THE EFFECTS OF INBREEDING AND LABORATORY-REARING ON A PYRAUSTID MOTH, *MIMORISTA PULCHELLALIS* DYAR (LEPIDOPTERA:PYRAUSTIDAE), IMPORTED FOR THE BIOLOGICAL CONTROL OF JOINTED CACTUS IN SOUTH AFRICA.

by

MARGARET DOROTHY WRIGHT

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Department of Zoology and Entomology
Rhodes University
Grahamstown
South Africa

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FRONTISPIECE

Stages in the life cycle of Mimorista pulchellalis Dyar on Opuntia aurantiaca Lindley.

A. Female adult.

B. Male adult.

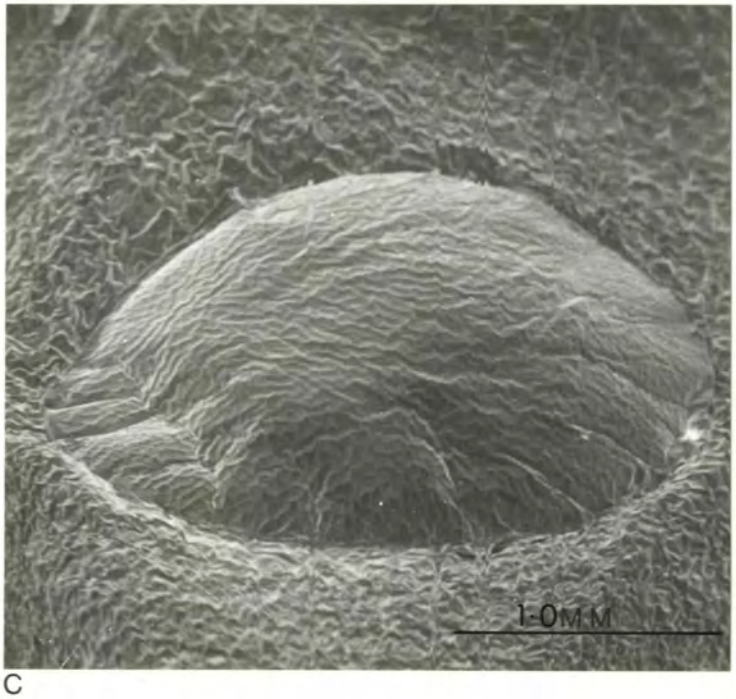
C. A flat, disc-shaped egg.

D. Each first instar larva penetrates the cactus cladode leaving a penetration window and small pile of frass.

E. The internal feeding damage of a cactus cladode by a late instar larva.

A

B



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ABSTRACT

Inbreeding was thought to be responsible for the loss in the second filial generation (F_2) of Amalafrida leithella Dyar, Cactoblastis mundelli Heinrich, Nanaia sp. Heinrich, Sigelgaita sp. Heinrich and Sigelgaita transilis Heinrich in the laboratory. This pre-empted the investigation of the effects of inbreeding on another cactophagous moth, Mimorista pulchellalis Dyar, an established biological control agent of jointed cactus in South Africa.

Initially three populations were set up. A randomly-mating control (OUT 1) population, and a sibmating experimental (IN 1) population, consisted of laboratory-reared stock. A second experimental population (KR 1) comprised a small number of field-collected randomly-mating individuals which recreated the conditions under which the five above-mentioned species were lost.

The inbreeding depression of fifteen fitness components was assessed. The mean values of each component in each generation of treatments IN 1 and KR 1 were compared with those of OUT 1. In addition the mean values of IN 1 were regressed against the coefficients of inbreeding since inbreeding depression is linear with respect to the probability of two genes at any locus being homozygous through ancestry.

The component egg viability was important because a reduction in OUT 1, IN 1 and KR 1 in the F_2 resulted from mated females producing no viable eggs. Duplicate treatments OUT 2, IN 2 and KR 2 were set up to confirm whether this was a general F_2 phenomenon.

Assessment of the fitness components prevented a direct evaluation of the numbers of offspring produced. However a hypothetical estimate of population size and growth rate was made using the percentage survival calculated from life-table analysis. Although not statistically demonstrable in the component analysis, life table analysis indicated

that egg viability suffered an inbreeding depression and affected population fitness. It was also evident that treatments OUT 1 and 2 were fitter than treatments IN 1 and 2 and KR 1 and 2 with respect to population fitness.

Thus, inbreeding, resulting from sibmating and introduction of a small number of individuals to a laboratory, caused a decrease in numbers of offspring produced and population growth rate. This is important in relation to the demise of the cactophagous Lepidoptera and to future biological control programmes.

CHAPTER 1

INTRODUCTION

Opuntia aurantiaca Lindley (jointed cactus) is a low-growing, succulent plant. It has almost cylindrical cladodes covered in sharp, barbed spines and tiny glochids, and produces attractive, small, yellow flowers and reddish, club-like fruits. Reproduction is vegetative, and the cladodes which are easily dislodged, root readily and if buried, form woody tubers or storage organs (Zimmermann 1981).

The exact details of the history of O. aurantiaca in South Africa are not known but a comprehensive review of all the particulars available is provided by Moran et al. (1976) and Moran and Annecke (1979). The plant is indigenous to Uruguay and Argentina and is thought to have been imported to South Africa via England in 1843 or perhaps earlier. Collectors could obtain the plant from the Cape Town Botanical Gardens from 1848 to 1858. Because of its drought resistant and ornamental properties, it was grown in dry areas such as the Eastern Cape and the Karoo. At first jointed cactus was cultivated in rockeries, cemeteries and hedgerows, but by the 1890's it had encroached on other areas.

In 1892, MacDonald described it as a noxious weed that ought to be removed: since then various forms of control have been implemented which have been discussed by Moran and Annecke (1979) and Zimmermann and Moran (1982). Control was essential as large portions of grazing land were being invaded by the jointed cactus, replacing nutritive plants and also preventing livestock from reaching the edible plants. The sharp spines are injurious to animals while wool entangled with cladodes depreciates in value (Zimmermann 1981).

The first attempt at biological control commenced in 1936 when the cochineal insect, Dactylopius austrinus De Lotto was released on jointed cactus. During the 1930's Cactoblastis cactorum Berg was released on Opuntia fiscus-indica (Linnaeus) Miller, but the insect has

subsequently attacked jointed cactus (Robertson 1985). Initially these insects, especially D. austrinus, had a large impact on the weed but because there was regrowth from the damaged plants, the cochineal insect was, by 1946, considered to have failed as a biological control agent (Slabber 1964). As a consequence further biological control was abandoned and herbicidal control was reintroduced.

Biological control attempts have since been renewed because of the high cost of chemical control and its limited efficiency (Moran and Annecke 1979). For this reason several additional natural enemies, mainly Lepidoptera, have been imported to South Africa from South America. Of these, only one has been successfully established, namely, the pyraustid moth, Mimorista pulchellalis Dyar (Nieman 1983). Tucumania tapiacola Dyar was also released on jointed cactus but failed to establish (Hoffmann 1982), even though it had done so in Australia (Dodd 1940).

Considerable problems have been encountered in the laboratory-rearing of other species of cactophagous Lepidoptera for the biological control of jointed cactus. These species were; Amalafrida leithella Dyar, Cactoblastis mundelli Heinrich, Nanaia sp. Heinrich, Sigelgaita sp. Heinrich, and Sigelgaita transilis Heinrich. Attempts were made to rear these species in the laboratory for the purpose of host-specificity tests, to obtain a large number for mass-release, and to eliminate any possible disease from the stock. Initially, the laboratory- rearing went well, but in the second filial generation (F_2) each species suffered a decrease in numbers and, except for three shipments of Nanaia sp., entire cultures were lost (Appendix 1).

The aim of the present study was to determine the cause of the demise of these insects in the laboratory. Because laboratory-rearing is an integral part of most programmes the present investigation is particularly relevant to future biological control projects.

There is also considerable concern about the effects of laboratory-rearing on the establishment and success of biological control

programmes in the field (Myers and Sabath 1981, Mackauer 1981). One cause for this concern occurred in the early 1970's when mass-production of the screwworm fly, Cochliomyia hominivorax Coquerel, was aimed at rearing the maximum number of flies at the lowest possible cost (Bush 1975). This goal was achieved but the flies reared had been, inadvertently, selected for the temperature and light conditions of the laboratory. When they were released in the field these male flies were no longer competitive against the wild males and this led to the failure of the screwworm eradication programme (Bush and Neck 1976).

Since then a much broader approach to the evaluation of biological control has been taken. Both Chambers (1975) and Huettel (1976) have suggested that the success of biological control depends on how well an insect functions in its intended role, either in the laboratory or in the field. This success is determined by comparing the insect's performance to a standard, usually derived from observations of the insect under natural conditions. The extent of the success is, in turn, related to the fitness of the insect or its inherent ability to perform a task. Therefore, this task may be related to increasing numbers for mass-production or how well the insect performs at biological control in the field.

In some way, the failure of the cactophagous species A. leithella, C. mundelli, Nanaia sp., Sigelgaita sp., and S. transilis, to be reared in the laboratory, was a result of a decrease in fitness. There are several factors which may influence the fitness of laboratory-reared insects: (a) disease (Myers and Sabath 1981), predation or parasitism (Moran and Zimmermann 1984), and (b) artificial laboratory conditions such as temperature, humidity, light, diet, crowding and maintenance schedule (Mackauer 1976, Mackauer 1981, Zimmermann and Moran 1982, Simmonds 1964).

(a) Disease, predation or parasitism

The probability of disease, predation or parasitism being responsible

for the loss of the populations of the cactophagous Lepidoptera used for biological control was rejected. There was no evidence of these in any of the insect stock (Hoffmann pers. comm.). It would also have been unlikely that either one of these factors would have operated on all of the species, A. leithella, C. mundelli, Nanaia sp., Sigelgaita sp., and S. transilis in the F_2 generation, on separate occasions.

(b) Artificial laboratory conditions

In the founder populations, small numbers of A. leithella, C. mundelli, Nanaia sp., Sigelgaita sp., and S. transilis were imported, on each occasion (Appendix 1). Financial, ecological and practical constraints hindered the collection of larger numbers. Thus, these populations had gone through a "genetic bottleneck" as they consisted of a few individuals from an originally larger population (Myers and Sabath 1981). The amount of genetic variability lost, as a consequence, depends on the size of the "bottleneck", the rate of population growth and the mutation rate (Nie et al. 1975). Also the degree of naturally occurring inbreeding in the original population would influence the loss of genetic variability because inbreeding leads to genetic uniformity (Falconer 1981). Therefore, a natural population with a high degree of inbreeding would lose less genetic variability. Once within the laboratory a population is subjected to artificial environmental conditions. Two modifications can result from this: (i) non-permanent physiological and behavioural changes or (ii) more permanent genetic changes (Mackauer 1976).

