	Chi-square	1.26	.24	3.66	.15	0.0	
1-3	Observed	5	11	13	21	4	54
	Expected	8.3	9.0	11.0	18.1	7.6	
	Chi-square	1.31	.44	.36	.46	1.71	
4	Observed	11	12	15	9	2	49
	Expected	7.5	8.1	10.0	16.4	6.9	
	Chi-square	1.64	1.88	2.50	3.34	3.47	
4.5	Observed	20	17	31	37	21	126
	Expected	19.4	20.9	25.7	42.3	17.8	
	Chi-square	.02	.73	1.09	.66	.57	
5	Observed	5	6	4	27	13	55
	Expected	8.4	9.1	11.2	18.5	7.8	
	Chi-square	1.38	1.05	4.63	3.91	3.46	
TOTAL		49	53	65	107	45	319

TABLE 5.14 Contingency table for Cairns flies showing the observed and expected numbers of pairs for all possible combinations of mates. The data is from samples of copulating flies collected from population cages C1 and C2 during weeks 7-9. The total Chi-square is 37.23 with 16 degrees of freedom ($P < .005$).

MALE COLOUR- FORMS		FEMALE COLOUR-FORMS					TOTAL
		0	1-3	4	4.5	5	
0	Observed	9	5	6	9	5	34
	Expected	4.0	8.2	7.3	10.7	3.8	
	Chi-square	6.25	1.24	.37	.27	.37	
1-3	Observed	5	25	22	18	5	75
	Expected	8.9	18.1	16.1	23.6	8.3	
	Chi-square	1.71	2.63	2.16	1.33	1.31	
4	Observed	11	16	16	18	8	69
	Expected	8.2	16.7	14.8	21.7	7.6	
	Chi-square	.95	.03	.10	.63	.02	
4.5	Observed	15	33	26	55	9	138
	Expected	16.3	33.4	29.7	43.4	15.2	
	Chi-square	.10	.01	.46	3.10	2.53	
5	Observed	4	11	10	17	14	56
	Expected	6.6	13.5	12.0	17.6	6.2	
	Chi-square	1.03	.47	.33	.02	9.81	
TOTAL		44	90	80	117	41	372

The contingency tables are summarized in Table 5.15. This table shows that in samples of copulating flies from Brisbane and Cairns population cages, each grade of male mated with females of their own kind, either more frequently than expected, or with a frequency similar to the expected. In addition, some grades of males mated more frequently than expected with females similar to their own colour-form. The only exception was the grade 4 males, which in both Cairns and Brisbane samples, mated more frequently than expected with grade 0 females (Table 5.15).

TABLE 5.15 Summary of contingency tables on the frequency of homogamic matings by the colour-forms in population cages during weeks 7-9.

MALE COLOUR- FORMS	FEMALE COLOUR-FORMS					MALE COLOUR- FORMS	FEMALE COLOUR-FORMS				
	0	1-3	4	4.5	5		0	1-3	4	4.5	5
0	+	0	-	0	0	0	+	-	0	0	0
1-3	-	0	0	0	-	1-3	-	+	+	-	-
4	+	+	+	-	-	4	+	0	0	0	0
4.5	0	0	+	0	0	4.5	0	0	0	+	-
5	-	-	-	+	+	5	-	0	0	0	++

Explanation of symbols:

	Chi-square
0 Observed similar to expected	< .90
- Observed less than expected	> .89
= Observed less than expected	> 8.99
+ Observed greater than expected	> .89
++ Observed greater than expected	> 8.99
+++ Observed greater than expected	> 17.99

In samples from both Brisbane and Cairns population cages, the observed number of homogamic matings of grades 0 and 5 exceeded the expected. The contingency table for the Brisbane flies (Table 5.13) shows that among grade 0 flies, 5.4 homogamic matings were expected and 8 were observed. Among grade 5 flies, 7.8 homogamic matings were expected and 13 were observed. The contingency table for the Cairns flies (Table 5.14) shows that 9 homogamic matings by grade 0 flies were observed when only 4 were expected. Similarly, for grade 5 flies, 14 homogamic matings were observed and only 6.2 were expected.

Weeks 12-18. Again a deviation of the observed from the expected was found for certain combinations of mates in the samples collected from Cairns and Brisbane population cages. A total Chi-square of 36.23 was found in the Brisbane contingency table (Table 5.16) and 58.59 in the Cairns contingency table (Table 5.17). Both were significant ($P < .005$).

Similar differences between the observed and the expected were found in both contingency tables. Males of grades 0, 1-3, 4, and 4.5 each mated more frequently than expected with females of grades 0, 1-3, 4, and 4.5, and less frequently than expected with grade 5 females. There was only one exception, the grade 4 males in the Brisbane population cages mated less frequently than expected with grade 0 females. Grade 5 males mated less frequently than expected with alien females and more frequently than expected with their own females.

TABLE 5.16. The contingency table for Brisbane flies showing the observed and expected numbers of pairs of all possible combinations of mates. The data is from samples of copulating flies collected from population cages B1 and B2 during weeks 12-18. The total Chi-square is 36.23 with 16 degrees of freedom ($P < .005$).

MALE COLOUR- FORMS		FEMALE COLOUR-FORMS					TOTAL
		0	1-3	4	4.5	5	
0	Observed	2	3	4	8	4	21
	Expected	2.5	3.7	3.2	6.5	5.1	
	Chi-square	.10	.13	.20	.35	.24	
1-3	Observed	17	15	16	23	9	80
	Expected	9.3	13.9	12.3	24.9	19.6	
	Chi-square	6.40	.09	1.11	.14	5.73	
4	Observed	3	10	8	18	9	48
	Expected	5.6	8.3	7.4	14.9	11.8	
	Chi-square	1.21	.35	.05	.64	.66	
4.5	observed	23	32	25	52	38	170
	Expected	19.8	29.6	26.1	52.9	41.6	
	Chi-square	.51	.19	.05	.02	.31	
5	Observed	6	16	14	35	47	118
	Expected	13.8	20.5	18.1	36.7	28.9	
	Chi-square	4.41	.99	.93	.08	11.34	
TOTAL		51	76	67	136	107	437

TABLE 5.17. The contingency table for Cairns flies showing the observed and expected numbers of pairs for all possible combinations of mates. The data is from samples of copulating flies collected from population cages C1 and C2 during weeks 12-18. The total Chi-square is 58.59 with 16 degrees of freedom ($P < .005$).

MALE COLOUR- FORMS		FEMALE COLOUR-FORMS					TOTAL
		0	1-3	4	4.5	5	
0	Observed	3	2	4	10	1	20
	Expected	1.6	2.2	2.6	7.3	6.3	
	Chi-square	1.23	.02	.75	1.00	4.46	
1-3	Observed	7	5	12	16	9	49
	Expected	3.9	5.5	6.4	17.8	15.5	
	Chi-square	2.46	.05	4.91	.18	2.73	
4	Observed	4	10	8	15	7	44
	Expected	3.5	4.9	5.7	16.0	13.9	
	Chi-square	.07	5.31	.93	.06	3.42	
4.5	Observed	14	22	24	65	38	163
	Expected	12.9	18.3	21.2	59.1	51.5	
	Chi-square	.09	.75	.37	.59	3.54	
5	Observed	11	16	16	72	100	215
	Expected	17.1	24.1	28.0	77.9	67.9	
	Chi-square	2.18	2.72	5.14	.45	15.18	
TOTAL		39	55	64	178	155	491

Among grade 0 flies in the sample from Brisbane population cages, 2 homogamic matings were observed and 2.5 were expected (Table 5.16). However, among grade 5 flies, 47 homogamic matings were observed when only 28.9 were expected. Similarly, in the Cairns contingency table (Table 5.17) 3 homogamic matings by grade 0 flies were observed and only 1.6 were expected. But 100 homogamic matings by grade 5 flies were observed when only 67.9 were expected.

The two contingency tables are summarized in Table 5.18.

Weeks 25-26. To analyse samples of copulating flies collected from the Brisbane population cages in a contingency table, it was necessary to combine grades 0, 1-3, and 4. The total Chi-square for this contingency table (Table 5.19) was 6.34 and was non-significant ($P > .10$). Thus each observed number was similar to the expected.

Grades 0, 1-3, 4, and 4.5 were combined to analyse samples of copulating flies from the Cairns population cages. The total Chi-square was 12.79 (Table 5.20) and was significant ($P < .005$). Table 5.20 shows that the grade 5 males mated more than expected with grade 5 females and less than expected with alien females.

A summary of both contingency tables is given in Table 5.21.

TABLE 5.18 Summary of contingency tables on the frequency of homogamic matings by the colour-forms in the population cages during weeks 12-18.

BRISBANE						CAIRNS					
MALE COLOUR- FORMS	FEMALE COLOUR- FORMS					MALE COLOUR- FORMS	FEMALE COLOUR-FORMS				
	0	1-3	4	4.5	5		0	1-3	4	4.5	5
0	0	0	0	0	0	0	+	0	0	0	-
1-3	+	0	+	0	-	1-3	+	0	+	0	-
4	-	0	0	0	0	4	0	+	+	0	-
4.5	0	0	0	0	0	4.5	0	0	0	0	-
5	-	-	-	0	++	5	-	-	-	0	++

See Table 5.15 for explanation of symbols.

TABLE 5.19 The contingency table for Brisbane flies showing the observed and expected numbers of pairs of all possible combinations of mates. The data is from samples of copulating flies collected from population cages B1 and B2 during weeks 25-26. The total Chi-square is 6.34 with 4 degrees of freedom and is not significant ($P > .10$).

MALE COLOUR- FORMS		FEMALE COLOUR-FORMS			TOTAL
		0-4	4.5	5	
0-4	Observed	25	16	37	78
	Expected	22.2	14.4	41.4	
	Chi-square	.35	.18	.47	
4.5	Observed	16	20	47	83
	Expected	23.6	15.3	44.1	
	Chi-square	2.45	1.44	.19	
5	Observed	73	38	129	240
	Expected	68.2	44.3	127.5	
	Chi-square	.34	.90	.02	
TOTAL		114	74	213	401

TABLE 5.20 The contingency table for Cairns flies showing the observed and expected numbers of pairs of all possible combinations of mates. The data is from samples of copulating flies collected from population cages C1 and C2 during weeks 25-26. The total Chi-square is 12.79 with 1 degree of freedom ($P < .005$).

MALE COLOUR- FORMS		FEMALE COLOUR-FORMS		
		0-4.5	5	TOTAL
0-4.5	Observed	38	52	90
	Expected	24.2	65.8	
	Chi-square	7.31	2.69	
5	Observed	73	248	321
	Expected	86.8	234.3	
	Chi-square	2.04	.75	
TOTAL		111	300	411

TABLE 5.21 Summary of contingency tables on the frequency of homogamic matings by the colour-forms in the population cages during weeks 25-26.

BRISBANE				CAIRNS			
MALE COLOUR- FORMS	FEMALE COLOUR-FORMS			MALE COLOUR- FORMS	FEMALE COLOUR-FORMS		
	0-4	4.5	5		0-4.5	5	
0-4	0	0	0	0-4.5	+	-	
4.5	-	+	0	5	-	0	
5	0	-	0				

See Table 5.15 for explanation of symbols.

In conclusion, the frequency of some combinations of matings deviated significantly from the expected in the Cairns and Brisbane population cages in weeks 7-9 and 12-18 and only in the Cairns population cages only in weeks 25-26. The most important deviations were the greater than expected number of homogamic matings by grades 0 and 5 in weeks 7-9 and by grade 5 flies in weeks 12-18 and 25-26.

5.43 The influence of different times of mating of the colour-forms on the frequency of homogamic matings.

The samples of copulating flies from both localities collected in weeks 7-9 showed that grade 0 flies tended to mate earlier in the day than grades 1-3, 4, and 4.5. Grade 5 flies tended to mate latest in the day. These differences in time of mating are correlated with the observed frequency of some combinations of mates. The number of homogamic matings by grades 0 and 5 was greater than expected.

The samples of copulating flies from the Brisbane and Cairns population cages in weeks 12-18 and from the Cairns population cages in weeks 25-26 showed that grades 0, 1-3, 4, and 4.5 each mated at a similar time but earlier in the day than grade 5 flies. Grade 5 flies mated more frequently than expected among themselves and less frequently than expected with other colour-forms.

The sample of copulating flies collected from the Brisbane population cages in weeks 25-26 showed that all colour-forms were mating with similar frequencies at the same time. There was little difference between the observed and the expected frequency of all combinations of mates.

Thus, at times in the history of the population cages, some colour-forms or groups of colour-forms were partially isolated from each other due to differences in their time of maximum frequency of mating.

Section 5.5 THE CORRELATION BETWEEN TIME WHEN SELECTIVE DIFFERENCES BETWEEN COLOUR-FORMS WERE FOUND AND CHANGE IN PROPORTION OF COLOUR-FORMS IN POPULATION CAGES.

Introduction. Selective differences between colour-forms were found in proportions that mated, rates of birth, and frequency of some combinations of mates. In this section I showed how the time when these selective differences were found in population cages was correlated with the change in proportions of colour-forms in the same population cages.

Figure 5.02 shows the rate of change in proportions of three categories of colour-forms, grade 0, a combined category of grades 1-4.5, and grade 5. The percentage of each category given is the mean estimated from the two replicated population cages.

A three week period of development is required at 25°C from egg to adult. This interval of time would be required before any of the above selective differences would affect the proportions of adult colour-forms in population cages. Selective differences were measured in population cages during weeks 3, 7-10, 12-19, 22, and 25-28. Figure 5.02 shows the time when these examinations were carried out and also the time that selective differences, if found, would influence the proportions of adult colour-forms in population cages.

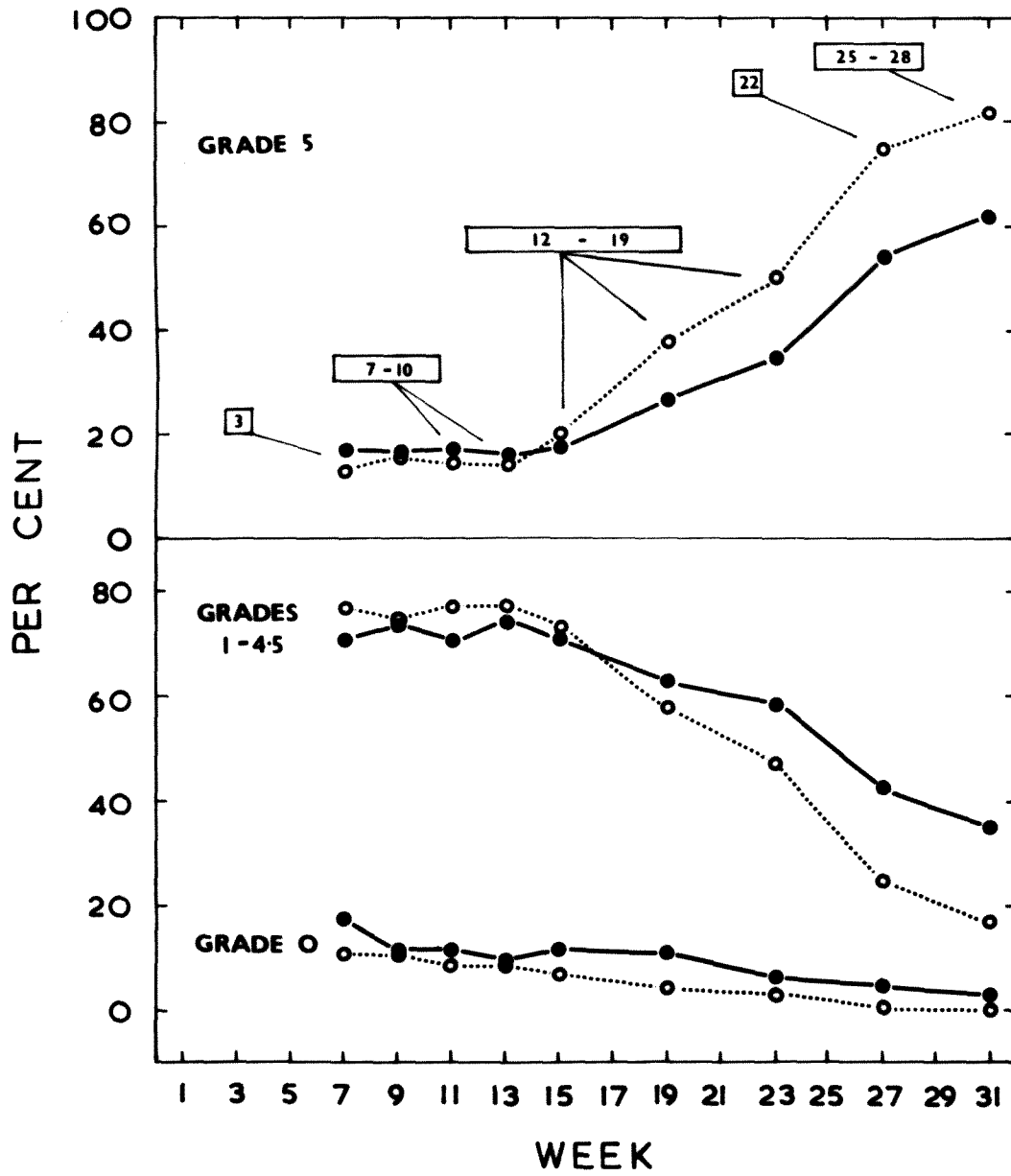
FIGURE 5.02

The rate of change in proportion of grade 0, grades 1 to 4.5, and grade 5 flies during the early history of the population cages. The percentages of each of these categories of colour-forms given is the mean from the two replicated population cages of each locality.

solid circles - mean of B1 and B2

hollow circles - mean of C1 and C2

Examinations for selective differences were completed during the weeks indicated in the rectangles. The time that selective differences, if found, would influence the proportions of adult colour-forms in the population cages are indicated by the lines from each rectangle.



Week 3. The survival of larvae was examined for selective differences between colour-forms. Selective differences were not found.

Weeks 7-10. There were no differences in the proportion that mated or in the rates of births between colour-forms. There was however, a greater than expected number of homogamic matings by grades 0 and 5 flies. This would tend to produce more grades 0 and 5 and fewer grades 1-3, 4, and 4.5 among the flies emerging in population cages in weeks 11 and 13. However, such increases were not found in the population cages at this time (Figure 5.02).

Weeks 12-19. Selective differences were found favouring the grade 5 flies. A higher percentage of grade 5 flies mated than any of the other colour-forms. Although grade 5 females produced the highest number of viable eggs per female per unit of time, only the difference between grade 5 and grade 0 was significant. The observed frequency of homogamic matings by grade 5 flies greatly exceeded the expected. The selective advantages were correlated with an increase in proportion of grade 5 flies in population cages (Figure 5.02).

The survival of larvae, examined in week 18, revealed no selective differences.

Week 22. The survival of larvae in very crowded densities was examined. No selective differences were found.

Weeks 25-28. Again selective differences were found favouring the grade 5 flies. Birth rates were examined in the Brisbane population cages only. Grade 5 females had a higher birth rate than did grades 0, 1-3, and 4.5. Grade 4 females were omitted from the analysis. A slightly greater than expected frequency of homogamic matings by grade 5 flies was found in the Cairns population cages only. A higher proportion of grade 5 flies mated than any of the other colour-forms in both Cairns and Brisbane population cages.

In summary, grade 5 flies were at a selective advantage because they had the highest birth rate, the highest proportion that mated, and a greater than expected number of homogamic matings. The time when these selective advantages were found correlates well with the time when the grade 5 flies increased in proportion in the population cages.

Section 6. ANALYSIS OF SELECTIVE DIFFERENCES
 BETWEEN COLOUR-FORMS.