(i) If the artificial conditions fall within the range to which the insects are already suited, the populations do not change in major ways. If not, individuals may change behaviourally or physiologically to suit the laboratory conditions. Mackauer (1981) describes this as acclimatization although it cannot be easily distinguished from selection. Selection involves a change in gene frequency (Wright 1969), but it is not always known if the proportions of genes change during acclimatization (Mackauer 1981). Since all or most of the individuals have the capacity to acclimatize the consequences are not

severe. Therefore, these non-permanent changes were probably not responsible for the above-mentioned loss of the cactophagous Lepidoptera populations.

(ii) It appeared likely that the factor responsible was genetic, particularly because small numbers of individuals had been introduced to the laboratory. Random genetic drift and inbreeding are related to population size (Falconer 1981, Mackauer 1981), and their effects cannot easily be distinguished from that of a third genetic factor selection.

Genetic drift results from a limited number of genotypes being chosen, at random, from a larger population (Falconer 1981). This may lead to a genotype being successfully established within the laboratory which is representative of a minor portion of the naturally occurring population. However, selection acts to eliminate those genotypes, which are the least suited to the environmental conditions, and thus influences the direction of the genetic change (Mackauer 1981). Selection, for example, was responsible for the change in the frequencies of the genes which determined the flight patterns in C. hominivorax. This, subsequently, affected the ability of the mass-produced males to find and so mate with the wild females (Bush and Neck 1976). Drift is unlikely to have operated repeatedly in all of the laboratory founder populations and always in the F_2.

Of the three genetic factors, inbreeding was thus thought most likely to have caused the loss of the A. leithella, C. mundelli, Nanaia sp., Sigelgaita sp., and S. transilis populations. Inbreeding is defined as the mating of individuals that are closely related to each other by ancestry (Falconer 1981). It results in a change of gene frequency causing an increase of homozygotes at the expense of heterozygotes. This may produce an inbreeding depression or a decrease in the fitness of an individual, as deleterious genes are often recessive.

The fitness of an individual is described as "its relative ability to contribute to the next generation" (Falconer 1981). This is not only

in terms of the numbers of offspring, but also their quality. The ability of an individual depends on all of its morphological, physiological and behavioural attributes, such as size, the success of mating, fertility and viability. Any change in the fitness of an individual will be related to a change in the mean values of these traits, particularly those associated with reproduction (Falconer 1981). Both the fitness components and the consequent fitness of individuals are assessed relative to a population mean.

A change in the fitness of individuals would affect the size of a population and its growth rate, the population fitness. Careful consideration has to be given to the fitness of a population because it is affected by environmental conditions (Falconer 1981). So for the comparison of two populations, either in the field or the laboratory, the environmental conditions have to be the same. To summarize, inbreeding causes a decrease in the mean values of the fitness components, in the fitness of an individual and consequently, in the fitness of a population.

The rate at which inbreeding depression occurs depends on the system of mating, the degree of the ancestral relationship of the mated individuals and the population size. In a small population the chances of mating with a close relative are increased. With knowledge of the mating regime and the population size, the inbreeding coefficient (F) or the likelihood of two genes at a locus in an individual being homozygous, through ancestry, can be calculated. The inbreeding depression in a fitness component, is linearly related to the calculated inbreeding coefficients (Falconer 1981).

As mentioned previously, the loss of the cactophagous Lepidoptera populations was thought to be attributable to the small numbers of founder individuals that had been imported. Further, the decrease in population fitness that occurred in the F_2 made the other possibilities appear unlikely. The initial population (base population, F_0) placed in the laboratory was the product of random matings or outbreeding. With a further reduction in size, because of selection against those

individuals not suited to the laboratory conditions and no recruitment the F_1 progeny were likely to mate with a close relative. These considerations initiated the investigation of the effects of inbreeding on the pyraustid moth, M. pulchellalis.

M. pulchellalis was chosen for the investigation instead of the other cactophagous Lepidoptera, A. leithella, C. mundelli, Nanaia sp., Sigelgaita sp., and S. transilis, because it had been successfully laboratory-reared and established in the field. M. pulchellalis was obviously less affected by the factor, thought to be inbreeding, which was responsible for the loss of the above species. This success of M. pulchellalis was a prerequisite for the study envisaged because it allowed an investigation of the effects of inbreeding for more than two generations. C. cactorum also had the same credentials as M. pulchellalis but the latter species was chosen for several reasons. M. pulchellalis has approximately six generations per year in comparison to the two generations of C. cactorum (Robertson 1985). This enabled the study of high levels of inbreeding, over more generations, in the available time. In addition C. cactorum produces eggsticks and the larvae are gregarious while M. pulchellalis lays single, flat, disc-like eggs on a cactus cladode (see frontispiece, c) and one larva is found per plant (Nieman 1983). This enabled greater accuracy to be achieved when counting the number of eggs and larvae.

The history of M. pulchellalis in South Africa is presented in Fig 1. It describes, briefly, the importation of one hundred and twenty-eight larvae, collected in June 1978 from Parana, Argentina. The larvae were transported in plastic vials, with artificial medium or cladodes of the host plant to Rhodes University, Grahamstown. Of these, one hundred and eighteen survived and then emerged as adults. A culture was maintained in Grahamstown and a supplementary culture, which originated from this stock, was maintained at the Weeds Laboratory, Uitenhage.

Permission for the release of this species was given after host-specificity tests had been satisfied and the first large scale releases were made on the Andries Vosloo Kudu Reserve, over the period March

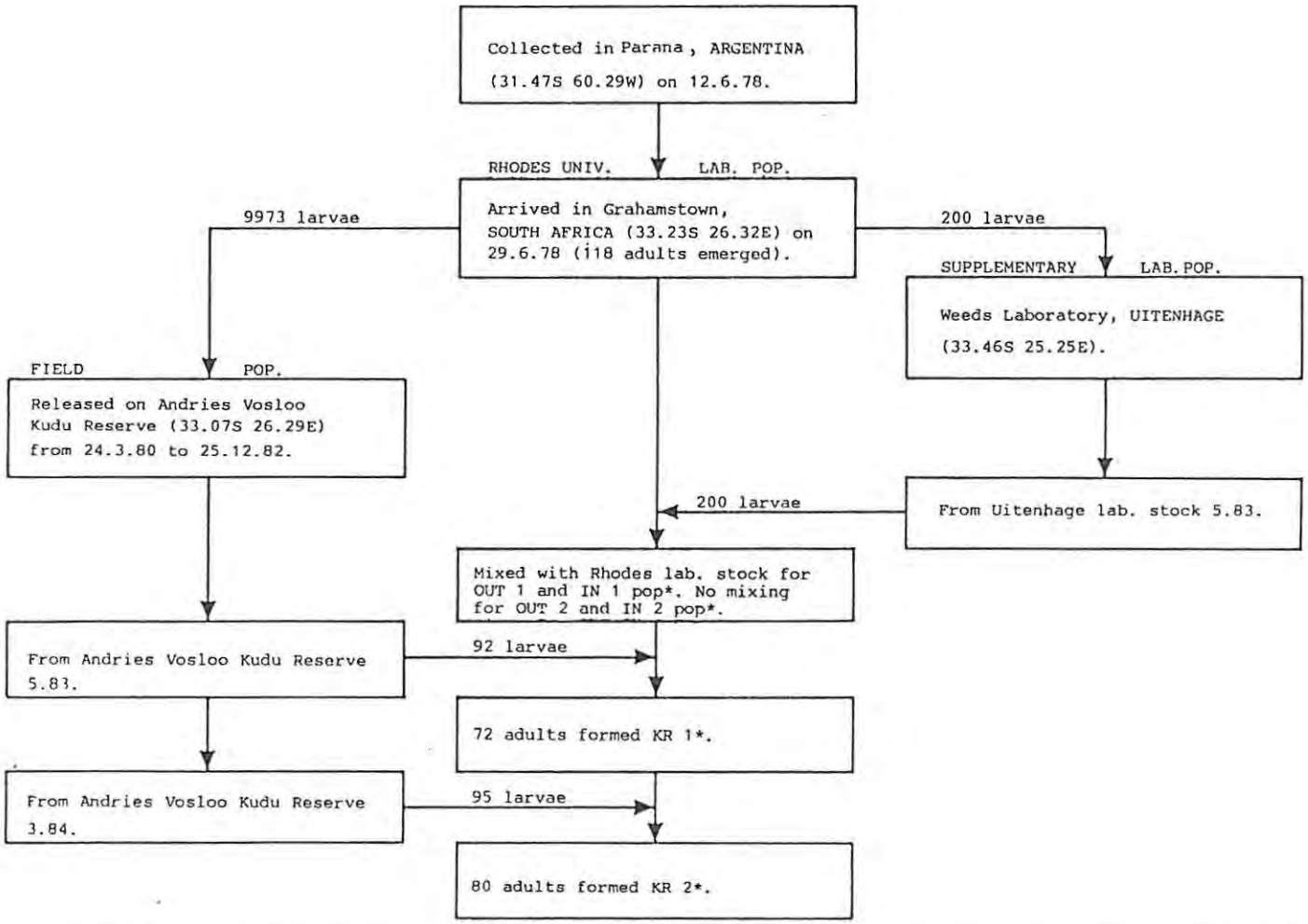


Fig 1. The history of M. pulchellalis in South Africa. Larvae were imported from Parana, Argentina to Rhodes University, Grahamstown (Rhodes Univ. lab. pop.). A supplementary culture was established at the Weeds Laboratory, Uitenhage (Supplementary lab. pop.). Releases were made in the Andries Vosloo Kudu Reserve (Field pop.). 200 larvae from Uitenhage were mixed with the Rhodes stock from which the OUT 1 and IN 1 populations were derived. Approximately 11 months later, the OUT 2 and IN 2 populations were initiated from Rhodes stock, except that Uitenhage stock was not used this time. KR 1 and KR 2 consisted of individuals collected on the Andries Vosloo Kudu Reserve.
* see Chapter 2, Fig 2 for further explanation.

1980 to December 1982. Subsequently, Nieman (1983) has reported that the moth is successfully established in the field. The individuals had been established for at least three generations and at most thirteen by the start of the present study, May 1983. It is therefore assumed that they would have adapted to the field conditions.

In order to present an outline of the investigation of inbreeding on M. pulchellalis, the reasons for using certain methods have to be explained. These reasons depend upon some of the early results obtained, which are presented here. An interpretation of the terminology and the basic principles is also given. A detailed description, however, is provided in Chapters 2 and 3.

The availability of two laboratory cultures and a field population of M. pulchellalis enabled the following three treatments or populations to be set up.

(i) The randomly-mating, OUT 1 population was initiated from a laboratory stock. It was established to provide a control against which the two experimental populations, IN 1 and KR 1, could be compared.

(ii) The IN 1 population was derived from the same laboratory stock. In this population a close system of mating, full-sibmating, was employed. This ensured that matings took place between individuals, more closely related, than the average relationship of the individuals in the outbred (OUT 1) population (Falconer 1981).