6.0 INTRODUCTION.

The aim of this section is to describe some of the factors influencing the selective advantages possessed by grade 5 flies. It will be recalled (Section 5) that a higher proportion of grade 5 flies mated than any of the other colour-forms, they had the highest birth rate and they mated mostly amongst themselves.

My investigations were confined to (1) the way in which differences in the daily time of mating between colour-forms influenced the number of homogamic matings by grade 5 flies, and (2) the way in which changes in the environment of the population cages and differences in mating behaviour between colour-forms influenced the proportion of each colour-form that mated.

6.1 THE INFLUENCE OF DIFFERENCES IN DAILY TIME OF MATING
BETWEEN COLOUR-FORMS ON THE NUMBER OF HOMOGAMIC
MATINGS BY GRADE 5 FLIES.

Introduction. I showed in Section 5.42 that grade 5 flies in the population cages mated more frequently than expected among themselves and less frequently than expected with the other colour-forms. In addition, when the daily interval of mating was divided into three periods with approximate boundaries of time, the grades 0, 1-3, 4, and 4.5 flies mated mostly in the mid and late afternoon while the grade 5 flies mated mostly during the late afternoon and dusk.

The object of this section is to describe, in greater detail, differences in time of mating between colour-forms and determine how well these differences correlate with the frequency of all combinations of mates. If there is a close correlation then it is likely that the greater than expected frequency of homogamic matings by grade 5 flies is wholly dependent upon the differences in daily time of mating of the colour-forms.

Plan of experiment. I established an experimental cage containing some several thousand flies of an F2 generation from an interspecific cross between D. tryoni and D. neohumeralis. The percentage of each colour-form from a sample of 500 in this cage was estimated to be:

grade	0	6.6
grades	1-3	8.2
grade	4	17.2
grade	4.5	25.6
grade	5	42.4

These percentages were similar to those found in the Cairns population cages in week 19, when the frequency of homogamic matings by grade 5 flies greatly exceeded the expected.

All flies observed in copula were removed from the experimental cage for eight consecutive afternoons. Flies collected every 15 minutes were kept in separate batches to provide a frequency distribution of the time of mating for each colour-form. The captured flies were identified to colour-form and sex and were returned to the experimental cage at the end of each day's sampling.

Time of mating of the colour-forms. Figure 6.01 shows the differences in the frequency distribution of the time of mating of each colour-form. The time of maximum frequency of mating of grades 0, 1-3, and 4 was similar. Over 75% of each of these forms mated between 3.30 pm and 6:15 pm. Of the grade 4.5 flies, 71.3% mated between 4.45 pm and 6:15 pm, while 62.6% of the grade 5 flies mated between 6.00 pm and 6:30 pm. The frequency distribution of the time of mating of each of these three categories is compared in Figure 6.02. The number of each colour-form copulating at 15 minute intervals is given in Appendix K.

FIGURE 6.01

The percentage of each colour-form that mated in each 15 minute interval during the day in an experimental cage. The number of each colour-form sampled is given in parentheses.

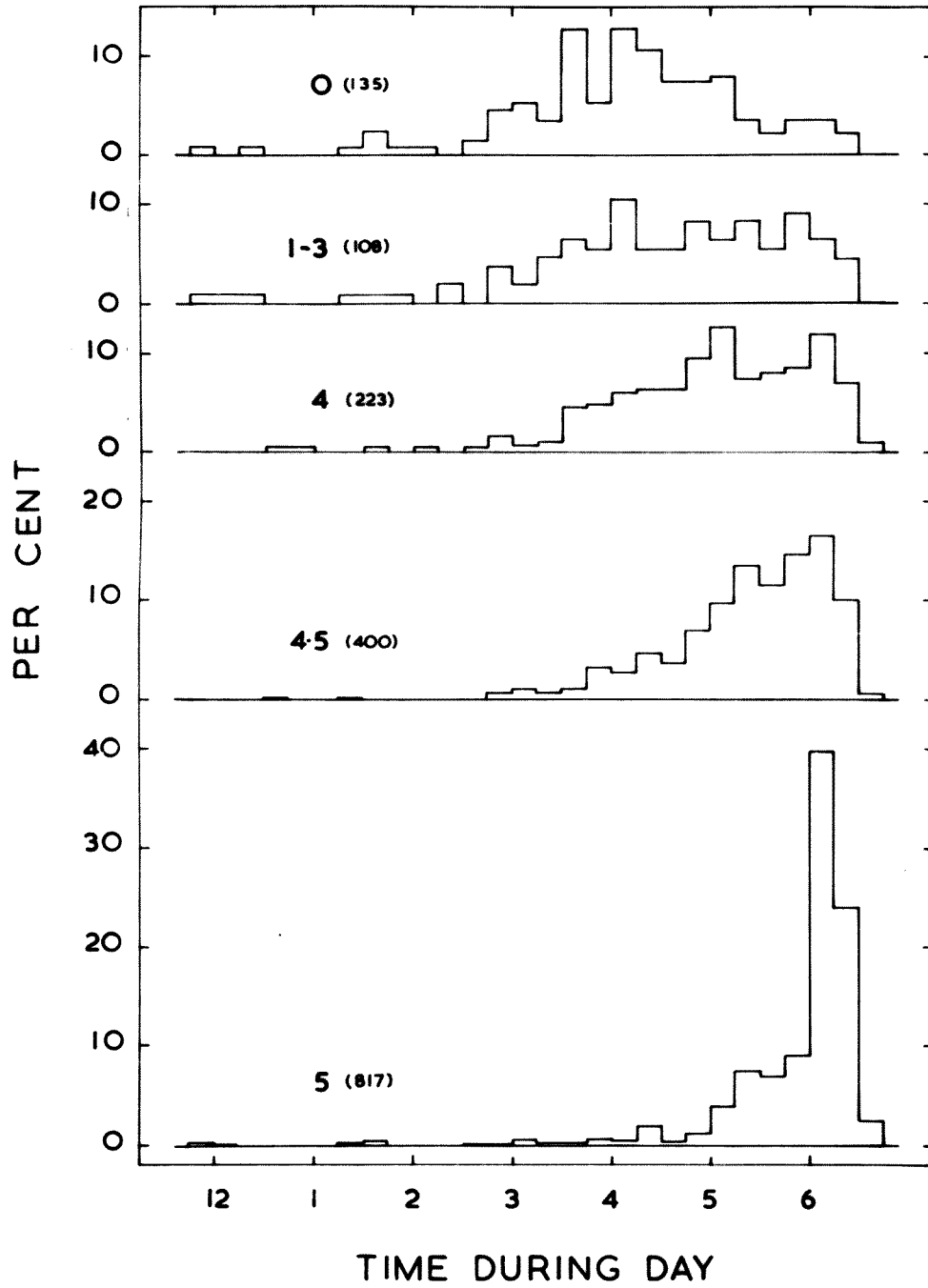
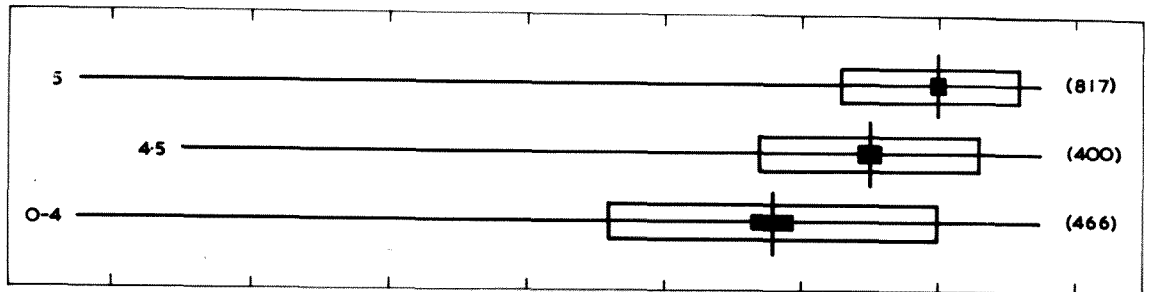


FIGURE 6.02

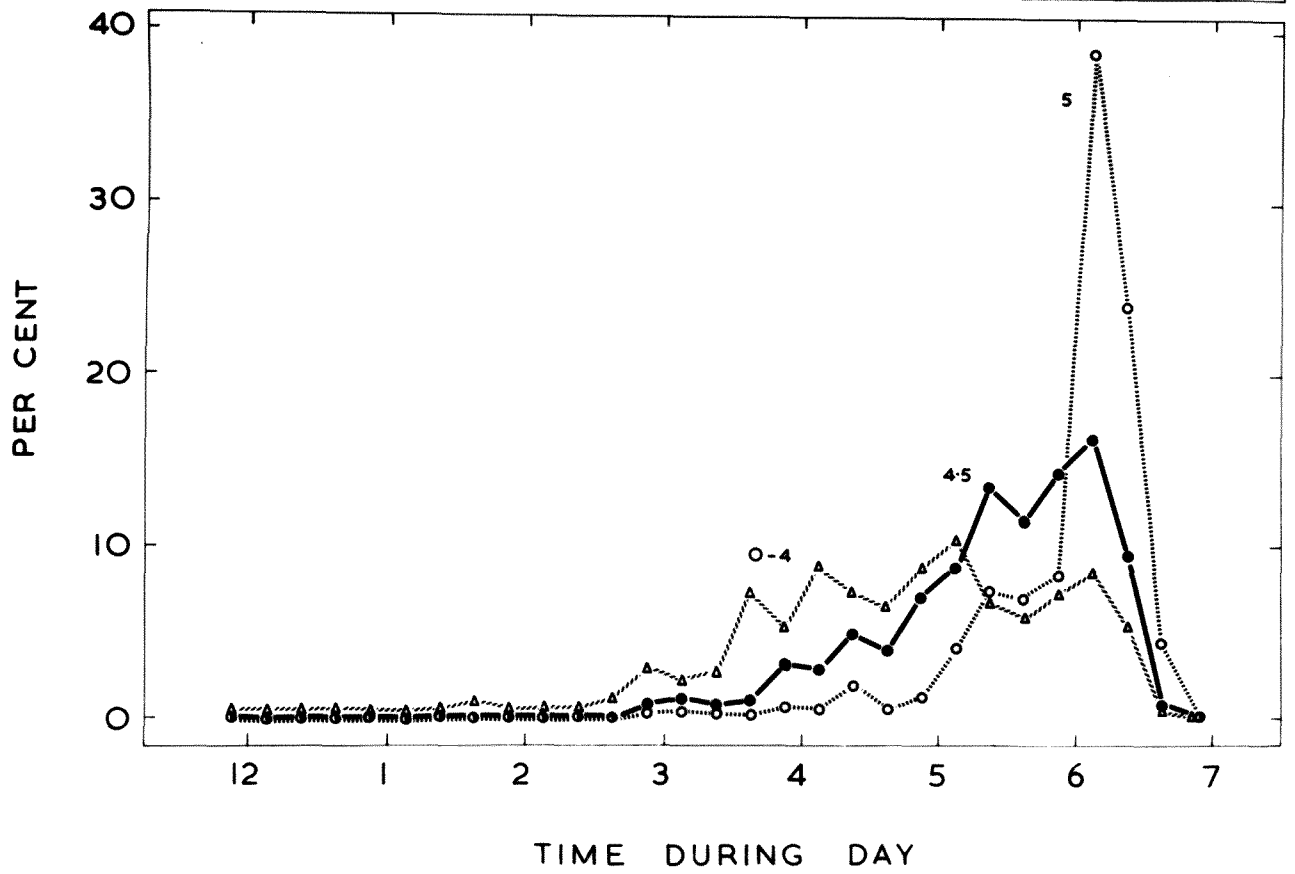
The differences in time of mating of three categories of colour-forms; grades 0-4, grade 4.5 and grade 5.

- A. The mean time of mating (vertical line) and the range in time of mating (horizontal line) during the day. The hollow rectangle represents one standard deviation either side of the mean. The solid rectangle represents 95% confidence limits on the mean.
- B. Percentage of each category that mated in each 15 minute interval during the day. The differences in time of mating between categories show why the number of homogamic matings by grades 0-4 and 4.5 slightly exceeded the expected, and why the number of homogamic matings by grade 5 greatly exceeded the expected.

A



B



Frequency of all combinations of mates. The observed frequency of some combinations of mates deviated from the expected as indicated by the significant total Chi-square of 142.70 ($P < .005$). The contingency table is given in Table 6.01 and is summarized in Table 6.02.

Table 6.02 shows that grades 0, 1-3, and 4 mated in frequencies either similar to or greater than expected among themselves and with a frequency similar to or less than expected with grades 4.5 and 5. Grade 4.5 flies mated more frequently than expected among themselves and with a frequency similar to or less than expected with alien colour-forms. The observed number of homogamic matings by grade 5 flies greatly exceeded the expected.

In conclusion, the observed frequencies of matings as shown in the contingency table (Table 6.01) correlate well with the differences in the daily time of mating between colour-forms or groups of colour-forms. Therefore in this experimental cage and in the population cages, the greater than expected number of homogamic matings of grade 5 flies was dependent upon differences in the time of mating during the day between the colour-forms.

TABLE 6.01 Contingency table showing the observed and expected numbers of pairs for all possible combinations of mates. The total Chi-square is 142.70 with 16 degrees of freedom. ($P < .005$).

MALE COLOUR- FORMS		FEMALE COLOUR-FORMS					TOTAL
		0	1-3	4	4.5	5	
0	Observed	10	11	12	12	10	55
	Expected	5.0	3.5	6.9	13.4	26.2	
	Chi-square	5.00	16.07	3.77	.14	10.02	
1-3	Observed	11	9	8	5	18	51
	Expected	4.6	3.2	6.4	12.4	24.3	
	Chi-square	8.90	10.51	.40	4.42	1.63	
4	Observed	22	9	20	30	35	116
	Expected	10.6	7.4	14.5	28.2	55.4	
	Chi-square	12.26	.35	2.09	.11	7.51	
4.5	Observed	15	14	21	69	73	192
	Expected	17.5	12.2	23.9	46.7	91.6	
	Chi-square	.36	.27	.35	10.65	3.77	
5	Observed	18	10	43	87	262	420
	Expected	38.3	26.7	52.5	102.2	200.4	
	Chi-square	10.76	10.45	1.72	2.26	18.93	
TOTAL		76	53	104	203	398	834

TABLE 6.02 Summary of contingency table (Table 6.01).

MALE COLOUR- FORMS	FEMALE COLOUR-FORMS				
	0	1-3	4	4.5	5
0	+	++	+	0	-
1-3	+	++	0	-	-
4	++	0	+	0	-
4.5	0	0	0	++	-
5	=	=	-	-	+++

See Table 5.15 for explanation of symbols.

6.2 THE INFLUENCE OF CHANGES IN (1) DAILY PATTERN OF NATURAL ILLUMINATION AND (2) DENSITY OF ADULTS IN POPULATION CAGES ON THE DIFFERENCES IN PROPORTION THAT MATE BETWEEN COLOUR-FORMS.

6.20 Introduction.

The greatest selective difference between colour-forms appears to be in the proportions that mate. During weeks 7-9 a similar proportion of all colour-forms mated. But differences between colour-forms were found in population cages B1 and C1 in weeks 12-13 and in all four population cages in weeks 16-18 and 25-26. In every instance where such differences were indicated, a higher proportion of grade 5 flies mated than did any of the remaining colour-forms. Correlated with the presence of this selective advantage possessed by grade 5 flies is (1) an increase in density of adults in population cages and (2) a decrease in the intensity of natural illumination received by the population cages due to the approach of winter.

The object of this section was to determine if the above selective advantage possessed by grade 5 flies was dependent upon a particular density of adults or intensity of illumination. Of these two changes, I considered the change in pattern of illumination to be most important. Barton Browne (1957) has

shown that frequency of copulation and age at which copulation first occurs in D. tryoni were influenced by the intensity and length of daily illumination.

Based on the evidence of these changes in the population cages, I set out to test the hypothesis that differences in the proportion that mated between colour-forms were dependent upon either the density of adults, the daily pattern of illumination, or both.

A factorial experiment was designed to compare the proportion of D. tryoni and D. neohumeralis that mated at two levels of illumination and three levels of density of adults.

In addition, estimates were made of the mortality of the two species to determine if differential mortality could account for any differences between species in the proportion that mate.

6.21 Description of factors tested: daily pattern of illumination, density of adults, and species of fly.

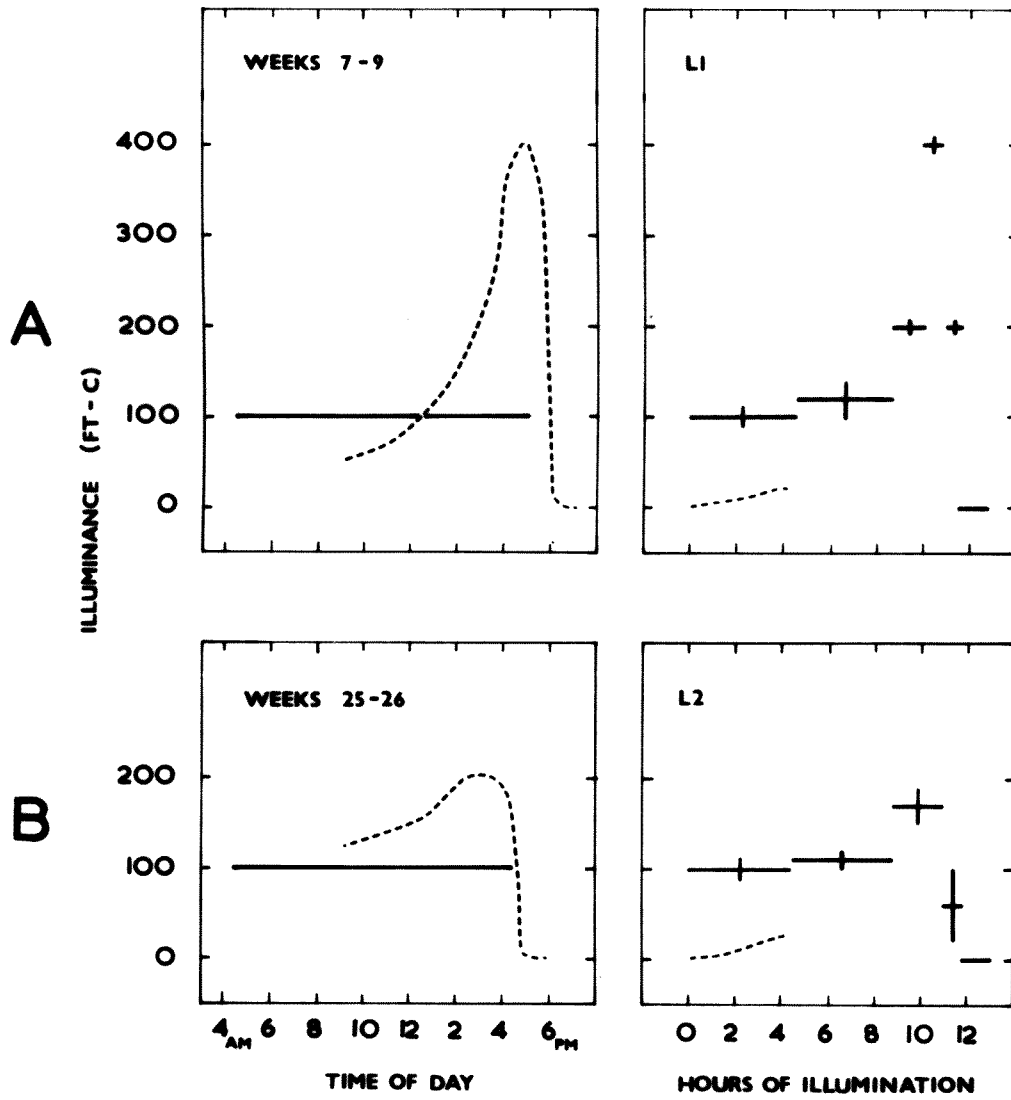
Daily pattern of illumination. Two daily patterns of artificial illumination were chosen. One pattern, called L1, reached a maximum intensity of illumination of 400 ft-c and resembled the pattern of illumination received by the population cages in weeks 7-9 (Figure 6.03A). The second pattern, L2, reached a maximum intensity of 200 ft-c and resembled the pattern of illumination received in weeks 25-26 by the population cages (Figure 6.03B).

FIGURE 6.03

Daily patterns of illumination received by the population cages in weeks 7-9 and 25-26, and by the experimental cages. The horizontal solid lines show the intensity of artificial illumination, the broken lines show the intensity of natural illumination, the vertical solid lines show the variation in intensity of artificial illumination received by different areas in the experimental cages.

- A. Showing the intensity of illumination received by the population cages in weeks 7-9 and by the experimental cages receiving the L_1 pattern of illumination.

- B. Showing the intensity of illumination received by the population cages in weeks 25-26 and by the experimental cages receiving the L_2 pattern of illumination.



The length of day during weeks 7-9 was about one hour longer than in weeks 25-26. This difference could not be included into the plan of the experiment as both patterns of illumination were given in the same room and each had to have the same length of day. The length of day for L1 and L2 was $11\frac{1}{2}$ hours followed by a 70 minute dusk.

As shown in Figure 6.03, a low intensity of natural illumination was present in patterns L1 and L2. This unavoidable source was less than 25 ft-c and was received only during the first $4\frac{1}{2}$ hours of each day.

Density of adults. A wide range of densities of adults was encountered when samples of copulating flies were removed from the population cages. Table 6.03 shows the estimated density of adults in each population cage when samples of copulating flies were taken. In view of these densities, three levels were chosen for the experiment: 0.5, 1.0, and 2.0 adults per cm^2 of cage surface.

Small cages, measuring 15 x 13 x 24.5 cm were used in the experiment. The required number for each density was 850, 1,700, and 3,400 adults per cage. These densities are referred to as D1, D2, and D3. The lowest density, D1, was to represent that density of adults found prior to week 10, while the two higher densities, D2 and D3 were to represent those found in the population cages in weeks 16-18 and 25-26 (Table 6.03).

TABLE 6.03 Estimated maximum density of adults in each population cage when the proportion of each colour-form that mated was determined.

Sampling period	Density of adults (number/cm ² of cage surface)			
	B1	B2	C1	C2
Weeks 7-9	0.61	0.60	0.61	0.62
Weeks 12-13	0.43	0.65	0.90	0.66
Weeks 16-18	0.92	1.15	1.85	1.56
Weeks 25-26	1.93	2.07	2.89	2.13

Species of fly. Equal proportions of D. tryoni and D. neohumeralis were used in an estimated 1:1 sex ratio. The two species were used because large numbers of the five colour-forms were difficult to obtain. Thus I expected D. tryoni to perform as grade 5 flies and D. neohumeralis to perform as grade 0 flies.

Both species were kept in the same experimental cage. The factorial design of the experiment was 2(species) x 2(sex) x 3(density) x 2(pattern of illumination). Each treatment was replicated four times. The design of the experiment is illustrated in Table 6.04.

The experiment was carried out at 25°C ($\pm 1^\circ$), and at a relative humidity of 70% ($\pm 15\%$).

TABLE 6.04 Design of experiment. Males and females of both species were kept in one cage. The six kinds of experimental cages were replicated four times.

Abbreviations: N = D. neohumeralis, T = D. tryoni.

L ₁ (bright pattern)	D ₁ (850/cage)	♂ N	approx. 212 flies
		♂ T	" " "
	D ₂ (1,700/cage)	♂ N	approx. 425 flies
		♂ T	" " "
	D ₃ (3,400/cage)	♂ N	approx. 850 flies
		♂ T	" " "
L ₂ (dim pattern)	D ₁ (850/cage)	♀ N	approx. 212 flies
		♀ T	" " "
	D ₂ (1,700/cage)	♀ N	approx. 425 flies
		♀ T	" " "
	D ₃ (3,400/cage)	♀ N	approx. 850 flies
		♀ T	" " "

6.22 Methods.

Large numbers of both species were obtained for the experiment from stocks kept in the laboratory for over a year and originally collected at Cairns. The flies for the experiment were from larvae reared at an optimal density. A sample of 500 adults was taken from each species to determine if either deviated from a 1:1 sex ratio. Table 6.05 shows that the sex ratio of the two species did not deviate significantly from the expected 1:1 ratio.

Following the estimation of the sex ratio, each sample of 500 adults was weighed and the average weight per fly of each species was determined. The total weight of each species necessary to give the correct density in each cage was determined from these weights. The required number of each species, as estimated by weight, was then placed into each cage.

Each cage was supplied with a similar amount of sugar, water, and protein hydrolyzate, regardless of the density of adults.

At the time the experimental cages were established, the flies were 1-7 days old; the oldest just approaching sexual maturity.

Copulation commenced after 9:30 am and continued until darkness at 5:10 pm. During this period the cages were searched at 10 to 15 minute intervals and all flies observed in copula were removed. Copulating flies were held in glass tubes for

TABLE 6.05 Number of males and females in a sample of 500 adults from each of the two species used in the experiment on proportion that mate. The sex ratio of each species did not deviate significantly from a 1:1 ratio.

Species	Females	Males	Chi-square	P
<u>D. tryoni</u>	246	254	1.35	> 0.10
<u>D. neohumeralis</u>	263	237	0.13	> 0.50

identification at the end of each day. They were returned to their cages early the following morning. Only one half of the total number of cages was sampled on any one day. Replicates 1 and 2 were sampled on alternate days to replicates 3 and 4.

The sampling continued over 36 consecutive days. Nearly all the pairs that copulated in the cages, when under observation, were recorded.

Dead flies were removed at 12 day intervals. The number of deaths in each experimental cage was estimated by dividing the total weight of dead flies by the mean weight per dead fly (from a sample of 100 dead flies). The percentage of each species by sex in a sample of 100 was multiplied by the total number of dead flies to provide an estimate of the total number of deaths of each species by sex for each experimental cage.

6.23 The percentage of *D. tryoni* and *D. neohumeralis* that mated.

The number of each species that copulated was expressed as a percentage of the original number of each species in the experimental cages. These percentages were transformed to $\sqrt{\text{percentage} + 0.5}$ for the analysis of variance. This analysis showed that the main effects of density and species were significant ($P < .005$). There was also a significant interaction between species and pattern of illumination ($P < .01$). The percentages and table of analysis of variance are given in Appendix L.

Table 6.06 shows that a higher percentage of all flies mated in D1 than in the remaining densities. Differences between transformed means were compared to the least significant difference based on the error variance. Only the difference between D1 and D3 was significant.

The percentage of the males and females of the two species that mated in each treatment is shown in Table 6.07. In every comparison between species, the percentage of D. tryoni that mated exceeded that of D. neohumeralis.

The significant interaction between species and pattern of illumination means that the difference between species in the percentage that copulated was dependent upon the pattern of illumination. This relationship is shown in Table 6.08. The percentage of D. tryoni copulating greatly exceeds that of D. neohumeralis in the highest intensity of illumination, L1. But in L2, the difference is not as great although a significantly higher percentage of D. tryoni copulated.

TABLE 6.06 Mean percentage of flies that copulated at each density.

Density	Percentage of flies copulating	Differences between means.	*
D1	13.1	D1 - D2	ns
D2	9.9	D2 - D3	ns
D3	6.3	D1 - D3	P < .01

* - from least significant difference based on error variance.

TABLE 6.07 The percentage of each species that copulated.

Each percentage is the mean of four replicates.

Pattern of illumination	Density	Sex	Species	
			<u>D. try.</u>	<u>D. neo.</u>
L1	D1	Males	19.7	9.6
		Females	20.9	7.4
	D2	Males	16.5	5.1
		Females	16.0	5.9
	D3	Males	9.6	3.1
		Females	9.8	3.7
L2	D1	Males	16.2	11.4
		Females	17.1	9.5
	D2	Males	13.1	7.0
		Females	13.2	7.5
	D3	Males	10.3	4.1
		Females	10.5	3.8

TABLE 6.08 Mean percentage of the number of each species
that copulated under each pattern of illumination.

Pattern of illumination	Species		Differences between species *
	<u>D. try.</u>	<u>D. neo.</u>	
L1	15.0	5.2	P < .001
L2	12.9	6.9	P < .02

* - from least significant difference based on error variance.

6.24 Mortality of *D. tryoni* and *D. neohumeralis* during the period of sampling for copulating flies.

An analysis of variance, identical to that used to analyse the percentage of each species mating, was completed on the percentage of each species that died during the period of sampling for copulating flies. For this analysis, the percentages were transformed to $\arcsin \sqrt{\text{percentage}}$. The percentages and the table of analysis of variance are given in Appendix M.

The analysis of variance showed that the main effects of density of adults and sex were significant ($P < .005$). In addition, there were significant interactions between pattern of illumination and density, density and species, and density, species, and sex.

This analysis was mainly concerned with differences between species. Therefore, the significant second order interaction between species, sex, and density is most important. This interaction indicates that there were differences between species but that they were restricted to one sex at certain densities.

Differences between species by sex and density are shown in Table 6.09. These differences were compared to the least significant difference based on the error variance. Only the difference between males of the two species was significant at the highest density, D 3. The percentage mortality of *D. neohumeralis* males was significantly greater than that of *D. tryoni* males.

TABLE 6.09. Mean percentage mortality of each species by sex and density.

Density	Sex	Species		Difference between species.	*
		<u>D. neo.</u>	<u>D. try.</u>		
D1	Male	50.7%	56.1%	ns	
	Female	40.8	42.0	ns	
D2	Male	53.5	63.2	ns	
	Female	50.4	46.8	ns	
D3	Male	86.7	71.0	P < .02	
	Female	59.5	64.3		

* - from least significant difference based upon the error variance.

ns = P > .05

In summary, a higher percentage of D. tryoni copulated than D. neohumeralis at all densities of adults and patterns of illumination. But the difference between species was reduced significantly at the lowest intensity of illumination. An examination of the mortality of the two species during the period of sampling for copulating flies revealed that at the highest density of adults only the mortality of D. neohumeralis males was significantly greater than D. tryoni males. The differences between species in the proportion that mated was not the result of a differential mortality of adults.

Relating these results to the population cages, it is likely that the higher proportion of grade 5 flies (resembling D. tryoni) that mated was not dependent upon either a particular density of adults or intensity of illumination. But the selective advantage due to the proportion that mated, possessed by grade 5 flies relative to grade 0 flies, was most likely reduced with the low intensity of illumination found in weeks 25-26.

6.3 THE SUCCESS OF MALES IN LOCATING FEMALES WILLING TO MATE AS A CAUSE OF THE DIFFERENCES IN THE PROPORTION THAT MATE BETWEEN SPECIES AND COLOUR-FORMS.

6.30 Introduction.

Experiments described in this section were undertaken to account for the differences in the proportion that mate between D. tryoni and D. neohumeralis and between the five colour-forms. These experiments showed how sexually active males locate females willing to mate in crowded laboratory populations, and how differences in the daily duration of sexual activity influenced the probability of a male locating a receptive female thus influencing the proportion that mate.

6.31 A comparison of the ability of males of D. tryoni and D. neohumeralis to locate females willing to mate.

Introduction. The greater success at mating of D. tryoni could be accounted for, if the males were able to locate their own receptive females more readily than D. neohumeralis. Receptive females are females willing to mate.

Sexual activity in the males of both species is marked by an audible "calling" (Myers, 1952; Gibbs, 1965). Monro (1953) has shown that in D. tryoni stridulation is produced by a rapid vibration of the wings causing the anal lobes of the wings to strike

along two rows of bristles on the third abdominal tergite. Myers (1952) has observed with D. tryoni that stridulation stimulates mature females to approach the "calling" males in the laboratory and after a variable pattern of movements, the male attempts copulation with the female.

In crowded population cages with one or more adults per 2 cm² of cage surface, this pattern of behaviour was not observed. Stridulating males were seen but females were rarely found to approach the "calling" males. These males, after a short period of stridulation, would either fly off to another part of the cage or would attempt copulation with a nearby adult, sometimes another male.

The hypothesis I tested was that in crowded laboratory populations, the males of both species locate receptive females by chance attempts at copulation with nearby adults, regardless of sex.

To test this hypothesis, two experimental cages were established; one contained several thousand D. tryoni and the other contained a similar number of D. neohumeralis. These cages were observed during the period of sexual activity. The sex ratio of the individuals upon which attempts at copulation were made was recorded. This sex ratio was compared to the sex ratio of a sample of adults taken at random from the same cage.

Results. Table 6.10 shows that the males of both species attempted to copulate with both males and females in a ratio that did not differ significantly from the sex ratio of the adults in the experimental cages. This information indicates that (1) the males of the two species were unable to distinguish between the sexes prior to an attempt at copulation, and (2) the females did not approach the "calling" males. If any of these events had occurred, there would have been more females among the individuals upon which attempts at copulation were made than among the adults in the experimental cage.

It appears likely that in crowded laboratory populations with a number of males stridulating at the same time in different locations of the cage that the females cannot locate any particular "calling" male. Therefore, in crowded populations, males locate females willing to mate by chance attempts at copulation.

TABLE 6.10 Comparison of the sex ratio of adults upon which attempts at copulation were made with the sex ratio of a sample of adults from the cage. The comparison was made for a cage of D. tryoni and a cage of D. neohumeralis.

<u>D. neohumeralis.</u>	Males	Females	Total
Males attempted copulation with the following number of each sex.	49	59	108
The following number of each sex was taken at random from the experimental cage.	83	90	173
Total	132	149	281

Chi-square = .09, P > .750

<u>D. tryoni.</u>	Males	Females	Total
Males attempted copulation with the following number of each sex.	45	40	85
The following number of each sex was taken at random from the experimental cage.	106	74	180
Total	151	114	265

Chi-square = 0.16, P > .500

6.32 Duration of daily sexual activity.

Introduction. If males locate receptive females by chance attempts at copulation, then any mechanism increasing the probability of a male locating a receptive female would increase the proportion of flies that mated. One such mechanism would be to have all the sexually active flies receptive at the same time during the day.

Consider the probability of a male locating by chance a receptive female in two populations, A and B. In population A, only 10% of the sexually active flies are receptive at any one time each day. In population B, all the sexually active flies are receptive at the same time. The probability of a male locating by chance a receptive female is much greater in population B than in population A. Mating in A would occur at a low frequency over an extended period of each day, while in population B, mating would occur at a high frequency over a short period.

Myers (1952) has shown that D. tryoni mated at dusk. Gibbs (1965) found that D. neohumeralis mated between 8:30 am and 2:00 pm, with the majority of copulations occurring between 9:30 am and 10:30 am and that D. tryoni copulated between 6:00 pm and 6:30 pm. The observations by Gibbs were made from samples of less than 25 pairs of each species.

This information suggests that the time when all D. tryoni flies are willing to mate coincides during a short period each day, and that perhaps individuals of D. neohumeralis are sexually active at different times from each other during the day. If this is so, then the probability of a male locating a receptive female is greater for D. tryoni than for D. neohumeralis. This could account for the higher proportion of D. tryoni that mated.

Therefore it becomes necessary to describe the duration of sexual activity during the day of D. tryoni, D. neohumeralis, and the five colour-forms. I used the duration of daily time of mating as an indication of the time of sexual activity.

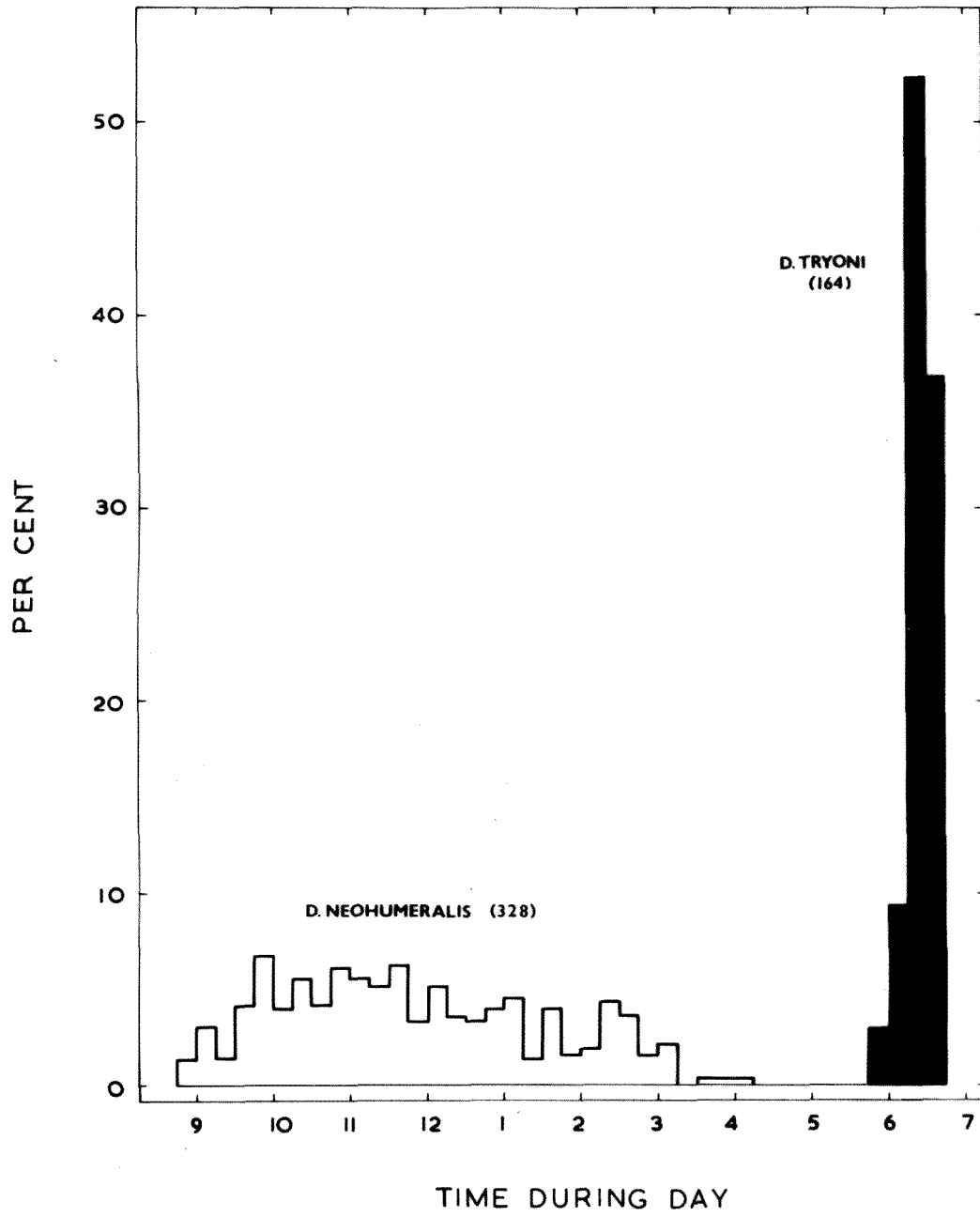
Several thousand of each species were established separately in two large experimental cages. Large numbers of copulating flies were collected over several days and the number of copulations was recorded at 15 minute intervals. Similar information for the five colour-forms was available from the large sample of copulating flies collected from the experimental cage of F2 generation adults described in Section 6.1.

Duration of daily sexual activity of D. tryoni and D. neohumeralis.