(iii) The founder stock for the KR 1 population was collected from the established field population in the Andries Vosloo Kudu Reserve. It was set up to investigate the effects of inbreeding resulting from the introduction of a small field population to a laboratory environment. This recreated the conditions under which the earlier unsuccessful attempts, to rear the other cactophagous moths in the laboratory, had taken place. Selection against those individuals not suited to the laboratory conditions, would result in a decrease in the effective population size and inbreeding would be intensified. Therefore, once

again the chances of mating with a close relative would be greater in this population than in the OUT 1 control population, although less than in the IN 1 population.

Treatments OUT 1, IN 1 and KR 1 each consisted of a number of sub-populations or lines (N) (Falconer 1981). A line was derived from a single mated female in the base population. At this stage it is necessary to distinguish between the use of "N" and "n". The former denotes the number of lines while the latter the number of generations. OUT 1 and IN 1 were maintained for nine generations and KR 1 was maintained for eight.

One way to detect an inbreeding depression is to evaluate all the descendants of a number of lines, in each generation, under various mating regimes. However, the measurement of the numbers of offspring does not allow any analysis of the inbreeding depression in the components of fitness. Consequently that fitness component which contributes most to the fitness of a population cannot be distinguished. For this reason it was decided to measure fifteen fitness components, in each generation, within each line. In order to do this, in the limited space with the available labour, the number of lines in each treatment was restricted to twenty. Unfortunately, the asynchronous emergence of male and female moths (Chapter 2), prevented the initiation of twenty lines in the base population in all of the treatments. This necessitated the increase of the number of lines in the next and subsequent generations. The fitness components, evaluated in each generation within each line, were representative of all the stages of the life cycle of M. pulchellalis. They were also relevant to the performance of this species as a biological control agent. The following components were measured: (i) mate success, (ii) number of eggs laid, (iii) egg viability, (iv) egg hatch, (v) female egg and (vi) male egg developmental periods (vii) larval penetration of the cactus cladode, (viii) larval and (ix) pupal survival, (x) female larval to adult and (xi) male larval to adult developmental periods (xii) virgin female and (xiii) mated female longevity, (xiv) female wing length, as a measure of size and (xv) female to male adult sex ratio.

The mean value of each fitness component was calculated, in each generation and for all generations combined in each treatment (see Chapter 2, Fig 2). Thus any change in fitness could be monitored. In the second filial generation (F_2) a reduction in the number of viable eggs occurred simultaneously in OUT 1, IN 1 and KR 1 (see Chapter 3). This decrease was brought about by a greater number of mated females laying no viable eggs. The reduction in egg viability is important because the decrease occurred in the same generation, the F_2, in which the A. leithella, C. mundelli, Nanaia sp., Sigelgaita sp., and S. transilis stocks were lost. To reaffirm whether the reduction in egg viability was a general F_2 phenomenon, duplicate treatments, OUT 2, IN 2 and KR 2, were set up.

Once the nature of the changes in the fitness components had been established, it was possible to determine what effect inbreeding would have on the numbers of offspring. As mentioned previously, the restriction of the number of lines to twenty, as a consequence of measuring the fitness components, precluded the possibility of calculating the total descendant production. However, the population sizes and growth rates could be simulated using life table analysis (Varley, Gradwell and Hassell 1973). A hypothetical estimate of the population fitness of M. pulchellalis was made using the mean values of mate success, number of eggs laid, egg viability, egg hatch, larval penetration and survival, and pupal survival that were calculated in the above-mentioned experiments. This analysis enabled the key factor, or that fitness component which contributed most to the fitness of a population to be determined. Comparisons of the size of each population were then made to establish the extent to which the fitness of the OUT 1 and 2 treatments differed from the IN 1 and 2 and KR 1 and 2 treatments, in terms of numbers. Such a difference would indicate an inbreeding depression.

The logistics of setting up the populations, the laboratory conditions and the associated problems are presented in Chapter 2. Also given are the ways in which the fitness components were measured, the fitness of the population calculated, and the statistical procedures used. The

results of the statistical analyses used to determine if inbreeding had affected the mean values of each fitness component are given in Chapter 3. Chapter 4 deals with the results of the life table analyses from which the fitness of each population was derived. Finally, a perspective of all the results is presented in Chapter 5.

CHAPTER 2

MATERIALS AND METHODS

This chapter deals with the (2.1) experimental and (2.2) statistical methods.

(2.1) Experimental methods

A diagrammatic synopsis of the programme undertaken in this research is shown in Fig 2. Initially three populations were instituted (Fig 1). Two populations, OUT 1 for outbreeding, and IN 1 for sibmating, were taken from laboratory stock. The stock used were progeny of Rhodes University laboratory stock crossed with Uitenhage Weeds Laboratory stock. The third population, KR 1, originated from individuals collected in the Andries Vosloo Kudu Reserve. The duplicate populations, OUT 2, IN 2, and KR 2, were started approximately eleven months later and were identical except that Uitenhage stock was not used in the founder populations in treatments OUT 2 and IN 2.

For the purpose of this research, a maximum of twenty lines (N) formed each treatment. A line consisted of the offspring of one breeding female. Each female was placed in a cage with two sibling males to decrease the possibility of male sterility or size incompatibility being the cause of a failure to mate. Because the females only mate once (Nieman 1983) no genetic discrepancy would occur as a result of a female being inseminated by two males. The individuals which were mated in the OUT 1 and 2, and KR 1 and 2 populations, were randomly chosen. Those mated in the IN 1 and 2 populations were siblings, to ensure a greater degree of inbreeding than in the outbred populations. In each of these populations, mating had been random in the base populations. Therefore only in the F_1 would sibmating take place in IN 1 and 2.

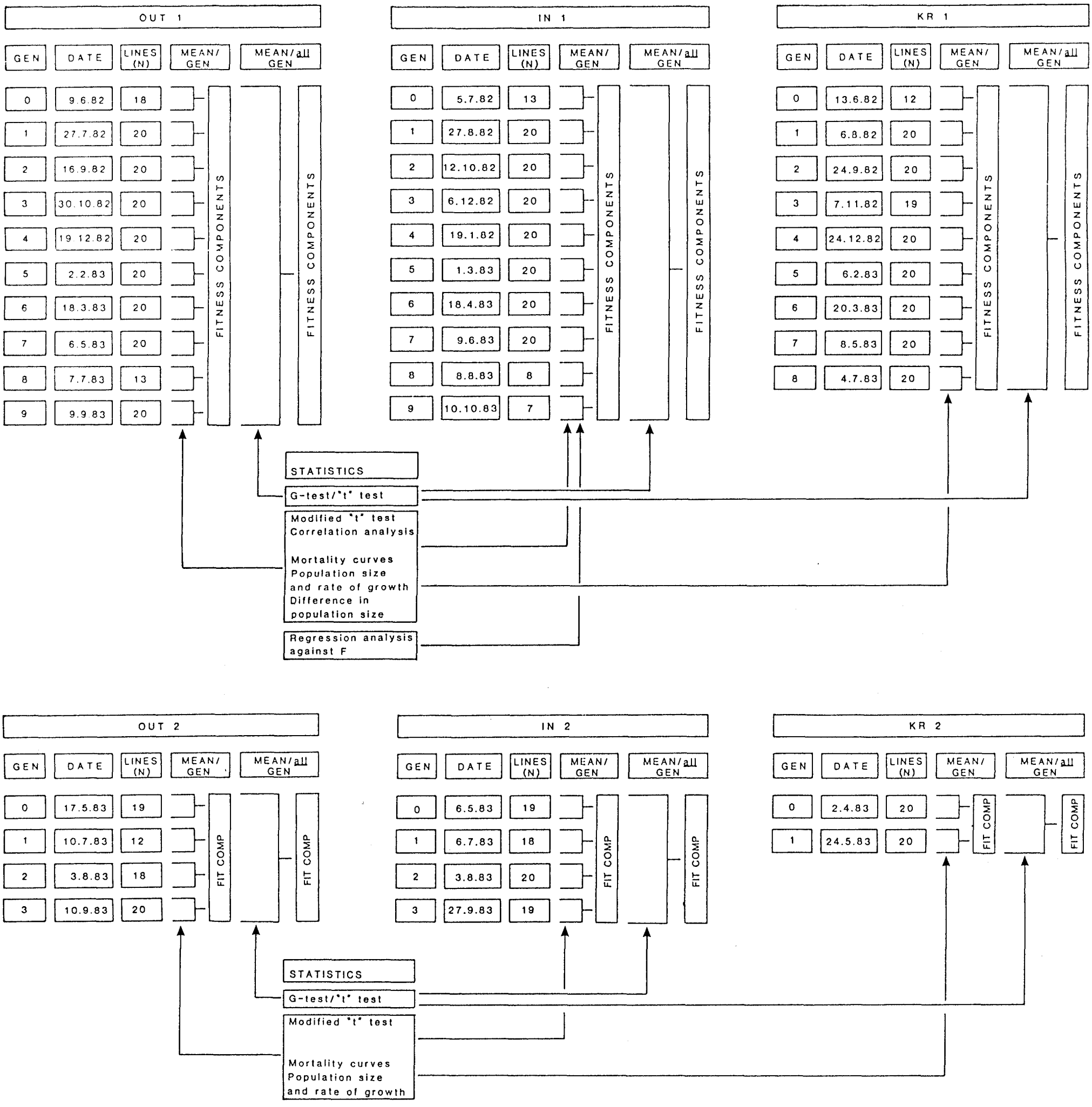


Fig 2. A synopsis of the OUT 1 and 2, IN 1 and 2, and KR 1 and 2 populations of M. pulchellalis. The generations (Gen.), date on which each generation started, and the number of lines in each generation are shown. An indication of what is meant by the mean values in each generation and for all generations combined of the fitness components is given. A list of statistical procedures used to compare the mean values and population fitness of treatments IN 1 and 2 and KR 1 and 2 with those of OUT 1 and 2 is also provided.

The experimental duration lasted several generations. OUT 1 and IN 1 were continued until larval penetration in the F_9. The rate of inbreeding decreases with time so by the F_9, IN 1 had an inbreeding coefficient of F= 0.859 (Falconer 1981). This means that there was an 86% chance of two genes, at any locus in an individual, being identical by descent. IN 1 suffered a reduction in the percentage egg viability in the F_8 (Fig 7). To see if this trend would continue, termination of the laboratory populations was delayed for a further generation. The KR 1 population was terminated earlier and the F_8 progeny were not mated. Data was collected from OUT 2 and IN 2 until larval penetration in the F_3 generation. This was in order to determine if the decrease in egg viability always occurred in the F_2 generation in all populations. KR 2 was discontinued after the F_1 adult emergence because it was considered a duplicate of OUT 2.