The number of each species copulating at fifteen minute intervals (Appendix N) was expressed as a percentage of the total number of that species that copulated. Figure 6.04 shows that D. neohumeralis mated between 8:45 am and 4:15 pm.

FIGURE 6.04

The percentage of D. tryoni and D. neohumeralis that copulated in each 15 minute interval during the day. Each species was kept in a separate cage. The number of pairs of each species sampled is shown in parentheses.



During this $7\frac{1}{2}$ hour period, no more than 7% of the copulations occurred in any one 15 minute interval. D. tryoni mated during a one hour period from 5:45 pm to 6:45 pm. Over 80% of the copulations occurred after 6:15 pm.

Barton Browne (1956) concluded that the daily period of sexual activity of D. tryoni was limited. When he subjected D. tryoni to a constant artificial dusk at the end of a $7\frac{1}{2}$ hour day, the frequency of "calling" males and attempts at copulation reached a maximum 10 to 20 minutes after the start of dusk and then decreased to a low frequency at the end of a total of 35 minutes. This suggests that an individual D. tryoni will remain willing to mate for a daily period of about 35 minutes.

As D. tryoni mate over a short interval of about an hour each day, the time of sexual activity of nearly all the receptive flies coincides. But D. neohumeralis mate over a $7\frac{1}{2}$ hour day period each day and it is likely that only a portion of those flies that are receptive on a particular day are sexually active at the same time. If the probability of a D. tryoni male locating a receptive female were greater than that of D. neohumeralis, then a higher percentage of the attempts by D. tryoni would be successful.

When the ability of the males of the two species to locate females was compared (Section 6.31), the number of successes by males of the two species was recorded. Table 6.11 shows the ratio of successful to unsuccessful attempts at copulations with

TABLE 6.11. Showing the ratio of successful to unsuccessful attempts at copulation by D. tryoni and D. neohumeralis males. Only attempts with females were considered. The two ratios differ by more than chance (Chi-square = 7.06, $P < .01$).

	Successful	Unsuccessful	Total
<u>D. neohumeralis</u>	1	80	81
<u>D. tryoni</u>	9	57	66
Total	10	137	147

females by the males of the two species. The two ratios differ from each other by more than chance ($P < .01$). Of the attempts made by D. tryoni males, 13.6% were successful. Only 1.2% of the attempts by D. neohumeralis were successful.

Duration of daily sexual activity of the five colour-forms.

Information on the duration of daily sexual activity of the five colour-forms was taken from the large sample of copulating flies collected from the experimental cage containing several thousand adults of an F2 generation from an interspecific cross (see Section 6.1). Only homogamic matings by three groups of colour-forms, grades 0-4, 4.5, and 5, were considered. Grades 0, 1-3, and 4 were combined because (1) each of these colour-forms mated at a similar time and (2) the homogamic matings by any of these colour-forms was scarce.

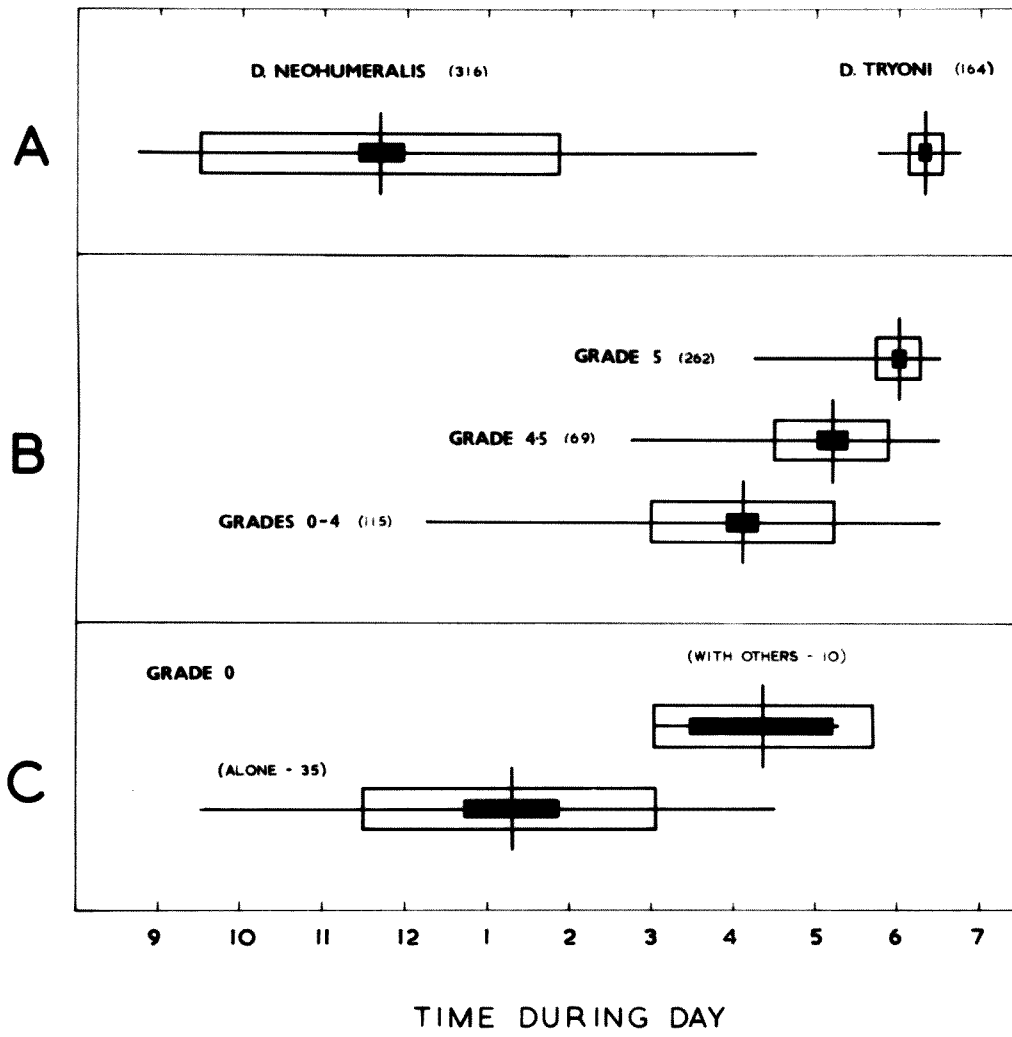
Figure 6.05 A and B show that the daily duration of mating of the grade 5 flies occurred over a restricted time, similar to D. tryoni. The daily duration of mating of grades 0-4 and 4.5 occurred over a longer period, but not to the extent of D. neohumeralis.

There is some evidence which suggests that the time of sexual activity of grades 0-4 precedes their time of mating during the day by several hours. Figure 6.05C shows that the mean time of mating of grade 0 flies when in a cage with other colour-forms was 4:15 pm, but in the absence of the other colour-forms, their mean time of mating was 1:15 pm. This suggests that when grade 0 flies are with the other colour-forms they are unable to locate a mate when

FIGURE 6.05

A comparison of the daily duration of time of mating of D. tryoni, D. neohumeralis, and the colour-forms. The range in time of mating is shown by the horizontal line; the mean time of mating is shown by the vertical line. The hollow rectangle shows one standard deviation either side of the mean. The solid rectangle indicates 95% confidence limits on the mean. The number of pairs sampled is shown in parentheses.

- A. Daily duration of time of mating of D. tryoni and D. neohumeralis.
- B. Daily duration of time of mating of grades 0-4, 4.5, and 5, when together in the same cage.
- C. Daily duration of time of mating of grade 0 when in a cage with the remaining colour-forms, and when in a cage without the other colour-forms present.



they first become sexual active in the day. Only those that remain sexually active later in the day succeed in finding a mate.

Appendix O gives the number of pairs copulating at 15 minute intervals for grades 0-4, 4.5, 5, grade 0 in the presence of other colour-forms (6.6% of the population), and grade 0 flies without any other colour-forms in the population.

To summarize, the daily duration of sexual activity is restricted for D. tryoni and for grade 5 flies in the presence of other colour-forms. The daily time of sexual activity of most of these flies must, as a result, coincide. The probability of a sexually active D. tryoni or grade 5 male locating a receptive female by chance attempts at copulation is greater than for D. neohumeralis and grades 0 to 4.5. These latter flies are sexually active over a longer period each day and thus a lower proportion are sexually active at any one time.

Duration of daily sexual activity of adults in population cages B1, B2, C1, and C2, after 96 weeks of selection.

If a restricted time of mating provides a selective advantage in crowded laboratory populations, then those adults in the population cages in week 96 should mate within a restricted period of each day.

Several thousand newly emerged adults from the Brisbane population cages were placed into one large cage and a similar number from the Cairns population cages was placed into a second

cake. In addition, several thousand D. tryoni were placed into a third cage so that the time of mating of the individuals from the population cages from both localities could be compared to that of D. tryoni.

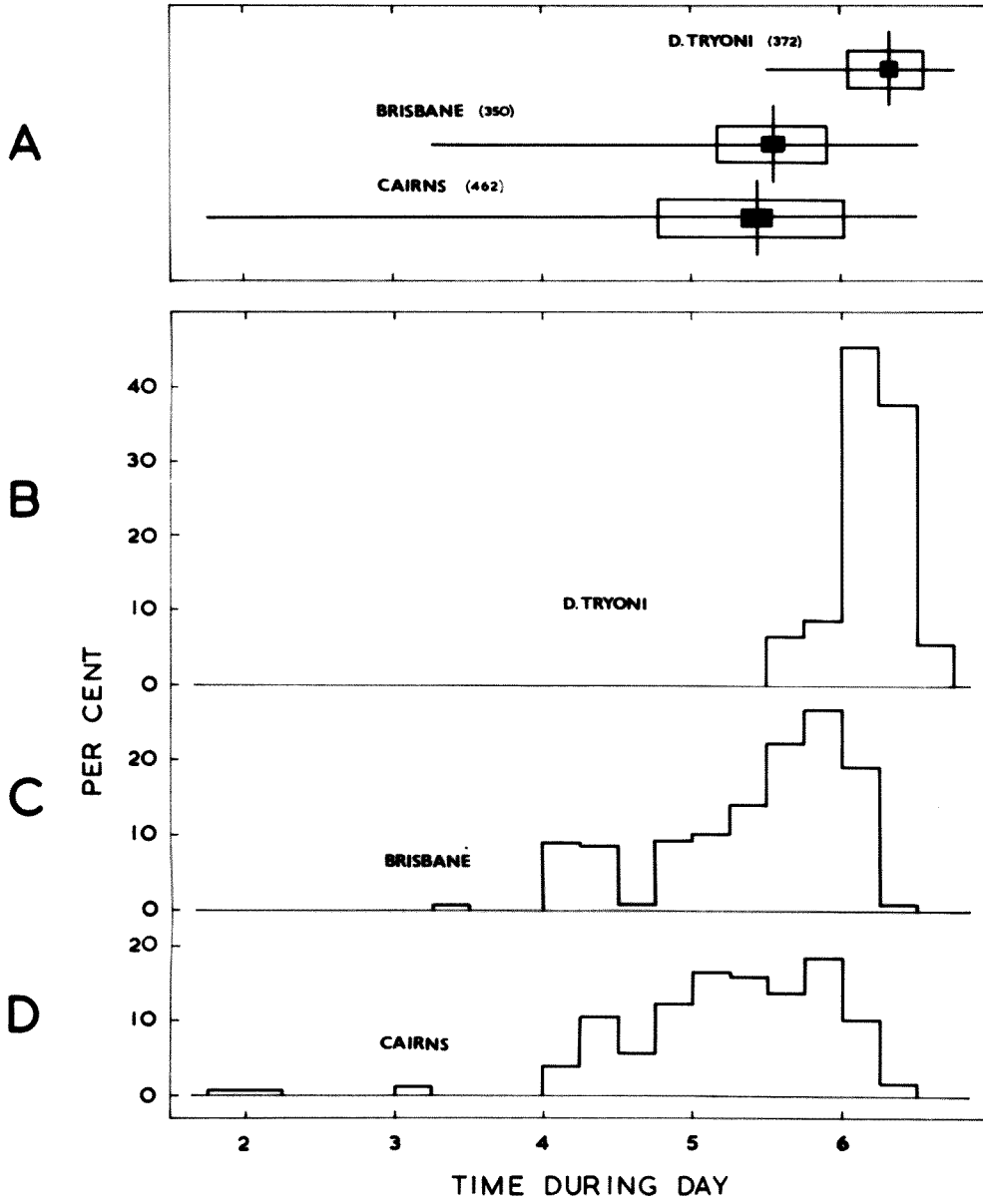
The mean time of mating of the flies from the population cages from each locality was similar (Figure 6.06) but occurred some 45 minutes earlier in the day than that of D. tryoni. The flies from the population cages did mate within a restricted period of time but not to the same extent as D. tryoni. There were no apparent differences in the time of mating during the day between colour-forms in the population cages.

The number of matings, at 15 minute intervals, of D. tryoni and the flies from the population cages from both localities in week 96 is given in Appendix P.

FIGURE 6.06

Daily duration of time of mating of D. tryoni and flies from the Brisbane and Cairns population cages in week 96.

- A. The mean time of mating (vertical line) and range in time of mating (horizontal line) during the day of D. tryoni and flies from the Brisbane and Cairns population cages. The hollow rectangles are one standard deviation either side of the mean. The solid rectangles indicate 95% confidence limits on the mean.
- B. The percentage of D. tryoni that mated in each 15 minute interval during the day.
- C. The percentage of flies from the Brisbane population cages that mated in each 15 minute interval during the day.
- D. The percentage of flies from the Cairns population cages that mated in each 15 minute interval during the day.



Section 7.

DISCUSSION.

Differences in mating appear to be the greatest source of selective differences between colour-forms in the population cages. Following is a discussion of (1) the four components of mating: time of mating, frequency of homogamic mating, method of locating a mate, and proportion that mate, and (2) how the selective differences resulting from mating are influenced by environment.

7.1 TIME OF MATING.

Differences in time of mating between colour-forms were found in the population cages. Generally, the mean daily time of mating of grade 0 flies was earlier than that of grades 1-3, 4, and 4.5, while grade 5 flies mated latest during the day, at dusk. Gibbs (1965) found that differences in daily sexual activity between D. tryoni and D. neohumeralis were responsible for maintaining the discreteness of the two species in natural populations.

In this section I intend to show that the time of mating of certain colour-forms varied during the history of the population cages. This is an important point to establish because the following section shows that differences in time of mating between colour-forms influenced the frequency of homogamic matings by grade 5 flies and ultimately affected the rate of selection in population cages.

Grade 5 flies in population cages mated most frequently during late afternoon and dusk. Early in the history of the population cages, grades 0 to 4.5 flies mated in early and late afternoon. But in weeks 25-26, the time of mating of these colour-forms was found to be later in the day. In the Brisbane population cages, grades 0 to 4.5 mated at a similar time to grade 5 flies. In the Cairns population cages, grades 0 to 4.5 mated later in the day, closer to that of grade 5 flies. The time of mating of grades 0 to 4.5 flies, in week 96, was entirely within the range of the time of mating of grade 5 flies in both Brisbane and Cairns population cages.

The change in time of mating during the day by grades 0 to 4.5 could be the result of (1) a decrease in proportion of these flies in the population cages and (2) the low probability of a male locating a receptive female (Section 6.32). As the proportion of grades 0 to 4.5 decreases in the population cages

so does the probability of a grade 0 to 4.5 male locating a female. It then becomes even more difficult for these colour-forms to locate a mate when they first become sexually active in the day. Only those individuals that remain sexually active later in the day will find a mate. In doing so, these flies approach the time of mating of grade 5 flies, and mate with them in an increasing proportion, as the proportion of grades 0 to 4.5 decreases in the population cage.

The time of mating of grades 0 to 4.5 is flexible. The mean daily time of mating of grade 0 flies differed by three hours depending on whether or not other colour-forms were present (Section 6.32). Gibbs (1965) found that the time of mating of individuals from an F1 generation (mostly grade 4 flies) from an interspecific cross was between 3:30 pm and 6:00 pm. When F1 males were confined with D. tryoni females in one cage and the reciprocal of this cross confined in another, nearly every mating occurred within the time when D. tryoni normally mated; between 6:00 pm and 6:30 pm.

The difficulty of grades 0 to 4.5 flies in locating a mate when in a low proportion in the population is analogous to situations in natural populations where individuals occur at the edge or beyond the range of distribution for their species. The feature in common to the population cages and to natural populations is that there is not an adequate number of mates of their own kind. In the absence of individuals of their

own kind they may be influenced by, and respond to, the mating behaviour of other closely related species (Mayr, 1963).

Therefore, the time of mating of each of the grades 0 to 4.5, when together with grade 5 flies in the same population cage, is variable and is dependent upon the proportion of each colour-form in the population cage.

7.2 FREQUENCY OF HOMOGAMIC MATINGS.

Any mechanism increasing the number of homogamic matings by grades 0 and 5 flies will increase the proportions of grades 0 and 5 and decrease the proportions of the intermediate colour-forms in the succeeding generation. In samples of copulating flies collected from the population cages, the number of homogamic matings, particularly by grade 5 flies, was greater than expected. The expected was based on the assumption that all flies in the sample mated at random. Differences in time of mating between colour-forms were the cause of such deviations. This was revealed in an analysis of the time of mating of the colour-forms and the frequency of all combinations of matings (Section 6.1).

In view of the selective differences in the proportion that mate and the rate of birth between colour-forms, the increased number of homogamic matings by grade 5 flies influenced

the rate of selection by increasing the proportion, in the succeeding generation, of those flies having the greatest selective advantage; grade 5.

The occurrence of an increased number of homogamic matings by grade 5 flies is dependent upon differences in time of mating between colour-forms. The latter, is influenced by the proportions of colour-forms in the population cage.

7.3 THE WAY MALES LOCATE FEMALES WILLING TO MATE.

A knowledge of how mates are chosen in the population cages is essential in understanding the selective advantage possessed by the grade 5 flies.

Apparently, in the population cages, males located receptive females by chance attempts at copulation with nearby adults, without discriminating between either sex or colour-form. I have shown that neither D. tryoni nor D. neohumeralis males were able to distinguish between females and other males of their own species (Section 6.31). Gibbs (1965) has concluded that discrimination between species by males of either species is very unlikely.

The inability of males to distinguish between females of closely related species and their own females occurs in Drosophila

(Mayr, 1946; Wallace and Dobzhansky, 1946). Thomas (1950) has shown that males of the genus Sarcophaga (Diptera) were unable to distinguish between females of their species and those of other species. But Miller (1950) has shown that the males of Drosophila affinis and D. algonquin were able to distinguish between females of either species before an attempt at copulation was made. In addition, males of some species of Drosophila are unable to distinguish between females and other males of their own species (Rendel, 1945; Wallace and Dobzansky, 1946; and Spieth, 1949).

It is likely that females in the population cages discriminated between certain forms of males. Gibbs (1965) has shown that in cages in which D. tryoni and D. neohumeralis were crossed with intermediate colour-forms and with each other, the percentage of females inseminated was dependent upon the difference in time of sexual activity between the males and females in each cross. Observations on mating behaviour in these crosses showed that the low proportion of females inseminated in some crosses was due to the vigorous resistance of females to attempts at copulation outside their normal time of sexual activity.

Barton Browne (1954) has shown that inseminated females of D. tryoni actively resist attempts at copulation. He concluded that females rarely copulated more than once, even in confined laboratory populations.

The discrimination of females against certain males of a particular genotype of the same species has been observed in Drosophila melanogaster (Sturtevant, 1915; Diederich, 1941; Merrell, 1949; Reed and Reed, 1950; Bastock, 1956; Barker, 1962), in D. subobscura (Rendel, 1945), and D. pseudoobscura (Tan, 1946). In such cases, females will prefer the wild type to those containing a particular sex-linked mutant gene. Evidence of females discriminating between their own males and males of another species in Drosophila is provided by Mayr (1950).