Environmental conditions are known to affect the fitness of a population (Falconer 1981). Therefore, both groups of populations were housed under identical conditions so that they could be compared. The conditions within each laboratory simulated those of early summer. They consisted of 14 hours daylight, at 25($\pm$1)^{o}C and a relative humidity of 45($\pm$10)%; followed by 10 hours darkness, at 17($\pm$1)^{o}C and a relative humidity of 90($\pm$10)%.

Fig 3. A cage in which a female and two male moths of M. pulchellalis were placed for breeding purposes. The female laid her eggs on a jointed cactus plant, O. aurantica, situated centrally in the cage.

The breeding individuals were housed in twenty-four cages (300x300x 300mm) held on metal stands (Fig 3). One side of each cage was fine mesh (0.5mm) while the other sides were clear perspex. The bottom was made of hardboard and was supported in runners so that it could be removed. The hardboard consisted of two halves with a central hole in which a plant was fitted.

Fig 4. The dried shrunken cladode of O. aurantiaca (top left hand side) indicates the presence of a pupa of M. pulchellalis. Recorded on the yellow label are the replicate code, number of the larva, and the dates on which the egg was laid and hatched.

The plants (Fig 4) were reared as follows. Cladodes of O. aurantiaca were collected from the Andries Vosloo Kudu Reserve, placed in seed trays in standardized potting soil and kept under constant

environmental conditions: 14 hours daylight at 27(±1°C), and a relative humidity of 50(±10)%; followed by 10 hours darkness, at 20(±1°C) and a relative humidity of 50(±10)%. Once the cladodes had sprouted and rooted they were transferred to tubes (180x100mm and 140x80mm) filled with potting soil. They were then kept under ambient conditions in a greenhouse. The plants were watered weekly and fertilized with "Wonder Potplant Food" (18% N, 12% P, 9% K, 0.01% Mn, 0.01% Zn, 0.003% B) at monthly intervals. Only plants with new growth were used for experimental purposes and after use they were returned to the greenhouse to recover.

Fifteen components of fitness were measured to assess the effects of inbreeding in M. pulchellalis. The first component to be measured in any generation was (i) mate success. Female moths which laid viable eggs were known to have mated. However, failure to lay viable eggs did not necessarily indicate that the female had not been mated. The female's abdomen was cut off and macerated in hot 10% KOH for 10 to 15 minutes. Then the bursa copulatrix was dissected and examined under a microscope to determine the presence of the spermatophore. If mating had taken place the brown chitinous spermatophore could be clearly seen, through the white wall of the bursa copulatrix (Ferro and Akre 1975).

(ii) The eggs laid by a female, on a cactus plant (see frontispiece, c) were counted. Each day the plant in the cage was removed and substituted with an unused plant. The number of eggs on the former plant was then recorded. After two days (iii) the viable eggs, which had turned dark grey, were counted; the non-viable eggs remained yellow and, subsequently, shrivelled. A few days later, the number of viable eggs which had (iv) hatched was counted by noting the number of flimsy transparent egg cases. Not all of the eggs hatched simultaneously so the observation was continued for up to three days or until it was certain that no more would hatch. Both the (v) female egg and (vi) male egg developmental periods were recorded. The sex of an egg could be determined by following the fate of each larva until adult eclosion. This was possible because the larvae were numbered when they penetrated

the cactus cladode. Each first instar larva penetrates the cactus cladode separately, leaving a penetration window and a small pile of frass (see frontispiece, d). With rare exceptions each larva could be associated with its window. Every window represented a successful (vii) larval penetration.

To maintain manageable numbers a maximum of twenty larvae, from each replicate were randomly chosen to represent a line. In the field only one M. pulchellalis larva is found living within a plant (Nieman 1983). Taking this into account and also to prevent food shortage, one larva was reared on a host plant in the insectary. Each larva was removed from the original plant and transferred to another plant with a damp paintbrush. This was not entirely unnatural because larvae had been observed to instinctively penetrate other cactus plants on depletion of their first food supply. Because it was known that most larvae penetrate the terminal flush of a plant (Nieman 1983) each larva transferred was placed in this position on the new host plant. The plant was then labelled with the larval number, the date on which the egg was laid and that on which the larva had hatched. If, after two days, the larva had not penetrated the cladode and there were still some progeny remaining on the initial plant the larva was replaced. Of those transferred 81% (SE = 0.001, N = 565) survived; an indication of the method's success.

All the host plants were checked regularly. If the food supply was insufficient the larva was moved to another plant. A dry and shrunken cladode indicated that the larva had pupated in the distal end of the plant (Fig 4) (Nieman 1983). The pupa was then carefully removed from its silken cocoon, placed on a piece of absorbent towel, in a glass vial which was closed with a gauze stopper. From the number of pupae collected (viii) the larval survival could be ascertained. The number of adults emerging gave an indication of (ix) pupal survival. The date on which this occurred was recorded so that the (x) female larval to adult and (xi) male larval to adult developmental periods, and eventually the (xii) virgin female and (xiii) mated female longevity, could be obtained. Then, (xiv) the length of the right wing of each

mated female was measured from the point of articulation with the thorax to the apical angle (Chapman 1971) as an indication of size. A pair of dividers was used for this purpose. Although this relationship has not been quantified in M. pulchellalis it has been recorded in Drosophila melanogaster Meigen (Robertson and Reeve 1952). Finally, (xv), the female to male adult sex ratio was also determined.

The mean values of each fitness component, for each generation within each line were calculated. IN 1 and 2 and KR 1 and 2 were statistically compared to OUT 1 and 2 in each generation to determine differences between treatments attributable to inbreeding. However, before discussing these tests the problems encountered in the course of the research should be mentioned.

As stated above the decision was made to transfer two hundred larvae from the Uitenhage Weeds Laboratory stock to reduce any effects of inbreeding in the Rhodes University laboratory stock that may have already occurred. Inbreeding leads to genetic uniformity, therefore the addition of new stock would increase the amount of genetic variability and eliminate the effects of inbreeding depression (Falconer 1981). This was done prior to the initiation of the OUT 1 and IN 1 populations. When the OUT 2 and IN 2 populations were set up, approximately eleven months later (Fig 2), the same procedure could not be implemented because all the Uitenhage Weeds Laboratory stock had been released to the field.

In some generations there were less than twenty lines in each treatment. This, for example, occurred in the base population of all the populations barring that of the KR 2. The problem arose primarily because of the asynchronous emergence of the male moths before the females. The developmental duration from egg to adult eclosion was on average shorter in male individuals ($\overline{x}$ = 46.0 days, SE = 1.07, N = 411) than in female individuals ($\overline{x}$ = 48.1 days, SE = 1.27, N = 420). To ensure a sufficient supply of male moths and, hence, increase the chances of obtaining the required number of lines, the male moths were kept at 15^{o}C, in continuous light. Those males of four days or older

were not used because age may have affected their virility. Virgin females, however, were kept in vials within the laboratory so that their longevity could be monitored. It is known that age affects a female's egg-laying ability (Nieman 1983) so females were not used when older than two days. All the available progeny were mated, from which twenty lines were randomly chosen.

The problem was exacerbated in IN 1 and 2 due to further restriction of sibmating. In some cases, a small number of progeny combined, with the asynchronous emergence, resulted in a loss of an entire line (Appendix 2). To make up the required number of lines in the next and subsequent generations, some of the existing lines were split into sublines. Two sibling females, randomly chosen from an existing line, each formed a separate line in the next generation.

A further two unrelated problems occurred. Firstly, two lines in IN 1 were lost in the F_3 because of egg parasitism (Appendix 2). The egg parasitoid Trichogramma sp. (Richards 1982) was however eliminated before the next generation. Secondly, a faulty light switch prevented the lights from switching off in the laboratory. The malfunction of the day:night regime on this occasion would almost certainly have inhibited mating amongst IN 1 individuals breeding in the F_7 generation. This is because female moths initiate calling at dawn after which mating takes place (Nieman 1983).

In spite of these problems, the required number of generations of OUT 1 and 2, IN 1 and 2 and KR 1 and 2 were successfully maintained and an adequate amount of data was collected. The data was analysed using the following procedures.

2.2 Statistical methods

The data was stored and analysed on a computer using the programme, Statistical Package for the Social Sciences (Nie et al. 1975).

The effects of inbreeding may be detected by comparing the changes in the fitness components in the different treatments. Initially, the mean values of each fitness component were calculated for each generation and for all generations combined (Fig 2). This was to determine whether, in each generation, any change in the mean values existed between treatments and which fitness component had been affected. The ways in which the mean values of the fitness components in each generation were calculated are summarized in Table 1. However, they will be briefly described below.

In the following cases each female or line contributed only a single result to the mean value of the fitness component for a particular treatment. These components were: number of eggs laid, egg viability, egg hatch, larval penetration, larval and pupal survival, wing length and sex ratio. Excluding the components number of eggs laid, wing length, and sex ratio, the result of the other above characters was calculated as a percentage of the number of individuals in the preceding stage. Thus, for the latter components, egg viability, egg hatch, larval penetration, larval and pupal survival, a mean percentage was calculated in each generation for each treatment.

The mean values for those characteristics that could have become biased because of the differing numbers of offspring evaluated per female were calculated as follows. The mean values in each generation of the female egg and male egg developmental periods, female larval to adult and male larval to adult developmental periods, and virgin female and mated female longevity, were calculated as a grand mean from the mean values of each line in each particular treatment. This was done because the offspring in each generation are not independent but belong, as siblings, to a particular line. The method used prevented a bias in the results by a line more heavily weighted, in terms of the number of offspring, than the other lines within a treatment.

Two statistical approaches were used to determine whether fitness had been affected. Firstly (a) the mean values of the fitness components for each generation and for all generations combined were compared

Table 1. The ways in which the mean values in each generation of the fitness components were derived in each treatment of *M. pulchellalis*. The maximum number of lines (N) in each treatment was twenty, and one female (f) represented a line, hence the notation; N_{f20}. Some calculations included only those females within a treatment that contributed a result (N_{fx}). For instance a mean value of the number of eggs laid did not include the number of eggs laid by unmated females.