To summarize, in the population cages, males attempted to copulate with flies without discrimination between either sex or colour-form. Females, however were highly selective and the majority only permitted copulation if they were virgin, mature, and were approached at a time during the day when they were receptive.

7.4 THE PROPORTION THAT MATE.

A higher proportion of grade 5 flies mated in the population cages than any of the other colour-forms. This however, did not occur until after week 15 in the history of the population cages. This selective advantage occurred independently of the changes in density of adults and the intensity of natural illumination. Similarly, Gibbs (1965) found that a higher proportion of D. tryoni mated than D. neohumeralis because three times as many D. tryoni females were inseminated.

I am unable to determine from the data on the proportion of each colour-form that mated whether a higher proportion of grade 5 flies actually mated or whether there were multiple matings by the same individuals. It would appear that multiple matings by any of the flies in the population cages, especially females, were rare because: (1) inseminated females actively repell attempts at copulation (Barton Browne, 1954), and (2) the frequency of flies copulating in the population cages was low; about 25 to 50 pairs a day. Therefore, it is very likely that any mechanism increasing the frequency of mating will almost directly increase the proportion that mate.

My hypothesis to account for the occurrence of this selective difference is that grade 5 flies mate over a relatively shorter daily period than the other colour-forms, increasing the probability of a male locating a receptive female by chance attempts at copulation.

Considered from the point of view of the females, the percentage of attempts at copulation occurring while the females were receptive was less for grades 0 to 4.5 than for grade 5 females. This is because the daily time of sexual activity of grade 5 flies coincides to a greater extent than that of the remaining grades. Because of the successful discrimination of the females against males attempting to copulate outside the daily period of receptiveness of the female, a lower proportion of grades 0 to 4.5 flies mated.

Other workers, in describing the selective differences between genotypes of the same species, have found differences in the proportion that mate between genotypes of Drosophila pseudoobscura (Wallace, 1948), D. persimilis (Spiess and Langer, 1961), and in Tribolium confusium (McDonald and Peer, 1961).

One feature of the data on the proportion that mate requires further clarification. In weeks 7-9 a similar proportion of all colour-forms mated, even though grade 5 flies mated over a restricted daily period. Possibly, grade 5 flies were at a slight disadvantage at this time in the history of the population cages. This disadvantage could be explained by the following information: (1) grade 5 flies were about 15% of the flies in each population cage, (2) grade 5 flies mate most frequently at dusk, and (3) all matings cease with the onset of darkness (Barton Browne, 1954).

Because grade 5 flies were low in proportion and mated at a time when few others were sexually active, they may have required a longer than normal time to find a mate. This time was not available as these flies mate just at dusk and mating does not occur in darkness. Hence, it may have been that fewer grade 5 flies found mates and the proportion that mated was reduced to a level similar to that of the other colour-forms. If this is so, the selective advantage possessed by the grade 5 flies in their frequency of mating is dependent upon the

proportions of colour-forms in the population.

Petit (1951) found that the selective advantage, resulting from a high frequency of mating by the wild type of D. melanogaster when compared with a mutant, was influenced by the proportion of wild to mutant males in the population. When the wild type males were less than 20% of the males, they were strongly preferred as mates. But when these males were in the majority, this preference was greatly reduced.

7.5 MATING A CAUSE OF SELECTIVE DIFFERENCES.

Two features of mating that resulted in selective advantages to grade 5 flies were (1) an increased number of homogamic matings and (2) a higher frequency of mating.

The importance of the process of mating as a selective force has been demonstrated by Merrell (1949). He found with Drosophila melanogaster that the females discriminated against males bearing one of several mutant sex-linked genes and favoured the wild type. Later, Merrell (1953) predicted the change in frequency of these mutant types when placed together with wild types in laboratory populations. These predictions were based on the relative ability of the wild type and mutants to mate. The observed changes were similar to the expected, indicating that selective mating was primarily responsible for the decline in frequency of the mutants. Similarly, Diederich (1941)

Reed and Reed (1950), Petit (1951), and Merrell and Underhill (1956) have shown that selective mating can result in changes of gene frequency in populations of D. melanogaster.

7.6 COMPONENTS OF ENVIRONMENT THAT INFLUENCE SELECTION IN POPULATION CAGES.

Two components of environment influenced the selective advantages possessed by grade 5 flies in population cages. Selective advantages resulting from mating were influenced by the intensity of natural illumination (i.e. a component of weather), and by the proportions of colour-forms in the population cages (i.e. other individuals).

The components of the environment, as classified by Andrewartha and Birch (1954), that are relevant in studies of laboratory populations of this sort are (1) weather, (light, humidity, and temperature), (2) food, and (3) other individuals of the same species.

(1) Weather. Moore (1952) found in populations of Drosophila melanogaster and D. simulans kept at 15°C, that D. simulans was favoured by selection. But at 25°C D. melanogaster was the successful species. Similarly, Birch (1953) showed that at 29.1°C, Calandra oryzae ("small strain") was the successful species when living together in maize with Rhizopertha dominica. The outcome of selection was reversed at 32.2°C. Birch (1961) founded populations with Dacus tryoni and D. neohumeralis at 25°C. Neohumeralis-like individuals were favoured by selection more than tryoni-like individuals. At

22°C the outcome of selection was reversed.

Dobzhansky and Spassky (1944) and Levitan (1951) found that selective differences between karyotypes of some species of Drosophila were influenced by temperature. Wright and Dobzhansky (1946) found that at 25°C, laboratory populations containing an equal proportion of the two gene arrangements ST and CH, ST increased to about 70% of the populations. But at 16.5°C there was little selective differences between the two and both remained in a similar proportion in the population.

Park (1954) found with populations containing Tribolium confusium and T. castaneum kept at 24°C and 30% relative humidity that T. castaneum was eliminated. At 34°C and 70% relative humidity T. confusium was eliminated.

(2) Resources. The outcome of selection between chromosomal types of Drosophila pseudoobscura in laboratory populations was influenced by the kinds of microorganisms used in the food of adults and larvae (Da Cunha, 1951). Merrell (1951) found that variations in quality of food in populations containing Drosophila funebris and D. melanogaster were sufficient to alter the selective differences between species.

Birch (1953) showed that when the sibling species of Calandra oryzae, the "large and small strains", were placed together in the same population in the laboratory the "small strain"

was eliminated when kept in maize, but the "large strain" was eliminated when the population was reared in wheat. Selective differences in survival of larvae between karyotypes of D. willistoni were influenced by the type of fruit in which the larvae were reared (Birch and Battaglia, 1957).

(3) Other individuals of the same species. The selective advantage of an individual is influenced by the number and kind of other individuals living in the same population. This has been shown with different genotypes of larvae of some species of Drosophila (Lewontin, 1955; Lewontin and Matsou, 1963), and of the house fly Musca domestica (Bhalla and Sokal, 1964; and Sokal and Sullivan, 1963). The longevity of adults of Tribolium castaneum was influenced by the density of adults and the proportion of other genotypes in the population (Sokal and Huber, 1963; and Sokal and Karten, 1964).

Birch (1955) found with D. pseudoobscura that the selective advantage possessed by the gene arrangement CH when together with ST was dependent upon uncrowded densities of larvae. When CH and ST were reared together in crowded densities of larvae, ST was at a selective advantage. When larvae were uncrowded, CH was superior to ST.

The fitness of individuals of D. melanogaster in populations containing six karyotypes was influenced by the proportion of karyotypes in the population. (Levene, Pavlovsky, and Dobzhansky, 1954 and 1958). For example, individuals homozygous for the CH gene arrangement had a fitness superior to those homozygous for AR in the presence of ST. In the absence of ST, AR were superior to CH. Spiess (1957), Parsons (1959), and Tobarí (1964) have also shown an influence of the proportions of karyotypes on selection in populations of Drosophila.

7.7 THE OUTCOME OF SELECTION IN POPULATION CAGES CONTAINING THE FIVE COLOUR-FORMS.

At the end of 99 weeks, the proportions of colour-forms in the population cages were similar. Grade 0 flies, resembling D. neohumeralis were estimated to be 0.1% or less of the adults, while grade 5 flies, resembling D. tryoni, were estimated to be more than 90% of the adults. Selection is directional, as classified by Mather (1953), as one extreme in the range of variation in colour had been selected for at the expense of the other colour-forms. These results are similar to those reported by Lewontin and Birch (in press).

Selection in crowded populations kept by Birch (1961) founded with 80% D. tryoni and 20% D. neohumeralis would be classified by Mather (1953) as stabilizing. Although grade 0 flies were selected over grade 5 flies, the intermediate colour-forms consisted of over 60% of the flies in each population. Similar results were reported by Van Valen (1963) for populations containing Drosophila persimilis and D. pseudoobscura.

The occurrence of disruptive selection in such populations would appear to be very unlikely. Such selective action would favour with grades 0 and 5. Populations of Drosophila polymorphic for a particular character, have been established and maintained through disruptive selection by Thoday (1958, 1960), Thoday and Boam (1959), Millicent and Thoday (1961) and Thoday and Gibson (1962).

For such a selective action to occur in the populations that I kept, is remote because grade 0 flies are at the greatest selective disadvantage.

In populations kept with a crowded density of adults at 25°C by Birch (1961), grade 0 flies, called "neohumeralis", were selected for over grade 5 flies, called "tryoni". This outcome is the reverse of that found by Lewontin and Birch (in press) and that reported in this thesis. The differences between these experiments were: (1) the locality of the flies used in the population cages; Birch (1961) used flies collected at Cairns, while Lewontin and Birch (in press) used flies collected at Brisbane, and (2) the way in which the populations were founded and maintained.

I founded and maintained populations from flies collected at Brisbane and Cairns in a manner similar to that of Lewontin and Birch (in press). The outcome of selection was the same in all populations and thus occurred independently of locality. The results were similar to those found by Lewontin and Birch (in press). Therefore, it is likely that the difference in outcome of selection between Birch (1961) and Lewontin and Birch (in press) was due to the way the populations were founded and maintained.

These differences were: (1) Birch (1961) established populations with D. tryoni and D. neohumeralis (either 20% or 80% D. tryoni). Populations kept by Lewontin and Birch (in press) were founded with F1 hybrids from an interspecific cross. (2) Populations kept by Birch (1961) received an artificial "day" illumination followed by a 30 minute period of artificial dusk. Populations kept by Lewontin and Birch (in press) received both natural and artificial "day" illumination with a natural dusk of about a hour in length.

It is most likely that different durations of dusk illumination received by population cages was responsible for the different outcomes of selection. In crowded populations kept by Birch (1961), a 30 minute period of dusk illumination may not have been of sufficient duration for grade 5 flies to mate at their maximum frequency. The duration of dusk illumination was twice as long in populations described in this thesis and by Lewontin and Birch (in press). Such a duration of dusk allowed the grade 5 flies more time to mate, providing these flies with a selective advantage over the other colour-forms. Therefore, the selective advantages from mating, possessed by the grade 5 flies, is dependent upon a suitable duration of dusk illumination.

The effect of the proportions of colour-forms on the duration of dusk illumination required for a maximum number of grade 5 flies to mate was discussed in Section 7.4. Possibly,

the density of adults also influences the optimal duration of dusk illumination required by grade 5 flies. This is indicated by the following information. When I observed the frequency of mating of D. tryoni and D. neohumeralis (Section 6.2), the majority of matings by D. tryoni did not occur until the last 30 minutes of a 70 minute period of dusk. These observations were made for crowded densities of adults. When Barton Browne (1957) observed the time of mating of D. tryoni, the majority of matings occurred during the first 30 minutes of dusk. These observations were made in uncrowded densities of adults. It is possible that the time required by D. tryoni and grade 5 flies to locate a mate is longer in crowded densities than in uncrowded densities.

Thus when Birch (1961) kept population cages with crowded densities of adults with only a 30 minute period of dusk illumination, selection was against grade 5 flies (called "tryoni") regardless of whether the populations were initiated with 20% or 80% D. tryoni. But when Birch (1961) kept population cages with uncrowded densities of adults with a 30 minute period of dusk, selection favoured the grade 5 flies even when the populations were founded with only 20% D. tryoni.

Section 8.

SUMMARY.

1. The aims of this study were to (a) describe and analyse some of the selective differences between neohumeralis-like and tryoni-like colour-forms to account for their relative frequencies in laboratory populations and (b) account for differences in the outcome of selection between crowded laboratory populations containing the five colour-forms kept at 25°C by Birch (1961) and by Lewontin and Birch (in press).

Colour-forms are produced in interspecific crosses between D. neohumeralis and D. tryoni. The range in variation of colour of flies in the F2 generation of the above cross is such that individuals resembling both species are formed as well as a continuous range of intermediate colour-forms.

To analyse the selective differences between colour-forms, four population cages were established with F1 hybrids from an interspecific cross. Two population cages were founded with adults whose parents originated from stocks collected at Brisbane, called B1 and B2. The remaining two population cages, called C1 and C2, were founded with adults whose parents originated from stocks collected at Cairns. Each population cage was initiated with 300 adults and was allowed to increase in number on a restricted consistent weekly amount of larval and adult food.

The approach taken in analysing selective differences between colour-forms was direct. These differences were measured in the

population cages at the time when the proportion of each colour-form was changing. Thus the results from this investigation can be used directly to explain the changes in proportion of each colour-form in these population cages as well as the final outcome of selection.

2. Weekly estimates of the density of first instar larvae and the number of adults in the population cages. These estimates were made to record the variation in density of larvae and adults during the early history of the population cages when the proportion of each colour-form was changing. In general, the density of adults and larvae increased in the population cages until about week 25. Following this period, the density of larvae and the number of adults in the population cages fluctuated; the density of larvae from the optimal to 45 times greater than optimal, and the number of adults from about 2,000 per population cage to 20,000.

3. Changes in proportion of each colour-form in the population cages. Estimates of the proportion of each colour-form were made at regular intervals in the population cages to determine the relative success of the colour-forms and the outcome of selection. The greatest change occurred between weeks 15 to 39. Grades 0, 1-3, 4, and 4.5 decreased in proportion while grade 5 flies increased in proportion. By week 99, grades

0 and 1-3 were rare, grades 4 and 4.5 were each less than 5%, while grade 5 flies composed 90 to 95% of the adults.

4. Selective differences between colour-forms. To account for the changes in proportion of each colour-form in the population cages, selective differences between these colour-forms were analysed by examining survival of larvae, proportion that mated, rate of birth, and frequency of homogamic mating. These examinations were made within the confines of the population cages.

(i) Survival of larvae. The ratio of the number of each colour-form from larvae reared at an optimal density was compared with the ratio of the number of each colour-form from larvae reared in the crowded densities of the population cages. This comparison was made for each population cage at several intervals in their history. The comparisons showed it was unlikely that a selective mortality of larvae occurred in the population cages.

(ii) Proportion that mate. Periodically, in each population cage, the ratio of the number of each colour-form in a sample of copulating flies was compared with the ratio of colour-forms in a random sample of adults. No differences were found in samples taken in weeks 7-9, indicating that each colour-form mated with a similar frequency. But differences between ratios in the above comparison were found in population

cages B1 and C1 in weeks 12-13, and in all population cages in weeks 16-18 and 25-26. In every instance where differences were indicated, the proportion of grade 5 flies in the sample of copulating flies exceeded their proportion in the population cage, usually in excess of 1.5 times. Therefore, the proportion of grade 5 flies that mated was higher than any of the other colour-forms at such times.

(iii) Birth rate. Birth rates of the colour-forms were estimated by removing females from the population cages and recording the number of viable eggs laid per female per unit of time. There were no differences in birth rates between colour-forms when measured in weeks 8 and 10. Differences were found between colour-forms in week 19. Grades 4.5 and 5 females from both localities had a higher birth rate than did grade 0 females. In week 28, only Brisbane females were sampled. Grade 5 females had a higher birth rate than did grade 4.5 and a combined category of grades 0 and 1-3. Grade 4 females were not included in this analysis. The high birth rate of the grade 5 females was undoubtedly influenced by the high proportion of those females that mated.

(iv) Frequency of homogamic mating. An analysis of the samples of copulating flies collected from the population cages showed that the number of homogamic matings by grade 5 flies was greater than expected in every sample with the exception of the sample of Brisbane flies collected in weeks 25-26.

Differences in time of mating between grades 0 to 4.5 and grade 5 accounted for this greater than expected frequency. Such an increased frequency of homogamic matings by grade 5 flies increased their proportion in the succeeding generation and thus influenced the rate of selection for grade 5 flies.

5. **Analysis of selective differences between colour-forms.**
Several experiments were completed and observations were made to obtain further information about mating in the populations containing the five colour-forms. The aim was to identify what influenced the number of homogamic matings by grade 5 flies and the difference in the proportion that mated between colour-forms.

(1) The influence of differences in time of mating between colour-forms on the frequency of homogamic matings by grade 5 flies. A detailed examination of the time of mating of each colour-form when together in an experimental cage revealed that there were differences mainly between the grades 0 to 4.5 and 5 flies. Hence there was partial reproductive isolation between grade 5 and the other colour-forms, resulting in a greater than expected frequency of homogamic matings by grade 5 flies.

(ii) The influence of an increase in density of adults and a decrease in the intensity of natural illumination on the differences in the proportion that mated between colour-forms. The time when the grade 5 flies mated with the highest frequency was correlated with an increase in density of adults and a decrease in the intensity of natural illumination. The results of a factorial experiment showed that the differences in the proportion that mated between colour-forms occurred independently of the changes in density of adults or the intensity of natural illumination. But the experiment did indicate that at the lowest intensity of illumination (equivalent to that occurring in weeks 20 to 30 -during winter) the selective differences between grades 0 and 5, in the proportion that mated, were reduced.

(iii) Differential success of males in locating females willing to mate as an influence on the proportion of colour-forms that mate. Following observations on the mating behaviour of D. tryoni, D. neohumeralis, and the five colour-forms, the following conclusions were reached. (a) Males of the two species and of the five colour-forms located females willing to mate by chance attempts at copulation with nearby flies regardless of sex or colour-form. (b) The time of sexual activity of D. tryoni and grade 5 flies is confined to a short daily period. D. neohumeralis and grades 0 to 4.5 have a longer daily period of sexual activity. Because the time of sexual activity of many

of the grade 5 and D. tryoni coincides, there is a greater probability of these males locating a receptive female; usually one of their own kind. Hence a higher proportion of grade 5 and D. tryoni mate.

6. The outcome of selection. The outcome of selection in crowded populations containing the five colour-forms at 25°C is similar to that reported by Lewontin and Birch (in press), but is the reverse to that reported by Birch (1961). It is concluded that differences in the duration of dusk illumination were responsible for the different outcomes of selection.

7. Differences in mating resulted in selective advantages for grade 5 flies in population cages. These advantages were not influenced by changes in density of adults or larvae. They were, however, influenced by the change in intensity of natural illumination and by the changes in proportion of each colour-form in the population cages.

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APPENDICES

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Appendix A.

Source and maintenance of stocks of Dacus tryoni and
D. neohumeralis used to found the population cages.

Source of flies for experiments - Brisbane. A variety of infested fruits including brazilian cherries (Eugenia uniflora L.), guavas (Psidium guajava L.), cherry guavas (Psidium cattleianum Sabine.), kumquats (Fortunella japonica (Thunb.), Swingle.), and lemons (Citrus limon L.) were collected from widely spaced areas of Brisbane on 10 March and 19-21 April, 1963. These fruits were packed in sawdust and returned to Sydney where both fruit and sawdust were placed in fine-meshed nylon cages and held at 25°C.

A total of 263 Dacus neohumeralis and 5,386 D. tryoni resulted from the collection of these fruits. These adults formed the basis of the Brisbane stocks.

Cairns. Infested kumquats, guava, and five-corners (Averrhoa carambola L.) were collected on 27-29 April and 19-22 June, 1963. These fruits were kept in a similar manner to those collected from Brisbane, and yielded 1,189 D. neohumeralis and 1,716 D. tryoni.

Maintenance of stocks. The two species from both localities were kept in large population cages measuring 35 x 33 x 43.5 cm. Adults and larvae were reared at uncrowded densities. Eggs were collected every 2-3 weeks, or when required. It was estimated that at no time did the number of inseminated females fall below 300 in any stock. Although D. neohumeralis from Brisbane were founded with only 263 adults, once the numbers were increased

in the succeeding generations, they were maintained in high numbers.

Every 4-8 months the stocks were examined for intermediate colour-forms. These were removed whenever found.

The stocks were kept throughout the duration of the project and were used on several occasions to produce adults or larvae for additional experiments.

Appendix B

Weekly estimates of the number of first instar larvae per ml of medium; weeks 1 to 55.

Table 1. Population cage Bl.

Week	Number of first instar larvae per ml. of medium for each collection of eggs (two collections per week; Monday and Wednesday).		Mean number of first instar larvae per ml. of medium.
	Monday	Wednesday	
1	0.0	0.2	0.1
2	1.4	2.0	1.7
3	3.8	1.2	2.5
4	3.4	1.8	2.6
5	1.6	0.2	0.9
6	0.6	0.2	0.4
7	0.2	0.4	0.3
8	1.2	0.6	0.9
9	2.8	0.4	1.6
10	0.8	*	0.8
11	1.0	0.8	0.9
12	1.6	2.8	2.2
13	3.2	1.6	2.4
14	3.2	0.0	1.6
15	3.6	2.0	2.8
16	5.6	5.6	5.6
17	8.0	8.0	8.0
18	10.6	9.2	9.9
19	*	*	*
20	9.8	9.8	9.8
21	16.4	15.0	15.7
22	16.2	16.2	16.2
23	20.9	19.6	20.2
24	15.0	9.0	12.0
25	9.6	6.4	8.0
26	8.6	3.0	5.8
27	4.0	3.2	3.6
28	*	*	*
29	3.4	3.4	3.4
30	6.6	10.4	8.5

* eggs were not collected from the population cages because many of the females were removed for examination of birth rates.

Table 1. Cont.

Week	Number of first instar larvae per ml. of medium for each collection of eggs (two collections per week; Monday and Wednesday).		Mean number of first instar larvae per ml. of medium.
	Monday	Wednesday	
31	7.4	12.2	9.8
32	17.6	12.6	15.1
33	12.2	4.4	8.3
34	2.8	1.2	2.0
35	6.6	8.4	7.5
36	7.4	5.0	6.2
37	4.4	7.6	6.0
38	14.4	17.0	15.7
39	16.4	15.0	15.7
40	12.8	18.4	15.6
41	16.4	12.2	14.3
42	10.2	10.4	10.3
43	8.2	5.4	6.8
44	5.6	8.4	7.0
45	13.4	20.4	16.9
46	17.6	15.2	16.4
47	16.0	16.0	16.0
48	19.8	24.2	22.0
49	14.6	19.0	16.8
50	15.0	13.6	14.3
51	11.6	7.6	9.6
52	13.8	5.0	9.4
53	4.0	4.0	4.0
54	8.6	8.6	8.6
55	16.8	11.2	14.0

Table 2. Population cage B2.

Week	Number of first instar larvae per ml. of medium for each collection of eggs (two collections per week; Monday and Wednesday).		Mean number of first instar larvae per ml. of medium.
	Monday	Wednesday	
1	0.0	0.2	0.1
2	2.2	1.8	2.0
3	3.4	1.4	2.4
4	3.2	1.8	2.5
5	1.6	0.2	0.9
6	1.4	0.4	0.9
7	0.8	2.4	1.6
8	1.6	1.4	1.5
9	3.4	1.4	2.4
10	1.0	*	1.0
11	1.0	0.4	0.7
12	2.0	3.0	2.5
13	3.6	2.0	2.8
14	6.8	0.0	3.4
15	1.6	1.2	1.4
16	2.0	1.0	1.5
17	4.0	10.2	7.1
18	15.0	16.4	15.7
19	*	*	*
20	11.2	11.2	11.2
21	14.6	9.2	11.9
22	12.6	12.6	12.6
23	16.8	14.0	15.4
24	14.0	7.0	10.5
25	7.6	7.6	7.6
26	9.0	7.6	8.3
27	4.8	5.4	5.1
28	*	*	*
29	0.8	2.0	1.4
30	7.2	8.4	7.8

Table 2. Cont.

Week	Number of first instar larvae per ml. of medium for each collection of eggs (two collections per week; Monday and Wednesday)		Mean number of first instar larvae per ml. of medium.
	Monday	Wednesday	
31	6.4	10.0	8.2
32	19.0	13.6	16.3
33	11.6	8.2	9.9
34	11.6	3.4	7.5
35	13.6	13.6	13.6
36	7.6	5.2	6.4
37	3.6	9.8	6.7
38	10.4	15.6	13.0
39	21.0	15.4	18.2
40	14.0	15.4	14.7
41	13.2	12.2	12.7
42	9.6	4.8	7.2
43	3.8	5.0	4.4
44	5.6	7.0	6.3
45	9.0	16.6	12.8
46	21.4	21.4	21.4
47	19.2	24.0	21.6
48	31.2	31.2	31.2
49	15.8	15.8	15.8
50	13.4	12.0	12.7
51	11.2	6.2	8.7
52	9.4	5.4	7.4
53	5.6	5.6	5.6
54	13.2	13.2	13.2
55	23.4	20.8	22.1

Table 3. Population Cage Cl.

Week	Number of first instar larvae per ml. of medium for each collection of eggs (two collections per week; Monday and Wednesday)		Mean number of first instar larvae per ml. of medium.
	Monday	Wednesday	
1	0.0	0.6	0.3
2	3.4	1.8	2.6
3	4.4	1.8	3.1
4	3.6	1.2	2.4
5	1.0	0.2	0.6
6	1.2	0.4	0.8
7	1.4	3.8	2.6
8	3.8	2.8	3.3
9	6.2	5.0	5.6
10	2.4	*	2.4
11	1.6	0.4	1.0
12	3.4	6.4	4.9
13	6.0	2.6	4.3
14	11.4	0.0	5.7
15	5.6	5.2	5.4
16	11.0	15.6	13.3
17	12.0	15.0	13.5
18	7.2	15.6	11.4
19	*	*	*
20	16.0	20.4	18.2
21	21.6	35.2	28.4
22	37.8	41.0	39.4
23	45.0	27.0	36.0
24	22.4	16.8	19.6
25	20.8	16.0	18.4
26	16.8	12.8	14.8
27	14.0	12.2	13.1
28	13.6	12.0	12.8
29	7.0	6.0	6.5
30	12.0	10.6	11.3

Table 3 Contd.

Week	Number of first instar larvae per ml. of medium for each collection of eggs (two collections per week; Monday and Wednesday)		Mean number of first instar larvae per ml. of medium.
	Monday	Wednesday	
31	3.6	7.2	5.4
32	10.8	10.8	10.8
33	28.0	19.6	23.8
34	25.0	9.2	17.1
35	12.0	14.8	13.4
36	6.2	6.2	6.2
37	8.2	7.0	7.6
38	9.6	13.2	11.4
39	17.6	21.4	19.5
40	30.8	28.0	29.4
41	24.2	20.2	22.2
42	11.6	6.4	9.0
43	8.6	8.6	8.6
44	6.2	7.4	6.8
45	11.2	13.4	12.3
46	23.0	25.6	24.3
47	19.8	25.0	22.4
48	30.8	46.0	38.4
49	22.4	13.6	18.0
50	12.4	13.6	13.0
51	11.2	13.6	12.4
52	13.0	8.6	10.8
53	9.2	9.2	9.2
54	8.4	12.6	10.5
55	22.4	19.8	21.1

Table 4. Population cage C2.

Week	Number of first instar larvae per ml. of medium for each collection of eggs (two collections per week; (Monday and Wednesday))		Mean number of first instar larvae per ml. of medium.
	Monday	Wednesday	
1	0.0	0.6	0.3
2	3.6	2.0	2.8
3	4.4	1.8	3.1
4	2.8	1.8	2.3
5	0.6	0.2	0.4
6	0.4	0.2	0.3
7	0.6	1.4	1.0
8	3.6	1.6	2.6
9	6.4	3.6	5.0
10	0.8	*	0.8
11	0.8	0.4	0.6
12	3.2	6.4	4.8
13	6.0	3.4	4.7
14	14.4	0.0	7.2
15	5.2	2.8	4.0
16	8.4	14.6	11.5
17	15.6	21.4	18.5
18	15.2	16.6	15.9
19	*	*	*
20	9.8	18.2	14.0
21	38.6	30.8	34.7
22	38.4	38.4	38.4
23	40.6	27.0	33.8
24	15.2	17.6	16.4
25	16.0	24.0	20.0
26	19.6	15.0	17.3
27	12.8	12.8	12.8
28	13.6	13.6	13.6
29	5.8	9.8	7.8
30	12.8	3.2	8.0

Table 4 Cont.

Week	Number of first instar larvae per ml. of medium for each collection of eggs (two collections per week; Monday and Wednesday)		Mean number of first instar larvae per ml. of medium.
	Monday	Wednesday	
31	0.0	0.0	0.0
32	2.8	8.2	5.5
33	31.6	21.0	26.3
34	25.8	9.6	17.7
35	18.4	20.0	19.2
36	11.6	5.8	8.7
37	12.4	13.6	13.0
38	7.8	9.0	8.4
39	12.2	14.6	13.4
40	20.8	27.4	24.1
41	15.8	27.8	21.8
42	12.6	11.4	12.0
43	8.4	14.0	11.2
44	11.2	9.8	10.5
45	9.6	16.6	13.1
46	14.2	16.6	15.4
47	13.2	19.8	16.5
48	19.3	25.2	22.3
49	16.0	16.0	16.0
50	10.6	13.0	11.8
51	15.0	17.4	16.2
52	19.6	20.8	20.2
53	13.2	11.8	12.5
54	0.0	8.2	4.1
55	15.0	23.2	19.1

Appendix C.

Weekly estimates of the number of adults in the
population cages; weeks 1 to 31.

Table 1. Population cage Bl.

Week	Number of newly emerged adults.	Number of adult deaths.	Escapes.	Error ¹	Number of adults in population cage.
1	500	14	10	0	476
2	0	13	10	0	453
3	0	34	10	0	409
4	0	25	10	0	374
5	1,417	75	10	191	1,515
6	1,210	350	10	163	2,202
7	1,470	600	200	198	2,674
8	580	650	200	78	2,326
9	200	700	200	27	1,599
10	180	215	200	24	1,340
11	756	120	200	102	1,674
12	770	250	200	104	1,890
13	*	300	200	0	1,390
14	783	190	200	105	1,678
15	2,115	170	200	284	3,139
16	1,990	625	200	268	4,036
17	215	645	200	29	3,377
18	800	775	200	108	3,094
19	1,550	600	200	208	3,636
20	1,580	315	200	213	4,488
21	4,090	1,561	400	550	6,067
22	*	1,480	200	0	4,387
23	3,930	1,953	400	529	5,435
24	7,120	6,524	400	958	4,673
25	8,270	8,243	200	1,112	3,388
26	10,912	3,980	400	1,468	8,452
27	7,275	7,255	400	978	7,014
28	6,475	3,181	400	871	9,119
29	5,022	5,050	400	675	8,014
30	3,575	4,180	400	481	6,528
31	*	1,966	400	0	4,162

1 - The error is 13.5% of the number of newly emerged adults. (See Table 3.01)

* - Eggs were not collected from the Brisbane populations in weeks 10, 19, and 28. Therefore there were no newly emerged flies in weeks 13, 23, or 31.

Table 2. Population cage B2.

Week	Number of newly emerged adults.	Number of adult deaths.	Escapes.	Error. ¹	Number of adults in population cage.
1	500	20	10	0	470
2	0	13	10	0	447
3	0	25	10	0	412
4	0	28	10	0	374
5	1,495	133	10	224	1,502
6	1,355	400	10	203	2,244
7	1,640	800	200	246	2,638
8	545	750	200	82	2,151
9	572	750	200	86	1,687
10	920	45	200	138	2,224
11	1,035	330	200	155	2,574
12	1,023	400	200	153	2,844
13	*	380	200	0	2,264
14	415	250	200	62	2,167
15	2,375	275	200	356	3,711
16	2,630	505	400	394	5,042
17	640	915	200	96	4,471
18	468	860	200	70	3,809
19	675	450	200	101	3,733
20	2,180	610	200	326	4,777
21	6,345	2,250	400	950	7,522
22	*	1,928	400	0	5,194
23	3,718	1,950	400	557	6,005
24	5,820	3,730	400	871	6,824
25	6,350	7,400	200	951	4,623
26	10,260	3,890	400	1,536	9,057
27	7,795	9,080	400	1,167	6,205
28	6,900	3,280	400	1,033	8,392
29	7,350	4,500	400	1,100	9,742
30	4,416	7,090	400	661	6,007
31	*	1,731	200	0	4,076

1 - The error is 15.0% of the number of newly emerged adults. (Table 3.01)

* - See explanation in Table 1, Appendix C.

Table 3. Population cage C1.

Week	Number of newly emerged adults.	Number of adult deaths.	Escapes.	Error ¹	Number of adults in population cage.
1	500	19	10	0	471
2	0	22	10	0	439
3	0	59	10	0	370
4	0	48	10	0	312
5	2,025	126	10	286	1,915
6	1,765	575	10	249	2,846
7	1,700	1,420	200	240	2,686
8	600	800	200	85	2,201
9	590	700	200	83	1,808
10	1,310	400	200	185	2,333
11	2,630	1,100	200	371	3,292
12	1,890	800	200	266	3,916
13	*	900	200	0	2,816
14	783	510	200	110	2,779
15	5,310	440	400	749	6,500
16	4,140	1,545	400	584	8,111
17	1,674	1,310	400	236	7,839
18	1,100	960	400	155	7,424
19	4,550	1,040	400	642	9,892
20	4,298	2,690	400	606	10,494
21	7,640	3,795	400	1,077	12,862
22	*	5,195	400	0	7,267
23	7,275	4,980	400	1,026	8,136
24	7,500	4,725	400	1,058	9,453
25	7,665	6,955	400	1,081	8,682
26	11,645	5,640	400	1,642	12,645
27	16,971	11,620	400	2,392	15,204
28	17,650	10,355	400	2,489	19,610
29	13,670	11,637	400	1,927	19,316
30	10,425	17,358	400	1,470	10,513
31	9,450	3,164	400	1,332	15,067

1 - The error is 14.1% of the newly emerged adults.
(See Table 3.01).

* - Eggs were not collected from the Cairns population cages during weeks 10 and 19. Therefore there were no newly emerged adults in weeks 13 or 22.

Table 4. Population cage C2.

Week	Number of newly emerged adults.	Number of adult deaths.	Escapes.	Error ¹	Number of adults in population cage.
1	500	18	10	0	472
2	0	36	10	0	426
3	0	45	10	0	371
4	0	41	10	0	320
5	2,115	102	10	326	1,997
6	1,540	675	10	237	2,615
7	1,535	1,000	200	237	2,713
8	365	750	200	56	2,072
9	230	800	200	35	1,267
10	830	400	200	128	1,369
11	1,875	390	200	289	2,365
12	1,425	470	200	220	2,900
13	*	700	200	0	2,000
14	500	820	200	77	1,403
15	4,665	365	200	719	4,784
16	4,365	1,265	400	673	6,811
17	700	1,550	400	108	5,453
18	1,175	2,135	200	181	4,112
19	4,185	780	400	645	6,472
20	4,970	2,115	400	766	8,161
21	7,775	3,585	400	1,198	10,753
22	*	3,470	400	0	6,883
23	5,495	5,100	400	847	6,431
24	11,200	7,890	400	1,726	7,215
25	9,180	9,175	400	1,415	5,405
26	9,295	3,940	400	1,432	8,928
27	13,050	10,280	400	2,011	9,287
28	16,475	16,021	400	2,539	6,802
29	15,875	7,950	400	2,446	11,881
30	11,000	18,164	400	1,695	2,622
31	12,475	0	400	1,922	12,775

1 - The error is 15.4% of the newly emerged adults.
(See Table 3.01)

* - See Table 3, Appendix C for explanation.

Appendix D.

Numbers of each colour-form found in the regular samples of adults from population cages; weeks 7 to 99.

Table 1.

Population cage B1

Week	Number of each colour-form					Total
	0	1-3	4	4.5	5	
7	59	69	62	143	67	400
9	70	115	82	233	100	600
11	57	68	53	157	65	400
13	50	69	64	157	84	424
15	63	82	63	210	82	500
19	57	56	48	183	156	500
23	72	105	101	312	410	1,000
27	22	62	55	211	650	1,000
31	15	40	22	86	337	500
35	30	64	109	151	646	1,000
39	11	35	69	129	756	1,000
43	20	54	76	127	723	1,000
47	14	37	76	151	722	1,000
51	10	55	62	125	748	1,000
55	9	37	53	146	755	1,000
59	7	34	79	100	780	1,000
67	6	25	59	74	836	1,000
75	2	21	35	53	889	1,000
83	12	32	43	73	840	1,000
91	2	19	26	52	901	1,000
99	1	6	33	59	901	1,000

Table 2.

Population cage B2

Week	Number of each colour-form					Total
	0	1-3	4	4.5	5	
7	43	73	86	126	72	400
9	59	117	96	231	97	600
11	39	73	47	177	78	414
13	28	66	60	147	99	400
15	50	71	57	222	100	500
19	49	94	62	181	114	500
23	59	145	121	383	292	1,000
27	65	133	92	273	437	1,000
31	14	43	43	115	285	500
35	15	33	67	196	689	1,000
39	6	31	65	143	755	1,000
43	7	14	48	116	815	1,000
47	4	20	48	101	827	1,000
51	2	15	46	85	852	1,000
55	4	8	25	75	888	1,000
59	0	4	28	38	930	1,000
67	0	11	15	23	951	1,000
75	1	6	11	19	963	1,000
83	0	8	17	43	932	1,000
91	0	1	23	40	936	1,000
99	1	4	18	67	910	1,000

Table 3.

Population cage C1

Week	Number of each colour-form					Total
	0	1-3	4	4.5	5	
7	37	85	87	129	62	400
9	51	123	100	200	126	600
11	26	54	65	196	65	406
13	23	58	65	163	96	405
15	27	55	55	252	111	500
19	14	39	39	185	223	500
23	17	54	60	345	524	1,000
27	6	21	48	159	766	1,000
31	3	23	17	131	826	1,000
35	1	20	27	89	863	1,000
39	0	13	35	68	884	1,000
43	0	11	28	47	914	1,000
47	0	5	19	51	925	1,000
51	0	2	24	58	916	1,000
55	0	3	19	52	926	1,000
59	0	3	11	40	946	1,000
67	0	5	14	41	940	1,000
75	0	2	19	25	954	1,000
83	0	6	25	38	931	1,000
91	1	2	20	30	947	1,000
99	0	4	15	50	931	1,000

Table 4.