FITNESS COMPONENT	CALCULATION OF MEAN VALUE
Mate success	$=\sum_{N_{f1}}^{N_{f20}}$ (females inseminated) $/N_{f20}$ x100
Number of eggs laid	$=\sum_{N_{f1}}^{N_{fx}}$ (no eggs laid) $/N_{fx}$
Egg viability	$=\sum_{N_{f1}}^{N_{fx}}\left(\frac{\text{no viable eggs}}{\text{no eggs laid}}\right)$ x100 $/N_{fx}$
Egg hatch	$=\sum_{N_{f1}}^{N_{fx}}\left(\frac{\text{no eggs hatched}}{\text{no viable eggs}}\right)$ x100 $/N_{fx}$
Female egg and male egg developmental periods	$=\sum_{N_{f1}}^{N_{fx}}\left(\frac{\text{no days}}{\text{no individuals}}\right)$ $/N_{fx}$
Larval penetration	$=\sum_{N_{f1}}^{N_{fx}}\left(\frac{\text{no larvae penetrated}}{\text{no eggs hatched}}\right)$ x100 $/N_{fx}$
Larval survival	$=\sum_{N_{f1}}^{N_{fx}}\left(\frac{\text{no pupae}}{\text{no larvae transfered}}\right)$ x100 $/N_{fx}$
Pupal survival	$=\sum_{N_{f1}}^{N_{fx}}\left(\frac{\text{no adults}}{\text{no pupae}}\right)$ x100 $/N_{fx}$
Female larval to adult and male larval to adult developmental periods	$=\sum_{N_{f1}}^{N_{fx}}\left(\frac{\text{no days}}{\text{no individuals}}\right)$ $/N_{fx}$
Wing length	$=\sum_{N_{f1}}^{N_{f20}}$ (total wing length) $/N_{f20}$
Sex ratio	$=\sum_{N_{f1}}^{N_{fx}}\left(\frac{\text{no female adults}}{\text{no male adults}}\right)$ $/N_{fx}$

between treatments. Secondly (b) a test was carried out to determine whether there was the expected linear relationship (Falconer 1981) between each of the fitness components of IN 1 and the corresponding coefficients of inbreeding. In the employment of these analyses some general considerations (c) were taken into account.

(a) The mean values for each fitness trait were compared for each generation between OUT 1 and IN 1 and between OUT 1 and KR 1. The same procedure was carried out in the second series of experiments. Any significant differences between the outbred, control populations and the sibmated and field-introduced experimental populations, resulting from inbreeding would thereby be detected. To allow for the different sample sizes and variances, a modified Student's "t" test was used (Snedecor and Cochran 1980):

$$t' = (\overline{X}_1 - \overline{X}_2) / \sqrt{S_1^2/N_1 + S_2^2/N_2}$$

(where s^2 = sample variance).

For the comparison of the mean values for all the generations combined both a Student's "t" test and a G-test of independence (Sokal and Rohlf 1981) were used. Treatments OUT 1 and 2 were compared with IN 1 and 2 and KR 1 and 2 as before. Student's "t" test was used for the female egg and male egg developmental periods and the female larval to adult and male larval to adult developmental periods, virgin female and mated female longevity, wing length and the sex ratio while the percentages of egg viability, egg hatch, larval penetration, and larval and pupal survival were tested using a G-test of independence.

(b) To test whether a linear inbreeding depression had occurred, the mean values in each generation of IN 1 were regressed against the corresponding inbreeding coefficients (F). The Null hypothesis was that the slope of the line was zero or that there was no functional relationship between the inbreeding coefficient and the values of the fitness component.

Modification of this method of analysis was required as regards the fitness components; female egg and male egg developmental periods (Figs 11 and 13) and female larval to adult and male larval to adult developmental periods (Figs 21 and 23). Here, the duration of each period progressively decreased from the base population until the F_4 or F_5, after which it increased again. This variation was identical for OUT 1, IN 1 and KR 1 as shown by the significant correlations (Table 2). This pattern is unlikely to be due to an inbreeding depression followed by a recovery because it occurred in OUT 1 in exactly the same way as in the experimental IN 1 and KR 1 populations. Therefore the factor responsible may be a genetic factor common to all three treatments or an environmental factor, because conditions were identical for the treatments. Three other significant correlations occurred amongst the fitness components but unlike the developmental periods the variation was not common to both OUT 1 and IN 1 and OUT 1 and KR 1.

This pattern of variation would influence the chances of obtaining a significant linear relationship between the mean values of IN 1 and the coefficients of inbreeding, if an inbreeding depression had occurred. To negate this effect a logarithm of the ratio of OUT 1 to IN 1 was taken in each generation. These logarithmic values were then regressed against the coefficients of inbreeding to determine if an inbreeding depression had occurred in the mean values of IN 1.

(c) The chance of making a type 1 error or falsely rejecting the Null hypothesis was taken into consideration and minimized as follows (Sokal and Rohlf 1981, Jones 1984). In the modified and unmodified Student's "t" tests, the G-test of independence and regression analysis, a conservative level of probability was used, $\alpha = 0.005$, with a two-tailed test. The significance levels for the modified Student's "t" test, at 20 degrees of freedom, and that for the Student's "t" test, at the relevant degrees of freedom, were obtained from Snedecor and Cochran (1980). The values for the G-test of independence, and the regression analysis, at the relevant degrees of freedom, were obtained from Zar (1974).

Table 2. A factor, independent of the treatments, may have influenced the pattern of variation in the mean values of each fitness component in *M. pulchellalis*. If so, the changes would occur simultaneously in the OUT 1 and IN 1 and OUT 1 and KR 1. The correlation coefficient (r), degrees of freedom and the significance at the level, α = 0.05, are given.

FITNESS COMPONENT	OUT 1 VS IN 1			OUT 1 VS KR 1		
	r	df	sig	r	df	sig
No of eggs laid	0.167	9	ns	0.54	8	ns
Egg viability	0.027	9	ns	0.716	8	sig*
Egg hatch	0.145	9	ns	0.154	8	ns
♀ egg development	0.81	8	sig*	0.95	8	sig*
♂ egg development	0.84	8	sig*	0.95	8	sig*
Larval penetration	0.459	9	ns	0.652	8	sig*
Larval survival	0.786	8	sig*	0.134	8	ns
Pupal survival	0.352	8	ns	0	8	ns
♀ larval to adult dev.	0.80	8	sig*	0.97	8	sig*
♂ larval to adult dev.	0.75	8	sig*	0.97	8	sig*
Virgin longevity	0.243	8	ns	0.350	8	ns
Mated longevity	0.415	8	ns	-0.055	7	ns
Wing length	0.471	9	ns	0.437	8	ns
Sex ratio	0.290	9	ns	0.603	8	ns

After the mean values of the fitness components had been measured and assessed, the effect of inbreeding on the population fitness of M. pulchellalis, was determined using life table analysis. (a) The mortality in each stage and its contribution to the total generation mortality of each treatment was derived. The total generation mortality was compared between the control OUT 1 and the experimental IN 1 and KR 1 populations. Following this, (b) the percentage survival was used to estimate the numbers of individuals in each generation and the growth rate of each population. The difference between OUT 1 and IN 1 and OUT 1 and KR 1 in the numbers of individuals in each generation was then determined. This was done to compare the relative fitness of each population.

(a) The mortality in each stage and the total generation mortality, that occurred in each population, was determined in the following way. Each population was initiated with a thousand theoretical individuals. Using the percentage mate success; mean number of eggs laid; and the percentages of egg viability, egg hatch, larval penetration and survival and pupal survival, the mortality of each stage was derived. The sex ratio was set at 1:1 because the differences in the mean values between populations were small (Fig 31). From this the "killing power" or the k-value of the mortality which operated at each stage was determined. This was done by calculating the difference between the logarithm at the start of a particular stage and at the end of that stage. An estimate of the total generation mortality (K), could be made from the addition of all the k-values (Varley, Gradwell and Hassell 1973).

In the OUT 1, IN 1 and KR 1 treatments the mortality in each stage was compared with the total generation mortality to determine the key factor which contributed to the population mortality. The total generation mortality was also compared between OUT 1 and IN 1 and OUT 1 and KR 1 to assess any difference in the total generation mortality curves. A two-tailed correlation test at $\alpha = 0.005$ and the relevant degrees of freedom (Zar 1974) was used in these analyses. The level of probability was set at $\alpha = 0.005$ in order to establish the key factor

contributing to the total generation mortality. Because only a hypothetical estimate of total generation mortality was made it was necessary to emphasis only the most biologically meaningful result. Such a key factor was established at this probability level. Furthermore, at the probability level α=0.05 the mortality of other fitness components was significantly correlated but not consistently so within each treatment.

(b) After this a hypothetical assessment of the increase in numbers of each population was made. To do this each population was theoretically started with a thousand individuals and the percentage survival in each generation, as calculated in the life table analysis, was used. The rate of increase of each population was also determined. The size of a population in each succeeding generation was compared with the previous generation to measure how much larger the population had grown. From this the rate of increase was established. Finally, the relative numbers of individuals in each generation, were compared between treatments. A ratio of the numbers of individuals in each generation of OUT 1 was made with IN 1 and KR 1 respectively. It could then be established whether the differences in numbers increased, decreased, or remained constant over a period. These results provided a comparison of the relative fitness of each population, from which the effects of inbreeding on the fitness of the sibmated (IN 1) and field-introduced (KR 1) populations could be deduced.

CHAPTER 3

FITNESS COMPONENTS

The effects of inbreeding on the mean values of each fitness component are discussed in this chapter. The results of each fitness component are presented in the following order: (i) mate success, (ii) number of eggs laid, (iii) egg viability, (iv) egg hatch, (v) female egg and (vi) male egg developmental periods, (vii) larval penetration, (viii) larval and (ix) pupal survival, (x) female larval to adult and (xi) male larval to adult developmental periods, (xii) virgin female and (xiii) mated female longevity, (xiv) wing length, and (xv) sex ratio.

The mean values of each component, in each generation and for all generations combined, are presented either as a table or a graph, together with a short description. Two methods were used to determine if an inbreeding depression had occurred in the sibmated (IN 1 and 2) and field-introduced (KR 1 and 2) populations of M. pulchellalis. Firstly, the mean values of each fitness component in each generation in OUT 1 and 2 were compared with those of IN 1 and 2 and KR 1 and 2 using a modified Student's "t" test. Either a G-test of independence or Student's "t" test was used to compare the values for all generations combined. Secondly, the mean values of each fitness component of IN 1 were regressed against the corresponding inbreeding coefficients.

No inbreeding depression could be demonstrated in any of these cases. Although not statistically demonstrable it was noticeable that there was a simultaneous decrease in egg viability in all three treatments OUT 1, IN 1 and KR 1 in the F_2 and F_7 generations. Examination of the data showed that this was due to a larger number of mated females producing no viable eggs. Although the two phenomena may be related this should not be confused with the demonstration of an inbreeding effect on egg viability, involving not only the F_2, by population modelling (see Chapter 4). An interpretation of these results will be discussed in Chapter 5.

(i) Mate success

The percentage of female moths that mated in each generation in all of the treatments (Table 3), was high. On only one occasion was the value as low as 75%. This occurred in the F_7 of IN 1. Another low value of 84.6%, was recorded in the F_8 of OUT 1. However the first of these decreases was probably caused by the failure of the lights in the insectary to switch off because of a faulty light switch mentioned in Chapter 2. The generations were slightly out of phase (Fig 2) and no mating was taking place in the OUT 1 and KR 1 populations at the time of the malfunction. Consequently, the mate success values of these treatments were not affected. Because the values were very high, and the changes minimal, it is clear inbreeding did not affect mate success.

Table 3. The percentage mate success in each generation (see Table 1 for calculation) in the various treatments of M. pulchellalis. The number of lines (N) in each generation are also given. The asterisk denotes the generation in which the lights in the insectary failed to switch off due to a faulty light switch.