Population cage C2

Week	Number of each colour-form					Total
	0	1-3	4	4.5	5	
7	49	85	90	130	46	400
9	76	139	106	208	72	600
11	45	63	69	175	51	403
13	49	75	47	168	64	403
15	51	85	67	202	95	500
19	26	63	67	185	159	500
23	41	74	74	342	469	1,000
27	3	24	39	197	737	1,000
31	3	10	30	136	821	1,000
35	1	12	32	83	872	1,000
39	1	15	28	64	892	1,000
43	2	21	28	59	890	1,000
47	1	9	19	64	907	1,000
51	0	4	21	77	898	1,000
55	1	8	17	53	921	1,000
59	1	1	25	51	922	1,000
67	0	7	5	38	950	1,000
75	0	1	9	21	969	1,000
83	0	0	8	25	967	1,000
91	0	0	8	32	960	1,000
99	1	2	12	36	949	1,000

Appendix E.

Description of experiments to determine an optimal density and method for rearing larvae.

EXPERIMENT 1.

Object. To compare the survival of larvae when reared in different containers and at different densities.

Factors tested. Two kinds of containers were tested: plastic cups and 150 ml glass flasks. Five densities were tested: 0.5, 1.0, 2.0, 4.0, and 8.0 eggs per ml of medium. There were ten replicates for each treatment.

Procedure. Each container was filled with 50 ml of medium consisting of the following proportions: 7.25 gm of dried carrot, 2.25 gm of dried yeast "Torafood", 37.75 ml of water, .025 gm of fungicide "Nipagin", and 0.3 ml of concentrated hydrochloric acid.

The necessary numbers of eggs for each container were counted out on a square of filter paper and placed on the surface of the medium in the container. Cotton wool stoppers were inserted into the necks of the flasks. On the third day after the eggs were laid, the number of unhatched eggs on each container were counted. The number of first instar larvae in each container was obtained by subtracting the number unhatched from the total number placed in the container. Five days later, the cotton wool stoppers were removed from the flasks and each flask and cup was placed into a carton with a layer of sawdust on the bottom. Each carton was covered with a gauze lid. Ten days later, when all larvae had left the medium and pupated in the sawdust, the pupae from each carton were counted and weighed.

Results. A heavy growth of yeast was found on the surface of the medium in the flasks. It was so extensive that the eggs could not be seen on the second day and the numbers of unhatched eggs could not be counted. As a result, the survival of larvae in the flasks could not be analysed.

The extensive growth of yeast was not present on the medium in cups. Both the survival from first instar larvae to pupae and the mean weight per pupa at the five densities were each analysed in a single factor analysis of variance.

There were no significant differences in the survival of larvae between the five densities (Table 1). However, there were significant differences in the average weight per pupa between densities (Table 2). These differences between densities were tested for significance using Duncan's New Multiple Range Test. The mean weight per pupa reared at each of the three lowest densities was significantly heavier than those reared at each of the two higher densities (Table 3).

Conclusions. Cups were superior to flasks. Survival from first instar larvae to pupae was not affected by the range of densities tested. But densities of 0.5, 1.0, and 2.0 eggs per ml of medium resulted in heavier pupae than the higher densities.

Table 1.

Table of analysis of variance. Survival of larvae.

Source of variation	df.	Sum Sq.	Mean Sq.	F	P
Density	4	322.77	80.7	1.53	>.10
Error	45	2,367.51	52.6		
Total	49	2,690.28			

For this analysis the percentages were transformed to arcsin per cent.

Table 2.

Table of analysis of variance. Mean weight per pupa (mg).

Source of variation	df.	Sum Sq.	Mean Sq.	F	P
Density	4	101.00	25.25	12.56	<.005
Error	45	90.67	2.01		
Total	49	191.67			



Table 3.

Survival of larvae and mean weight per pupa at five densities of larvae. Each figure is the mean of 10 replicates.

Level of density	Number of eggs/ml medium	Number first instar larvae /ml medium	Percentage survival of larvae.	Mean weight /pupa (mg).
D ₁	0.5	0.40	67.7	11.3*
D ₂	1.0	0.82	73.8	11.9*
D ₃	2.0	1.68	74.6	10.8*
D ₄	4.0	3.31	78.8	9.3↙
D ₅	8.0	6.63	70.9	8.0↙

* These figures are significantly higher than those indicated by the arrow (by Duncan's New Multiple Range Test).

EXPERIMENT 2.

Object. To compare the survival of larvae in different containers of medium, densities of larvae, depths of medium, and amounts of water in the medium.

Factors tested. Two types of containers were tested: The plastic cups used in Experiment 1, and shallow square plastic trays. Two levels of density were chosen: 0.5 and 1.0 eggs per ml of medium (these densities resulted in the heaviest pupae in Experiment 1). Two depths of medium were tested: 22mm (as in Experiment 1) and 11mm. Two quantities of water were used in the medium: The amount used in Experiment 1, and an increase of 20%.

Procedure. The procedure of counting the eggs, obtaining the number of first instar larvae, and rearing the larvae to the pupal stage were similar to those used in Experiment 1. The mean weight per pupa was not recorded.

Results. The design of the experiment was 2 X 2 X 2 X 2 factorial with five replicates (Table 4). The percentage survival of larvae was transformed to $\arcsin \sqrt{\text{percentage}}$.

The treatment means are given in Table 4. The analysis of variance table (Table 5) shows that the main effects of container, density of eggs, and amount of water are significant. In addition, significant first order interactions of container and density, container and amount of water, and density of eggs and amount of water are also significant.

Table 4.

Design of experiment and treatment means. Each figure is the mean of five replicates.

Container	Density of eggs	Depth of medium	Increase in amount of water	Survival of larvae
Cups	0.5 eggs/ml medium	11mm	Normal	68.2%
		22mm	20%	89.8
	1.0 eggs/ml medium	11mm	Normal	67.8
		22mm	20%	85.1
Trays	0.5 eggs/ml medium	11mm	Normal	71.9
		22mm	20%	84.6
	1.0 eggs/ml medium	11mm	Normal	78.2
		22mm	20%	86.6
	0.5 eggs/ml medium	11mm	Normal	75.4
		22mm	20%	86.3
1.0 eggs/ml medium	11mm	Normal	72.5	
	22mm	20%	78.6	
Trays	1.0 eggs/ml medium	11mm	Normal	72.3
		22mm	20%	71.0
	1.0 eggs/ml medium	11mm	Normal	67.0
		22mm	20%	56.9

Table 5.

Table of analysis of variance. Survival of larvae.

Source of variation	df.	Sum Sq.	Mean Sq.	F	P
Container	1	652.14	652.14	10.99	<.005
Density	1	289.60	289.60	4.88	<.05
Depth	1	182.75	182.75	3.08	ns
Water	1	768.12	768.12	12.94	<.005
<u>Interactions:</u>					
<u>1st Order</u>					
Cont. X Den.	1	284.97	284.97	4.80	<.05
Cont. X Depth	1	74.72	74.72	1.26	ns
Cont. X Water	1	476.33	476.33	8.02	<.05
Depth X Den.	1	62.01	62.01	1.04	ns
Depth X Water	1	103.06	103.06	1.74	ns
Den. X Water	1	314.73	314.73	5.30	<.05
<u>2nd Order</u>					
Cont. X Den. X Water	1	147.83	147.83	2.49	ns
Cont. X Den. X Depth	1	11.30	11.30	.19	ns
Cont. X Depth X Water	1	.00	.00	.00	ns
Den. X Depth X Water	1	.03	.03	.00	ns
<u>3rd Order</u>					
Cont. X Den. X Depth X Water	1	4.41	4.41	.07	ns
Error	64	3,798.92	59.36		
Total	79	7,170.92			

The treatment means by container, density, and amount of water (Table 6) were examined for significance. Significant differences, as shown by Duncan's New Multiple Range Test, are given in Table 6.

Table 6.

Treatment means of the survival of larvae by container, density of eggs, and amount of water.

Container	Density of Eggs	Amount of Water	Survival of Larvae
Cups	0.5 eggs/ml medium	Normal	68.0%
		20%	87.4
	1.0 eggs/ml medium	Normal	75.1
		20%	85.3
Trays	0.5 eggs/ml medium	Normal	74.0
		20%	82.7
	1.0 eggs/ml medium	Normal	69.7
		20%	64.0

Significant differences were determined by using Duncan's New Multiple Range Test. The mean indicated by the asterisk is significantly greater than those means indicated by the arrows.

Conclusions. Varying the depth of the medium did not influence the survival of larvae. Differences were found between containers but these were dependent upon density and amount of water. Differences between densities were significant but these were dependent upon container and amount of water. Differences between amounts of water were dependent upon container and density.

Description of optimal density and procedure of rearing larvae.

I chose to use cups containing 50 ml of medium at a depth of 22ml. The medium contained an additional 20% water. The number of eggs was 1 per ml of medium. This treatment gave the second highest survival of larvae in Experiment 2.

Appendix F.

Contingency tables comparing the ratio of the number of each colour-form reared as larvae at an optimal density with the ratio of the number of each colour-form reared as larvae in crowded densities. The comparisons are for each population cage during Weeks 3, 18, and 22.

Table 1.

Week 3

Population B1. Total Chi-square 5.69 ($P > .10$).

		Colour-form					
		0	1-3	4	4.5	5	Total
Crowded	Obs.	59	76	74	166	48	423
	Exp.	51.1	77.5	77.5	161.9	55.1	
	Chi-sq.	1.22	.03	.16	.10	.91	
Optimal	Obs.	30	59	61	116	48	314
	Exp.	37.9	57.5	57.5	120.1	40.9	
	Chi-sq.	1.65	.04	.21	.14	1.23	
Total		89	135	135	282	96	737

Population B2. Total Chi-square 5.84 ($P > .10$).

		Colour-form					
		0	1-3	4	4.5	5	Total
Crowded	Obs.	46	96	109	133	49	433
	Exp.	47.6	96.2	97.3	145.4	46.5	
	Chi-sq.	.05	.00	1.41	1.06	.13	
Optimal	Obs.	41	80	69	133	36	359
	Exp.	39.4	79.8	80.7	120.6	38.5	
	Chi-sq.	.06	.00	1.70	1.27	.16	
Total		87	176	178	266	85	792

Table 1. cont.

		Colour-form					
		0	1-3	4	4.5	5	Total
Population C2. Total Chi-square 19.21 ($P < .005$)							
Crowded	Obs.	41	74	99	120	41	375
	Exp.	39.8	86.5	100.2	121.5	27.1	
	Chi-sq.	.04	1.81	.01	.02	7.13	
Optimal	Obs.	34	89	90	109	10	332
	Exp.	35.2	76.5	88.8	107.5	23.9	
	Chi-sq.	.04	2.04	.02	.02	8.08	
Total		75	163	189	229	51	707

Population C1. Total Chi-square 16.21 ($P < .005$)

		Colour-form					
		0	1-3	4	4.5	5	Total
Population C1. Total Chi-square 16.21 ($P < .005$)							
Crowded	Obs.	55	105	78	154	38	430
	Exp.	53.0	103.2	90.5	156.8	26.5	
	Chi-sq.	.08	.03	1.72	.05	4.99	
Optimal	Obs.	37	74	79	118	8	316
	Exp.	39.0	75.8	66.5	115.2	19.5	
	Chi-sq.	.10	.04	2.35	.07	6.78	
Total		92	179	157	272	46	746

Table 2.

Week 18

Population B1. Total Chi-square 3.25 ($P > .50$).

		Colour-form					
		0	1-3	4	4.5	5	Total
Crowded	Obs.	27	58	32	154	163	
	Exp.	31.0	56.7	36.3	145.9	164.1	434
	Chi-sq.	.52	.03	.51	.45	.01	
Optimal	Obs.	31	48	36	119	144	
	Exp.	27.0	49.3	31.7	127.1	142.9	378
	Chi-sq.	.59	.03	.58	.52	.01	
Total		58	106	68	273	307	812

Population B2. Total Chi-square 12.18 ($P < .025$).

		Colour-form					
		0	1-3	4	4.5	5	Total
Crowded	Obs.	31	49	38	138	112	
	Exp.	26.3	56.0	43.1	148.3	94.3	368
	Chi-sq.	.84	.88	.60	.72	3.32	
Optimal	Obs.	24	68	52	172	85	
	Exp.	28.7	61.0	46.9	161.7	102.7	401
	Chi-sq.	.77	.80	.55	.65	3.05	
Total		55	117	90	310	197	769

Table 2. cont.

Population C1. Total Chi-square 5.65 ($P > .10$).

		Colour-form					
		0	1-3	4	4.5	5	Total
Crowded	Obs.	4	23	25	155	192	
	Exp.	5.6	22.8	31.2	161.4	178.1	399
	Chi-sq.	.46	.00	1.23	.25	1.08	
Optimal	Obs.	8	26	42	192	191	
	Exp.	6.4	26.2	35.8	185.6	204.9	459
	Chi-sq.	.40	.00	1.07	.22	.94	
Total		12	49	67	347	383	858

Population C2. Total Chi-square 6.96 ($P > .10$).

		Colour-form					
		0	1-3	4	4.5	5	Total
Crowded	Obs.	15	20	30	165	175	
	Exp.	10.6	23.8	34.9	167.6	168.1	405
	Chi-sq.	1.83	.61	.69	.04	.28	
Optimal	Obs.	6	27	39	166	157	
	Exp.	10.4	23.2	34.1	163.4	163.9	395
	Chi-sq.	1.86	.62	.70	.04	.29	
Total		21	47	69	331	332	800

Table 3.

Week 22

Population B1. Total Chi-square 2.82 ($P > .25$).

		Colour-form					
		0	1-3	4	4.5	5	Total
Crowded	Obs.	17	23	32	120	259	
	Exp.	16.3	26.0	28.7	113.8	266.2	451
	Chi-sq.	.03	.35	.38	.34	.19	
Optimal	Obs.	13	25	21	90	232	
	Exp.	13.7	22.0	24.3	96.2	224.8	381
	Chi-sq.	.04	.41	.45	.40	.23	
Total		30	48	53	210	491	832

Population B2. Total Chi-square 2.13 ($P > .50$).

		Colour-form					
		0	1-3	4	4.5	5	Total
Crowded	Obs.	37	57	56	135	138	
	Exp.	41.8	59.2	51.5	134.2	136.2	423
	Chi-sq.	.55	.08	.39	.00	.02	
Optimal	Obs.	45	59	45	128	129	
	Exp.	40.2	56.8	49.5	128.8	130.8	406
	Chi-sq.	.57	.09	.41	.00	.02	
Total		82	116	101	263	267	829

Table 3. cont

Population C1. Total Chi-square 9.54 ($P < .05$).

		Colour-form					
		0	1-3	4	4.5	5	Total
Crowded	Obs.	0	4	12	103	329	
	Exp.	1.5	7.3	15.6	93.2	330.4	448
	Chi-sq.	1.50	1.49	.83	1.03	.00	
Optimal	Obs.	3	11	20	88	348	
	Exp.	1.5	7.7	16.4	97.8	346.6	470
	Chi-sq.	1.50	1.41	.80	.98	.00	
Total		3	15	32	191	677	918

Population C2. Total Chi-square 6.74 ($P > .10$).

		Colour-form					
		0	1-3	4	4.5	5	Total
Crowded	Obs.	5	17	19	120	316	
	Exp.	5.0	16.0	28.1	121.8	306.1	477
	Chi-sq.	.00	.06	2.95	.03	.32	
Optimal	Obs.	5	15	37	123	295	
	Exp.	5.0	16.0	27.9	121.2	304.9	475
	Chi-sq.	.00	.06	2.97	.03	.32	
Total		10	32	56	243	611	952

Appendix G.

Contingency table comparing the ratio of the number of each colour-form reared as larvae in an optimal density with the ratio of the number of each colour-form reared as larvae in a crowded density. The comparison is a re-examination of the survival of larvae in similar conditions of density and proportion of colour-forms as was found in the Cairns population cages in Week 3.

Total Chi-square 3.21 ($P > .50$).

		Colour-form					
		0	1-3	4	4.5	5	Total
Crowded	Obs.	42	64	73	159	77	415
	Exp.	43.9	54.9	74.6	156.2	85.4	
	Chi-sq.	.08	1.51	.03	.05	.83	
Optimal	Obs.	129	150	218	450	256	1203
	Exp.	127.1	159.1	216.4	452.8	247.6	
	Chi-sq.	.03	.52	.01	.02	.13	
Total		171	214	291	609	333	1618

Appendix H.

Contingency tables comparing the ratio of the number of each colour-form in a sample of copulating flies with the ratio of the number of each colour-form in a random sample of adults from the population cage. The comparisons are for each population cage for samples collected in Weeks 7-9, 12-13, 16-18, and 25-26.

Table 1.

Weeks 7-9

Population Cage B1. Total Chi-square .11 ($P > .99$)

		Colour-form					Total
		0	1-3	4	4.5	5	
Popln. cage	Obs.	59	69	62	143	67	400
	Exp.	59.4	69.0	62.6	143.8	65.3	
	Chi-sq.	.00	.00	.01	.00	.04	
Mating	Obs.	53	61	56	128	56	354
	Exp.	52.6	61.0	55.4	127.2	57.7	
	Chi-sq.	.00	.00	.01	.00	.05	
Total		112	130	118	271	123	754

Population Cage B2. Total Chi-square 2.59 ($P > .95$)

		Colour-form					Total
		0	1-3	4	4.5	5	
Popln. cage	Obs.	43	73	86	126	72	400
	Exp.	43.3	69.6	84.2	135.1	67.8	
	Chi-sq.	.00	.17	.04	.61	.26	
Mating	Obs.	31	46	58	105	44	284
	Exp.	30.7	49.4	59.8	95.9	48.2	
	Chi-sq.	.00	.23	.05	.86	.37	
Total		74	119	144	231	116	684

Table 1. cont.

Population Cage C1. Total Chi-square 3.02 ($P > .50$).

		Colour-form					
		0	1-3	4	4.5	5	Total
Popln. cage	Obs.	37	85	87	129	62	
	Exp.	41.5	88.0	79.5	132.0	59.0	400
	Chi-sq.	.48	.10	.71	.07	.15	
Mating	Obs.	46	91	72	135	56	
	Exp.	41.5	88.0	79.5	132.0	59.0	400
	Chi-sq.	.48	.10	.71	.07	.15	
Total		83	176	159	264	118	800

Population Cage C2. Total Chi-square 1.00 ($P > .90$).

		Colour-form					
		0	1-3	4	4.5	5	Total
Popln. cage	Obs.	49	85	90	130	46	
	Exp.	45.0	86.1	89.3	133.2	46.5	400
	Chi-sq.	.36	.01	.01	.07	.01	
Mating	Obs.	35	76	77	119	41	
	Exp.	39.0	74.9	77.7	115.8	40.5	348
	Chi-sq.	.41	.02	.01	.09	.01	
Total		84	161	167	249	87	748

Table 2.

Weeks 12-13

Population Cage B1. Total Chi-square 12.41 ($P < .025$)

		Colour-form					Total
		0	1-3	4	4.5	5	
Popln. cage	Obs.	57	69	53	157	65	401
	Exp.	48.5	70.5	53.1	153.9	75.0	
	Chi-sq.	1.49	.32	.02	.06	1.33	
Mating	Obs.	7	24	17	46	34	128
	Exp.	15.5	22.5	16.9	49.1	24.0	
	Chi-sq.	4.66	.10	.06	.20	4.17	
Total		64	93	70	203	99	529

Population Cage B2. Total Chi-square 2.60 ($P > .50$).

		Colour-form					Total
		0	1-3	4	4.5	5	
Popln. cage	Obs.	39	73	47	177	78	414
	Exp.	35.5	73.9	50.9	173.5	80.2	
	Chi-sq.	.35	.01	.30	.07	.06	
Mating	Obs.	12	33	26	72	37	180
	Exp.	15.5	32.1	22.1	75.5	34.8	
	Chi-sq.	.79	.03	.69	.16	.14	
Total		51	106	73	249	115	594

Table 2. cont.

Population Cage C1. Total Chi-square 20.63 ($P < .005$).

		Colour-form					
		0	1-3	4	4.5	5	Total
Popln. cage	Obs.	26	54	65	196	65	406
	Exp.	20.4	57.1	63.4	183.3	81.8	
	Chi-sq.	1.59	.17	.04	.88	3.45	
Mating	Obs.	3	27	25	64	51	170
	Exp.	8.6	23.9	26.6	76.7	34.2	
	Chi-sq.	3.65	.40	.10	2.10	8.25	
Total		29	81	90	260	116	576

Population Cage C2. Total Chi-square 6.96 ($P > .10$).

		Colour-form					
		0	1-3	4	4.5	5	Total
Popln. Cage	Obs.	45	63	69	175	51	403
	Exp.	48.7	72.1	68.0	164.1	50.1	
	Chi-sq.	.28	1.15	.01	.72	.02	
Mating	Obs.	26	42	30	64	22	184
	Exp.	22.3	32.9	31.0	74.9	22.9	
	Chi-sq.	.61	2.52	.03	1.58	.04	
Total		71	105	99	239	73	587

Table 3.

Weeks 16-18

Population Cage B1. Total Chi-square 18.48 ($P < .005$).

		Colour-form					Total
		0	1-3	4	4.5	5	
Popln. cage	Obs.	63	82	63	210	82	500
	Exp.	54.8	87.0	63.8	193.3	101.2	
	Chi-sq.	1.11	.29	.01	1.44	3.64	
Mating	Obs.	22	53	36	90	75	276
	Exp.	30.2	48.0	35.2	106.7	55.8	
	Chi-sq.	2.23	.52	.02	2.61	6.61	
Total		85	135	99	300	157	776

Population Cage B2. Total Chi-square 11.17 ($P < .025$).

		Colour-form					Total
		0	1-3	4	4.5	5	
Popln. cage	Obs.	50	71	57	222	100	500
	Exp.	50.0	74.5	59.8	201.8	113.8	
	Chi-sq.	.00	.16	.13	2.02	1.67	
Mating	Obs.	28	45	36	92	77	278
	Exp.	28.0	41.5	33.2	112.2	63.2	
	Chi-sq.	.00	.30	.24	3.64	3.01	
Total		78	116	93	314	177	778

Table 3. cont.

Population Cage C1. Total Chi-square 72.17 ($P < .005$).

		Colour-form					Total
		0	1-3	4	4.5	5	
Popln. cage	Obs.	27	55	55	252	111	500
	Exp.	24.6	41.6	46.0	224.2	163.7	
	Chi-sq.	.23	4.32	1.76	n 3.45	16.96	
Mating	Obs.	12	11	18	104	149	294
	Exp.	14.4	24.4	27.0	131.8	96.3	
	Chi-sq.	.40	7.36	3.00	5.86	28.83	
Total		39	66	73	356	260	794

Population Cage C2. Total Chi-square 64.83 ($P < .005$).

		Colour-form					Total
		0	1-3	4	4.5	5	
Popln. cage	Obs.	51	85	67	202	95	500
	Exp.	41.3	67.4	58.9	188.7	143.8	
	Chi-sq.	2.28	4.60	1.11	.94	16.56	
Mating	Obs.	17	26	30	109	142	324
	Exp.	26.7	43.6	38.1	122.3	93.2	
	Chi-sq.	3.52	7.10	1.72	1.45	25.55	
Total		68	111	97	311	237	824

Table 4.

Weeks 25-26

Population Cage B1. Total Chi-square 61.30 ($P < .005$).

		Colour-form					
		0	1-3	4	4.5	5	Total
Popln. cage	Obs.	72	105	101	312	410	
	Exp.	64.4	100.0	101.7	266.7	467.4	1000
	Chi-sq.	.90	.25	.00	7.69	7.05	
Mating	Obs.	15	30	36	48	221	
	Exp.	22.6	35.0	35.3	93.3	163.6	350
	Chi-sq.	2.56	.71	.01	21.99	20.14	
Total		87	135	137	360	631	1350

Population Cage B2. Total Chi-square 67.63 ($P < .005$).

		Colour-form					
		0	1-3	4	4.5	5	Total
Popln. cage	Obs.	59	145	121	385	292	
	Exp.	55.8	135.0	109.5	338.8	360.9	1000
	Chi-sq.	.18	.74	1.21	5.77	13.15	
Mating	Obs.	22	51	38	109	232	
	Exp.	25.2	61.0	49.5	153.2	163.1	452
	Chi-sq.	.41	1.64	2.67	12.75	29.11	
Total		81	196	159	492	524	1452

Table 4. cont.

Population Cage C1. Total Chi-square 60.51 ($P < .005$).

		Colour-form					Total
		0	1-3	4	4.5	5	
Popln. cage	Obs.	17	54	60	345	524	1000
	Exp.	12.8	46.1	57.4	296.5	587.2	
	Chi-sq.	1.38	1.35	.12	7.93	6.80	
Mating	Obs.	1	11	21	73	304	410
	Exp.	5.2	18.9	23.6	121.5	240.8	
	Chi-sq.	3.39	3.30	.29	19.36	16.59	
Total		18	65	81	418	828	1410

Population Cage C2. Total Chi-square 110.34 ($P < .005$).

		Colour-form					Total
		0	1-3	4	4.5	5	
Popln. cage	Obs.	41	74	74	342	469	1000
	Exp.	31.9	66.6	64.4	280.4	556.7	
	Chi-sq.	2.60	.82	1.43	13.53	13.82	
Mating	Obs.	4	20	17	54	317	412
	Exp.	13.1	27.4	26.6	115.6	229.3	
	Chi-sq.	6.32	2.00	3.46	32.82	33.54	
Total		45	94	91	396	786	1412

Appendix I.

Tables of analysis of variance on the total number of eggs laid per female, the number of viable eggs per female and the percentage of eggs that hatched for the females of each colour-form. The data is from tests conducted during weeks 8 and 10, 19, and 28.

Table 1. Tables of analysis of variance; weeks 8 and 10.

Number of viable eggs per female.

Source of variation.	D.F.	Sum of Sq.	Mean Sq.	F	P
Locality	1	64.44	64.44	8.43	P < .025
Colour-form	4	58.37	14.59	1.91	ns.
Interaction	4	17.21	4.30	.56	ns.
Error	<u>10</u>	<u>76.37</u>	<u>7.64</u>		
	19	216.39			

Total number of eggs per female.

Source of Variation	D.F.	Sum of Sq.	Mean Sq.	F	P
Locality	1	85.70	85.70	6.40	P < .05
Colour-form	4	102.96	25.74	1.92	ns.
Interaction	4	28.58	7.15	.53	ns.
Error	<u>10</u>	<u>133.85</u>	<u>13.39</u>		
	19	351.09			

Survival of eggs.

Source of Variation	D.F.	Sum of Sq.	Mean Sq.	F	P
Locality	1	1,122.00	1,122.00	4.93	ns.
Colour-form	4	2,249.94	562.49	2.47	ns.
Interaction	4	740.62	185.16	.81	ns.
Error	<u>10</u>	<u>2,275.47</u>	<u>227.55</u>		
	19	6,388.03			

Table 2. Tables of analysis of variance; week 19.

Number of viable eggs per female.

Source of Variation	D.F.	Sum of Sq.	Mean Sq.	F	P
Locality	1	.01	.01	0.00	ns.
Colour-form	4	693.77	173.44	5.55	P < .025
Interaction	4	197.60	49.40	1.58	ns.
Error	10	312.63	31.26		
Total	19	1,204.01			

Total number of eggs per female.

Source of Variation	D.F.	Sum of Sq.	Mean Sq.	F	P
Locality	1	1.57	1.57	.08	ns.
Colour-form	4	465.74	116.44	5.97	P < .025
Interaction	4	113.91	28.48	1.46	ns.
Error	10	194.97	19.50		
Total	19	776.19			

Survival of eggs.

Source of Variation	D.F.	Sum of Sq.	Mean Sq.	F.	P.
Locality	1	.01	.01	.00	ns.
Colour-form	4	749.64	187.41	1.79	ns.
Interaction	4	667.34	166.84	1.59	ns.
Error	10	1,047.16	104.72		
Total	19	2,464.15			

Table 3. Tables of analysis of variance; week 28 (Brisbane population cages only).

Number of viable eggs per female.

Source of Variation.	D.F.	Sum of Sq.	Mean Sq.	F.	P.
Colour-form	2	73.11	36.56	43.52	P < .005
Error	3	2.51	.84		
Total	5	75.62			

Total number of eggs per female.

Source of Variation	D.F.	Sum of Sq.	Mean Sq.	F.	P.
Colour-form	2	45.42	22.71	32.44	P < .01
Error	3	2.09	.70		
Total	5	47.51			

Survival of Eggs.

Source of Variation	D.F.	Sum of Sq.	Mean Sq.	F.	P.
Colour-form	2	159.97	80.00	1.44	ns.
Error	3	166.87	55.62		
Total	5	326.84			

Appendix J.

The number of each colour-form copulating in three daily periods. The figures are from samples of copulating flies collected from the population cages in weeks 7-9, 12-18, and 25-26.

		Brisbane population cages				Cairns population cages			
Period		A	B	C	Total	A	B	C	Total
Colour- form									
Weeks	0	48	20	14	82	44	19	9	72
7-9	1-3	39	32	31	102	73	48	30	151
	4	39	37	26	102	50	52	33	135
	4.5	66	74	79	219	51	72	103	226
	5	11	35	48	94	14	17	51	82
Weeks	0	38	28	6	72	31	24	4	59
12-18	1-3	84	57	15	156	58	34	12	104
	4	49	50	16	115	54	35	19	108
	4.5	103	139	64	306	130	128	83	341
	5	30	108	87	225	90	144	136	370
Weeks	0	11	15	13	39	4	1	0	5
25-26	1-3	29	24	27	80	12	14	4	30
	4	21	21	33	75	17	12	11	40
	4.5	42	50	65	157	55	37	34	126
	5	100	104	249	453	114	246	261	621

Abbreviations: A = Early to mid-afternoon
 B = Late afternoon
 C = Dusk

Appendix K.

Number of each of the five colour-forms that mated at 15 minute intervals.

Time	Number of colour-forms				
	0	1-3	4	4.5	5
11:45-00 am	1	1			2
12:00-15 pm		1			1
15-30	1	1			
30-45			1	1	
45-00			1		
1:00-15					
15-30	1	1		1	1
30-45	3	1	1		3
45-00	1	1			
2:00-15	1		1		
15-30		2			
30-45	2		1		1
45-00	6	4	3	3	1
3:00-15	7	2	1	4	4
15-30	5	5	2	3	2
30-45	17	7	10	4	2
45-00	7	6	11	13	5
4:00-15	17	11	13	11	4
15-30	14	6	14	19	16
30-45	10	6	14	15	4
45-00	10	9	21	28	11
5:00-15	11	7	30	35	34
15-30	5	9	17	54	61
30-45	3	6	18	46	58
45-00	5	10	19	57	76
6:00-15	5	7	27	65	316
15-30	3	5	16	38	195
30-45			2	3	20
Totals	135	108	223	400	817

Appendix I.

Percentage of each species that copulated and table of analysis
of variance.

Table 1.

Percentage of the original number of each species that copulated.

			Replicates				
			R1	R2	R3	R4	Mean
L1	D1	♂ N	9.0	5.6	9.4	14.2	9.6
		♂ T	18.9	11.8	23.1	25.0	19.7
		♀ T	18.9	12.7	26.4	27.4	21.4
	D2	♂ N	4.7	3.5	6.4	5.6	5.1
		♂ T	14.4	20.7	12.7	18.1	16.5
		♀ T	13.6	19.8	13.2	17.4	16.0
	D3	♂ N	2.6	2.2	2.9	4.5	3.1
		♂ T	11.9	8.6	7.1	10.7	9.6
		♀ T	11.4	8.7	7.5	11.5	9.8
L2	D1	♂ N	11.3	13.2	9.9	11.3	11.4
		♂ T	7.1	11.3	26.9	19.3	16.2
		♀ T	8.0	11.8	27.8	20.8	17.1
	D2	♂ N	8.7	4.9	8.7	5.6	7.0
		♂ T	8.5	16.5	9.6	17.6	13.1
		♀ T	10.1	16.0	9.4	17.4	13.2
	D3	♂ N	6.4	3.9	3.6	2.5	4.1
		♂ T	11.3	7.9	11.9	10.0	10.3
		♀ T	12.4	8.1	11.9	9.6	10.5

Table 2.

Table of analysis of variance on the percentage of the original number of each species that copulated.

Source of Variation	D.F.	Sum of Sq.	Mean Sq.	F	P
<u>Main Effects:</u>					
Light	1	.02	.02	.07	ns.
Density	2	19.53	9.77	34.16	P<.005
Species	1	36.85	36.85	128.85	P<.005
Sex	1	.02	.02	.07	ns.
<u>Interactions:</u>					
<u>1st Order</u>					
L X Sp	1	2.17	2.17	7.59	P<.01
L X D	2	.31	.15	.52	ns.
L X Sex	1	.00	.00	.00	ns.
D X Sex	2	.11	.05	.17	ns.
D X Sp.	2	.04	.02	.07	ns.
Sp X Sex	1	.15	.15	.52	ns.
<u>2nd Order</u>					
L X D X Sp	2	.62	.31	1.08	ns.
L X D X Sex	2	.01	.00	.00	ns.
L X Sp X Sex	1	.00	.00	.00	ns.
D X Sp X Sex	2	.45	.22	.77	ns.
<u>3rd Order</u>					
L X D X Sp X Sex	2	.02	.01	.03	ns.
Error	72	20.61	.286		
Total	95	80.91			

Abbreviations: L = Light
D = Density
Sp = Species

Appendix M.

Percentage of the number of each species that died during the period of sampling for copulating flies and table of analysis of variance.

Table 1.

Percentage mortality of adults during the period of sampling for copulating flies.

		Replicates					
		R1	R2	R3	R4	Mean	
L1	D1	♂ N	42.9	43.9	43.9	60.8	47.9
		♂ T	46.7	59.0	59.4	59.0	56.0
	D2	♀ N	40.1	41.5	35.8	39.0	39.1
		♀ T	42.9	39.6	34.9	54.7	43.0
	D3	♂ N	58.4	58.1	51.1	60.5	57.0
		♂ T	62.8	64.9	54.6	87.3	67.4
	D1	♀ N	52.7	56.2	50.6	72.0	57.9
		♀ T	51.8	61.4	40.2	64.9	54.6
	D2	♂ N	91.5	65.3	93.1	86.1	84.0
♂ T		73.5	62.7	73.8	62.9	68.2	
D3	♀ N	62.4	59.3	49.8	60.0	57.9	
	♀ T	60.9	58.2	63.9	64.5	61.9	
L2	D1	♂ N	68.9	62.7	40.6	41.0	53.3
		♂ T	58.0	58.5	53.8	53.8	56.0
	D2	♀ N	44.3	42.9	31.6	51.4	42.6
		♀ T	49.1	40.6	34.0	40.1	41.0
	D3	♂ N	54.1	46.1	55.8	43.5	49.9
		♂ T	41.4	82.3	41.4	63.5	57.2
	D1	♀ N	39.3	42.1	52.9	36.5	42.7
		♀ T	35.3	78.8	35.8	55.3	51.3
	D2	♂ N	78.3	96.5	83.1	90.7	87.2
		♂ T	70.7	69.4	73.2	80.1	73.4
	D3	♀ N	50.5	63.6	58.1	72.2	61.1
		♀ T	35.3	78.8	35.8	55.3	51.3

Table 2.

Table of analysis of variance on the percentage mortality of adults during the period of sampling for copulating flies.

Source of Variation	D.F.	Sum.of Sq.	Mean Sq.	F	P
<u>Main Effects:</u>					
Light	1	7.06	7.06	.16	ns.
Density	2	3,275.05	1,637.52	38.01	P<.005
Species	1	4.16	4.16	.10	ns.
Sex	1	1,270.22	1,270.22	29.48	P<.005
<u>Interactions:</u>					
<u>1st Order</u>					
L X Sp	1	.02	.02	.00	ns.
L X D	2	272.64	136.32	3.16	P<.05
L X Sex	1	1.99	1.99	.05	ns.
D X Sex	2	215.76	107.88	2.50	ns.
D X Sp	2	274.28	137.14	3.18	P<.05
Sp X Sex	1	38.76	38.76	.90	ns.
<u>2nd Order</u>					
L X D X Sp	2	34.68	17.34	.40	ns.
L X D X Sex	2	1.20	.60	.01	ns.
L X Sp X Sex	1	28.36	28.36	.66	ns.
D X Sp X Sex	2	398.94	199.47	4.63	P<.025
<u>3rd Order</u>					
L X D X Sp X Sex	2	28.34	14.17	.33	ns.
Error	72	3,101.84	43.08		
Total	95	8,953.30			

Abbreviations: L = Light
D = Density
Sp = Species

Appendix N.

The number of D. neohumeralis and D. tryoni copulating at
15 Minute intervals during the day.

Number of pairs copulating

Time	<u>D. neohumeralis</u>	<u>D. tryoni</u>
8:45-00 am	5	
9:00-15	10	
15-30	5	
30-45	14	
45-00	22	
10:00-15	9	
15-30	16	
30-45	16	
45-00	18	
11:00-15	20	
15-30	17	
30-45	19	
45-00	11	
12:00-15 pm	17	
15-30	12	
30-45	11	
45-00	13	
1:00-15	15	
15-30	4	
30-45	13	
45-00	5	
2:00-15	6	
15-30	15	
30-45	12	
45-00	7	
3:00-15	12	
15-30		
30-45	1	
45-00	2	
4:00-15	1	
15-30		
30-45		
45-00		
5:00-15		
15-30		
30-45		
45-00		6
6:00-15		15
15-30		84
30-45		59
TOTALS	328	164

Appendix 0.

The number of homogamic matings, at 15 minute intervals of grade 0 alone in an experimental cage, grade 0 in the presence of other colour-forms, grades 0-4, grade 4.5, and grade 5. Flies in the latter three categories were all in the same experimental cage. All flies were from an F2 generation of an interspecific cross.

Number of pairs copulating

Time	0 (alone)	0 (with others)	0-4	4.5	5
9:30-45 am	1				
45-00	2				
10:00-15					
15-30					
30-45					
45-00	2				
11:00-15	1				
15-30	1				
30-45					
45-00	1				
12:00-15 pm	2				
15-30			1		
30-45					
45-00	6				
1:00-15	2				
15-30			1		
30-45	4		1		
45-00	2		1		
2:00-15	3		1		
15-30	3		1		
30-45	1		1		
45-00			6	1	
3:00-15	1	1	3	1	
15-30		2	5	1	
30-45		3	14		
45-00			7	2	
4:00-15	1		14	1	
15-30	2	1	12		1
30-45		2	12	7	
45-00			10	7	1
5:00-15		1	8	9	4
15-30			3	10	14
30-45			3	10	18
45-00			7	14	21
6:00-15			2	5	121
15-30			2	1	73
30-45					9
Totals	35	10	115	69	262

Appendix P.

The number of matings at 15 minute intervals of D. tryoni, and of the individuals from the Cairns and Brisbane population cages in week 96.

Number of copulating pairs			
Time	Brisbane population cages	Cairns population cages	<u>D. tryoni</u> (from Cairns)
1:45-00 pm		1	
2:00-15		1	
15-30			
30-45			
45-00			
3:00-15		2	
15-30	1		
30-45			
45-00			
4:00-15	6	8	
15-30	5	17	
30-45	1	12	
45-00	14	25	
5:00-15	15	35	
15-30	21	34	
30-45	36	29	12
45-00	44	41	16
6:00-15	30	22	80
15-30	2	4	68
30-45			10
Totals	175	231	186

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