POP 1	OUT		IN		KR	
Gen	%	N	%	N	%	N
F_0	100.0	18	100.0	13	100.0	12
F_1	100.0	20	100.0	20	100.0	20
F_2	95.0	20	100.0	20	100.0	20
F_3	100.0	20	100.0	20	100.0	19
F_4	95.0	20	100.0	20	100.0	20
F_5	100.0	20	100.0	20	100.0	20
F_6	95.0	20	85.0	20	95.0	20
F_7	90.0	20	75.0*	20	100.0*	20
F_8	84.6*	13	100.0	8	100.0	20
F_9	100.0	20	100.0	7		
POP 2						
F_0	95.0	19	100.0	19	100.0	20
F_1	100.0	12	94.4	18	85.0	20
F_2	100.0	12	100.0	20		
F_3	95.0	20	84.2	19		

(ii) Number of eggs laid

The number of eggs laid ranged from 29.1 to 37.3 for all generations combined in each treatment (Table 4). In the IN 1 treatment more eggs were laid in the base population than in any succeeding generation (Fig 5) however the number laid varied amongst these generations. In spite of this decrease in IN 1 there were no significant differences between the treatments in the number of eggs laid for all generations combined (Table 5) or in each generation (Fig 6). In addition there was no significant correlation between the number of eggs laid in each generation in IN 1 and the inbreeding coefficients ($r = 0.336$, $n = 10$). Therefore, inbreeding did not cause a significant reduction in the number of eggs laid.

Table 4. The mean number of eggs laid by mated females for all generations combined in various treatments of M. pulchellalis. The standard error (X1) and the number of lines (N) are given for all generations combined.

	OUT			IN			KR		
POP	MEAN %	SE	N	MEAN %	SE	N	MEAN %	SE	N
1	36.0	1.17	184	32.6	1.28	160	37.3	1.24	170
2	31.4	1.76	68	29.8	1.19	75	29.1	2.52	37

Table 5. The differences, in the mean number of eggs laid for all generations combined, were determined between treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 and KR 1, and OUT 2 was compared with IN 2 and KR 2. The t-value, degrees of freedom, and the significance at the probability level, α = 0.005, are given.

	OUT vs IN			OUT vs KR		
POP	t-value	df	sig	t-value	df	sig
1	1.98	342	ns	0.78	352	ns
2	0.61	141	ns	0.76	103	ns

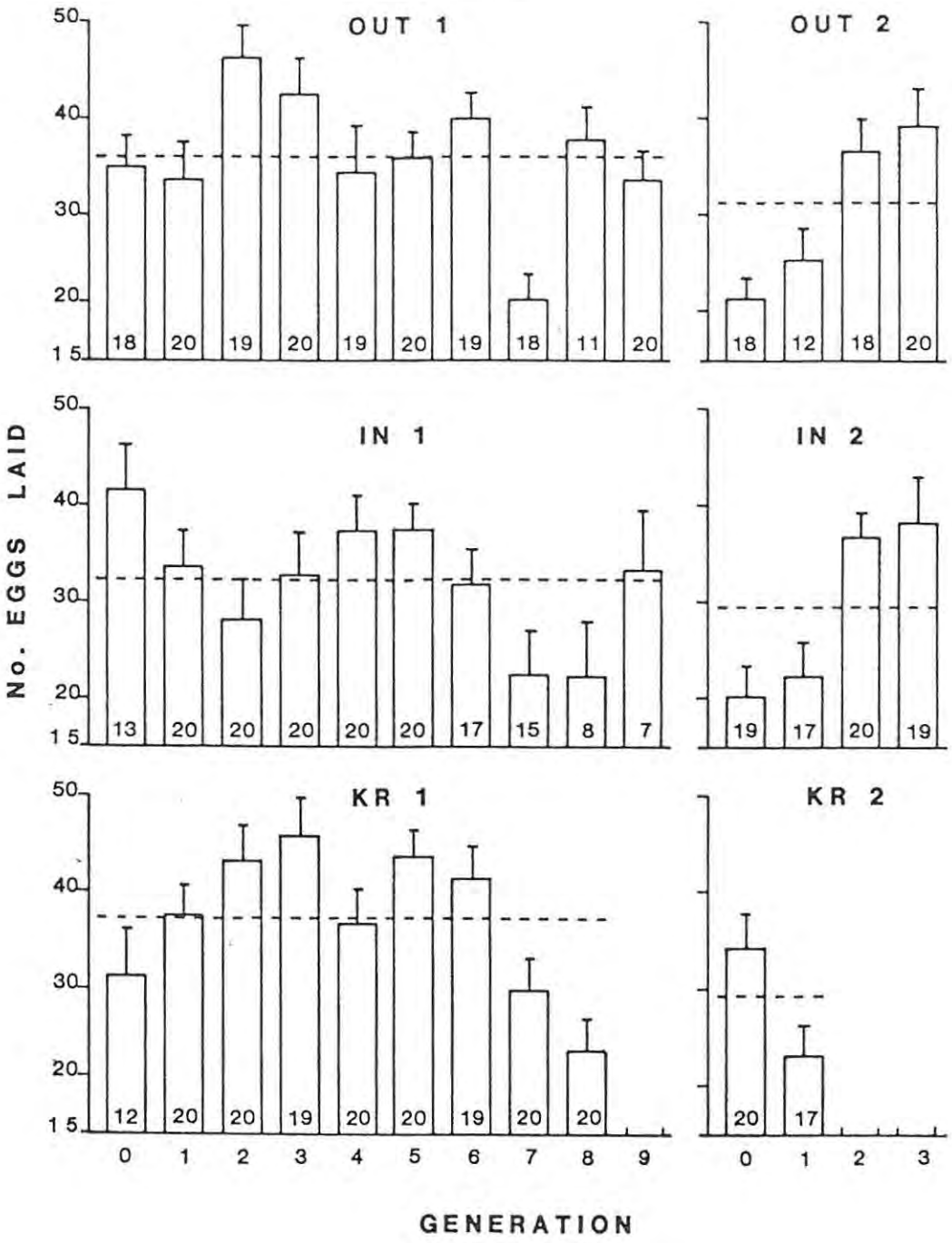


Fig 5. The mean number of eggs laid by mated females in each generation (see Table 1 for calculation) in the various treatments of M. pulchellalis. The mean for all generations combined (- - - -), the number of lines in a generation from which the mean value was derived, and standard error (X1, vertical line) are also shown.

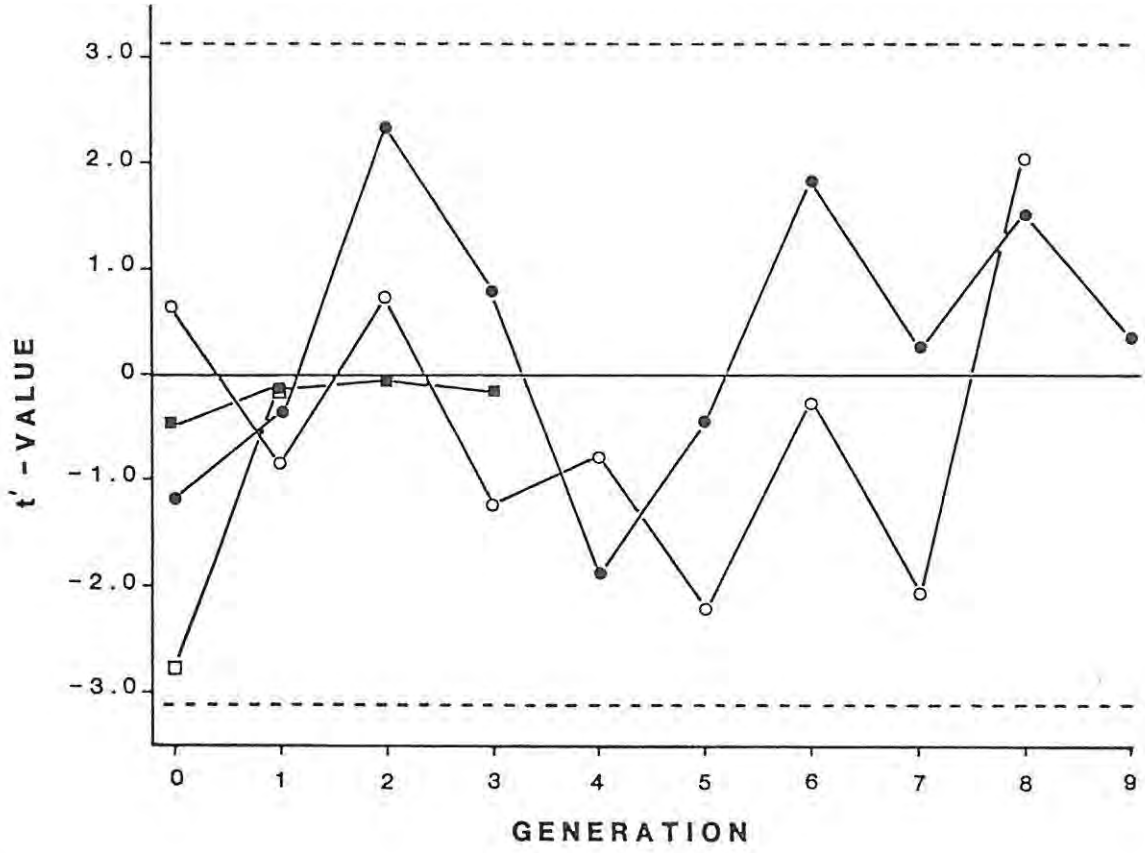


Fig 6. The differences in the mean number of eggs laid in each generation were determined between treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 (●——●) and KR 1 (○——○) and OUT 2 was compared with IN 2 (■——■) and KR 2 (□——□). The t'-values are depicted and the level of probability ($t'_{0.005(2),20} = \pm 3.153$) is shown as two horizontal dotted lines.

(iii) Egg viability

The mean percentage of viable eggs laid by mated females for all generations combined ranged from 71.4% in IN 1 to 78.4% in KR 2 (Table 6). A simultaneous reduction in the percentage egg viability occurred in the F_2 and F_7 in the OUT 1, IN 1 and KR 1 treatments (Fig 7, hatched histogram bars). In the first of these generations IN 1 only produced 32.7% viable eggs. A wide range is evident because in the F_9, 96.5% of the eggs were viable in the IN 1. In spite of the very low values in some generations there were no significant differences between the treatments for all generations combined (Table 7), and only one significant difference, between OUT 1 and IN 1 in the F_8 generation (Fig 8). Neither was there a linear inbreeding depression in egg viability within IN 1 since there was no significant correlation ($r=0.234$, $n=10$) between these values and the inbreeding coefficients.

Egg viability differed from the other fitness components in a way that was later shown to have great significance in the demonstration of a fitness decrease (Chapter 4). This difference was due to the fact that the reduction of the percentage egg viability in the F_2 of OUT 1, IN 1 and KR 1 resulted from a greater number of mated females laying no viable eggs as did the reduction in the F_7 of OUT 1 and KR 1 (Table 8). A result for IN 1 was unobtainable because of the failure of the day:night regime in the insectary at this time. In the consideration (Chapter 5) of whether this loss of fitness, due to reduced egg viability, could be attributed to inbreeding depression the following three factors were considered: (a) the quality of food is known to affect oocyte maturation (Engelmann 1970), (b) an insufficient quality and quantity of sperm produced by inbred males (Maynard Smith 1956) or (c) the susceptibility of the embryonic stage of development to an inbreeding depression (Oliver 1981).

Table 6. The mean percentage egg viability for all generations combined in the various treatments of M. pulchellalis. The standard error (X1) and the number of lines (N) are given for all generations combined.

	OUT			IN			KR		
POP	MEAN %	SE	N	MEAN %	SE	N	MEAN %	SE	N
1	77.3	0.02	184	71.5	0.03	160	75.5	0.02	170
2	77.8	0.04	68	71.4	0.04	75	78.4	0.05	39

Table 7. The differences, in the mean percentage egg viability for all generations combined, were determined between treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 and KR 1, and OUT 2 was compared with IN 2 and KR 2. The G-statistic, degrees of freedom and the significance at the probability level, α = 0.005, are given.

	OUT vs IN			OUT vs KR		
POP	G-statistic	df	sig	G-statistic	df	sig
1	0.897	2	ns	0.097	2	ns
2	1.097	2	ns	0.112	2	ns

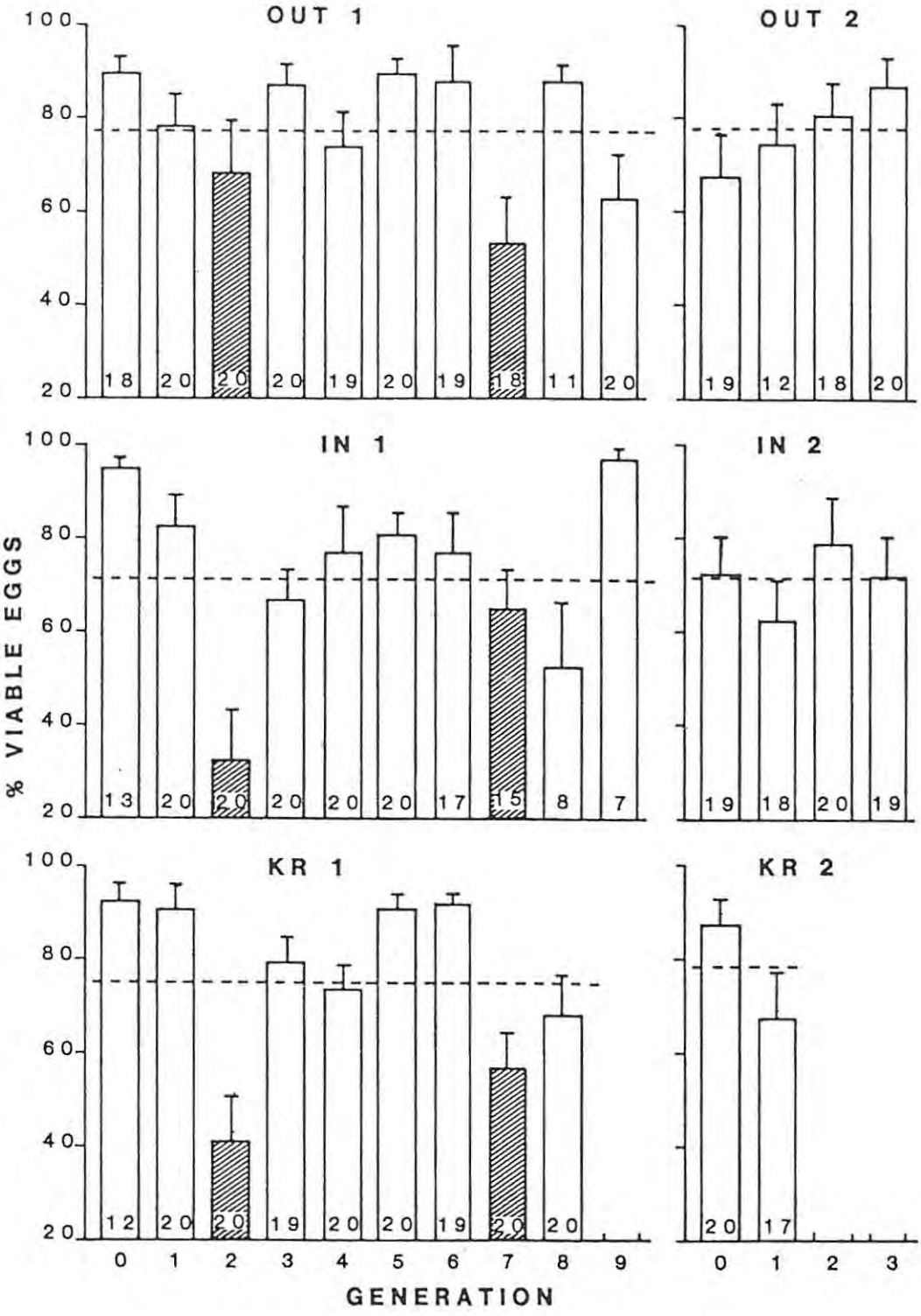


Fig 7. The mean percentage egg viability in each generation (see Table 1 for calculation) in the various treatments of M. pulchellalis. The mean for all generations combined (- - -), the number of lines in a generation from which the mean value was derived, and standard error (X1, vertical line) are shown. Hatched bars indicate the simultaneous reductions of the mean values in the F_2 and F_7 of OUT 1, IN 1 and KR 1.

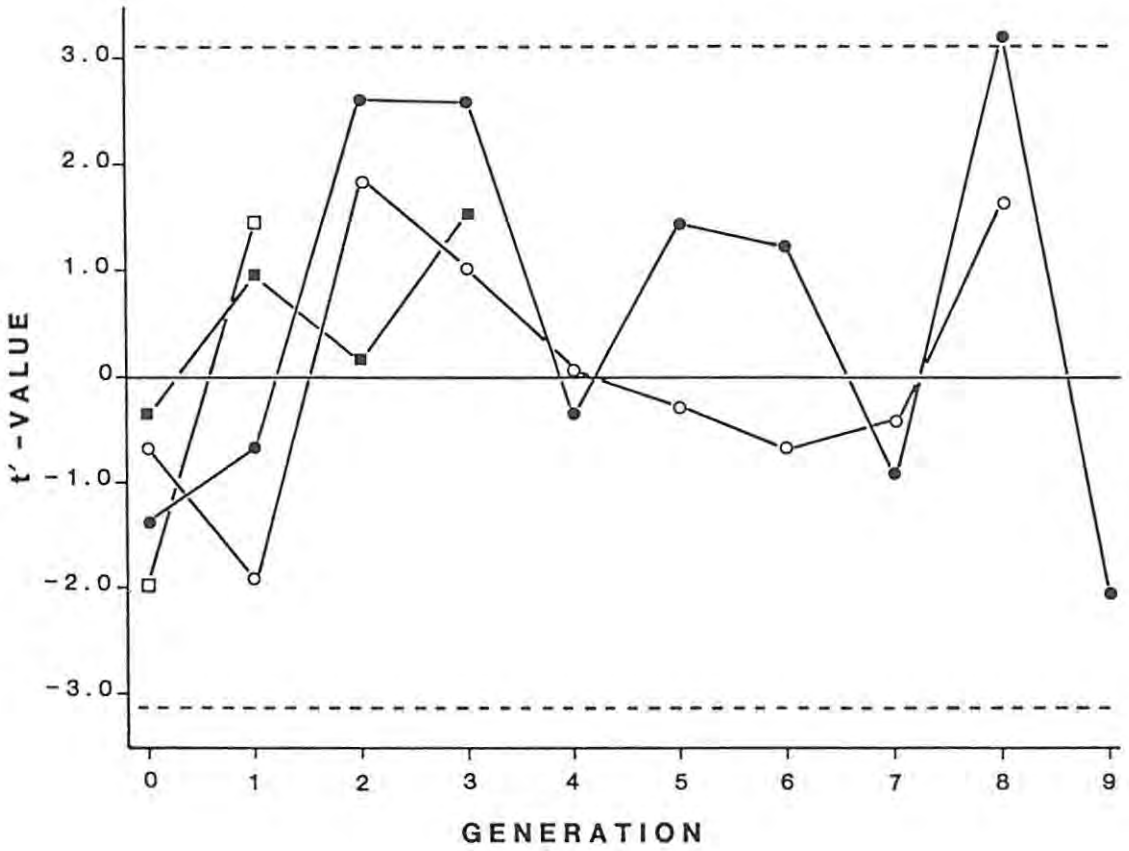


Fig 8. The differences in the mean percentage viable eggs in each generation were determined between treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 (● — ●) and KR 1 (○ — ○) and OUT 2 was compared with IN 2 (■ — ■) and KR 2 (□ — □). The t'-values are depicted and the level of probability ($t'_{0.005(2),20} = \pm 3.153$) is shown as two horizontal dotted lines.

Table 8. The percentage of inseminated females that produced no viable eggs in each generation in the various treatments of M. pulchellalis. The asterisk denotes the generation in which the lights in the laboratory failed to switch off due to faulty light switch.

POP 1	OUT		IN		KR	
GEN	%	No	%	No	%	No
F_0	0	18	0	13	0	12
F_1	0	20	0	20	0	20
F_2	21.0	19	55.0	20	30.0	20
F_3	0	20	0	20	0	19
F_4	5.3	19	10.0	20	5.0	20
F_5	0	20	0	20	0	20
F_6	5.5	18	5.3	19	0	20
F_7	27.8	18	*	*	20.0	20
F_8	0	13	12.5	8	20.0	20
F_9	20.0	20	0	7		
POP 2						
F_0	11.1	18	15.8	19	0	20
F_1	8.3	12	17.6	17	17.6	17
F_2	0	12	5.0	20		
F_3	0	19	0	19		

(iv) Egg hatch

The percentage of eggs that hatched in each population for all generations combined ranged from 89.5 to 93.7% (Table 9). Only in one generation (F_7:IN 1) did the percentage drop below 80% (Fig 9, hatched histogram bars). However, there was no significant difference in this or any other generation in egg hatch (Fig 10). Neither was there a significant difference between treatments for all generations combined (Table 10). In IN 1 an inbreeding depression was not evident in the mean values since there was no significant linear relationship ($r=0.14$, $n=10$) between the percentage egg hatch in each generation and the inbreeding coefficients. These results show that inbreeding did not result in a demonstrable reduction in the percentage of eggs that hatched.

Table 9. The mean percentage egg hatch for all generations combined in the various treatments of M. pulchellalis. The standard error (X1) and the number of lines (N) are given for all generations combined.

	OUT			IN			KR		
POP	MEAN %	SE	N	MEAN %	SE	N	MEAN %	SE	N
1	92.9	0.01	169	90.1	0.01	143	90.8	0.01	155
2	93.6	0.02	64	91.6	0.02	65	89.5	0.02	34

Table 10. The differences, in the mean percentage egg hatch for all generations combined, were determined between treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 and KR 1, and OUT 2 was compared with IN 2 and KR 2. The G-statistic, degrees of freedom and the significance at the probability level, α = 0.005, are given.

	OUT vs IN			OUT vs KR		
POP	G-statistic	df	sig	G-statistic	df	sig
1	0.325	2	ns	0.290	2	ns
2	0.301	2	ns	1.111	2	ns

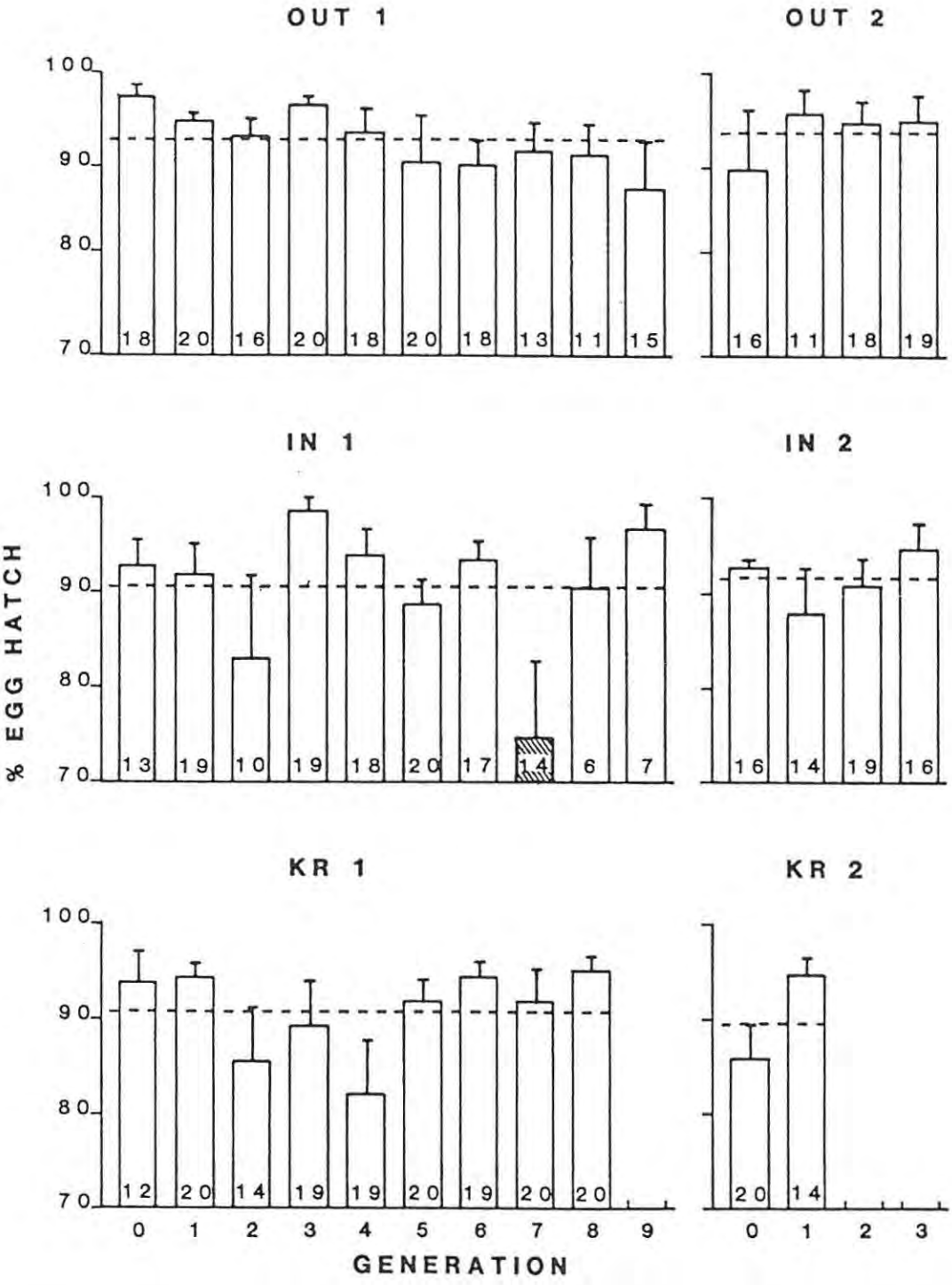


Fig 9. The mean percentage egg hatch in each generation (see Table 1 for calculation) in the various treatments of M. pulchellalis. The mean for all generations combined (---), the number of lines in a generation from which the mean value was derived, and standard error (X1, vertical line) are also shown. Hatched bars indicate a value lower than 80%.

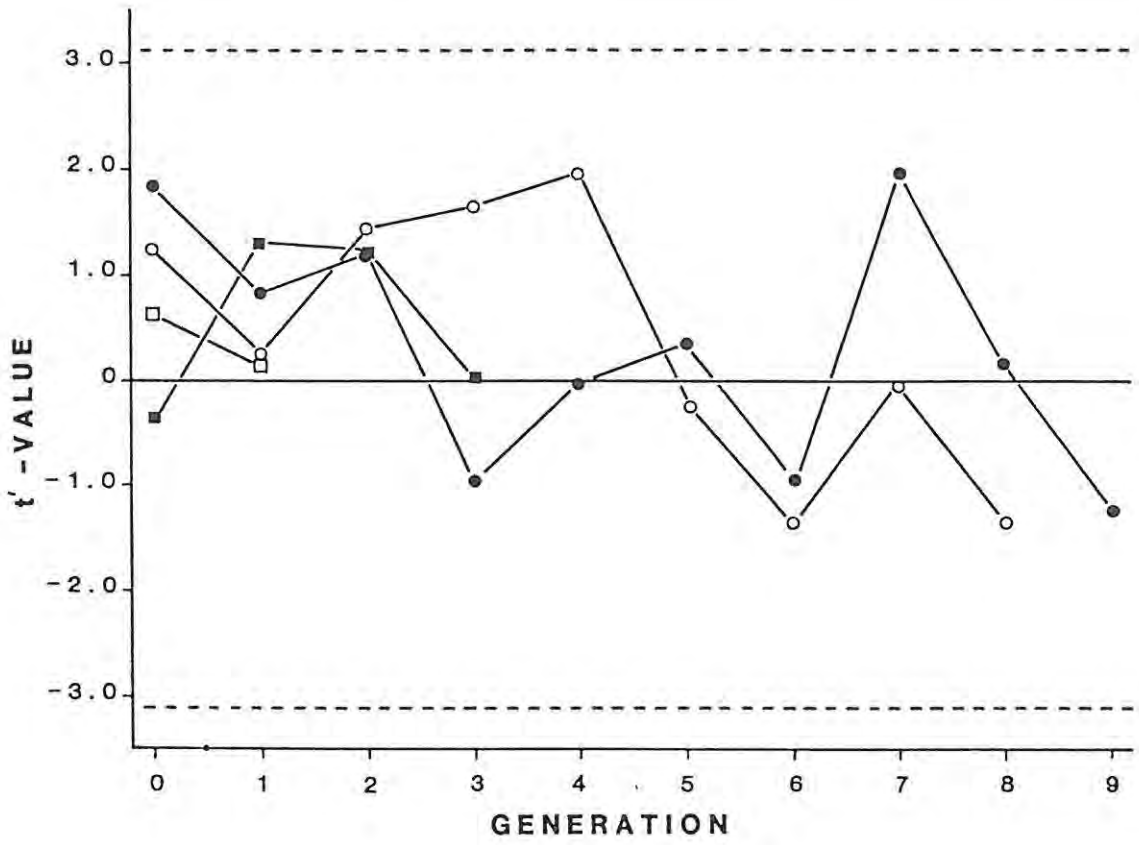


Fig 10. The differences in the mean percentage egg hatch in each generation were determined between treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 (●——●) and KR 1 (O——O) and OUT 2 was compared with IN 2 (■——■) and KR 2 (□——□). The t'-values are depicted and the level of probability ($t'_{0.005(2),20} = \pm 3.153$) is shown as two horizontal dotted lines.

(v) Female egg and (vi) Male egg developmental periods

The female egg and male egg developmental periods for all generations combined ranged from 8.33 to 9.72 days and 8.36 to 9.11 days, respectively (Table 11). A striking feature is that the developmental periods, in all treatments but KR 2, decreased until the F_4 or F_5 after which they increased (Figs 11 and 13). This was probably due to an influence independent of the treatments which was discussed earlier in Chapter 2.

Inbreeding did not produce any significant differences between the treatments, in the female egg and male egg developmental periods in each generation (Figs 12 and 14). There was one significant difference in the female egg and one significant difference in the male egg developmental periods for all generations combined (Table 12). In both situations the significant difference occurred between OUT 2 and IN 2. No progressive inbreeding depression was evident in the female egg or male egg developmental periods of IN 1. The correlations between the mean values in each generation of the female egg ($r=0.441$, $n=9$) and male egg ($r=0.412$, $n=9$) developmental periods and the inbreeding coefficients were not significant. Therefore, the duration of these developmental periods did not differ greatly as a result of inbreeding.

Table 11. The mean female egg and male egg developmental periods (days) for all generations combined in the various treatments of M. pulchellalis. The standard error (X1) and the number of lines (N) are given for all generations combined.

		OUT			IN			KR		
	POP	MEAN	SE	N	MEAN	SE	N	MEAN	SE	N
Egg dev	1	8.6	0.08	148	8.5	0.1	124	8.5	0.08	148
	2	8.3	0.14	40	8.4	0.16	47	9.7	0.15	29
Egg dev	1	8.6	0.08	146	8.9	0.09	122	8.5	0.08	143
	2	8.4	0.14	40	8.5	0.17	45	9.1	0.16	31

Table 12. The differences, in the mean female egg and male egg developmental periods (days) for all generations combined, were determined between treatments of M. pulchellalis. Four comparisons (for each component) were made; OUT 1 was compared with IN 1 and KR 1, and OUT 2 was compared with IN 2 and KR 2. The t-value, degrees of freedom, and the significance at the probability level, $\alpha = 0.005$, are given.

		OUT vs IN			OUT vs KR		
	POP	t-value	df	sig	t-value	df	sig
Egg dev	1	0.75	270	ns	-0.86	294	ns
	2	-0.54	85	ns	-4.53	67	sig
Egg dev	1	0.73	342	ns	0.75	287	ns
	2	-0.80	83	ns	-3.48	69	sig

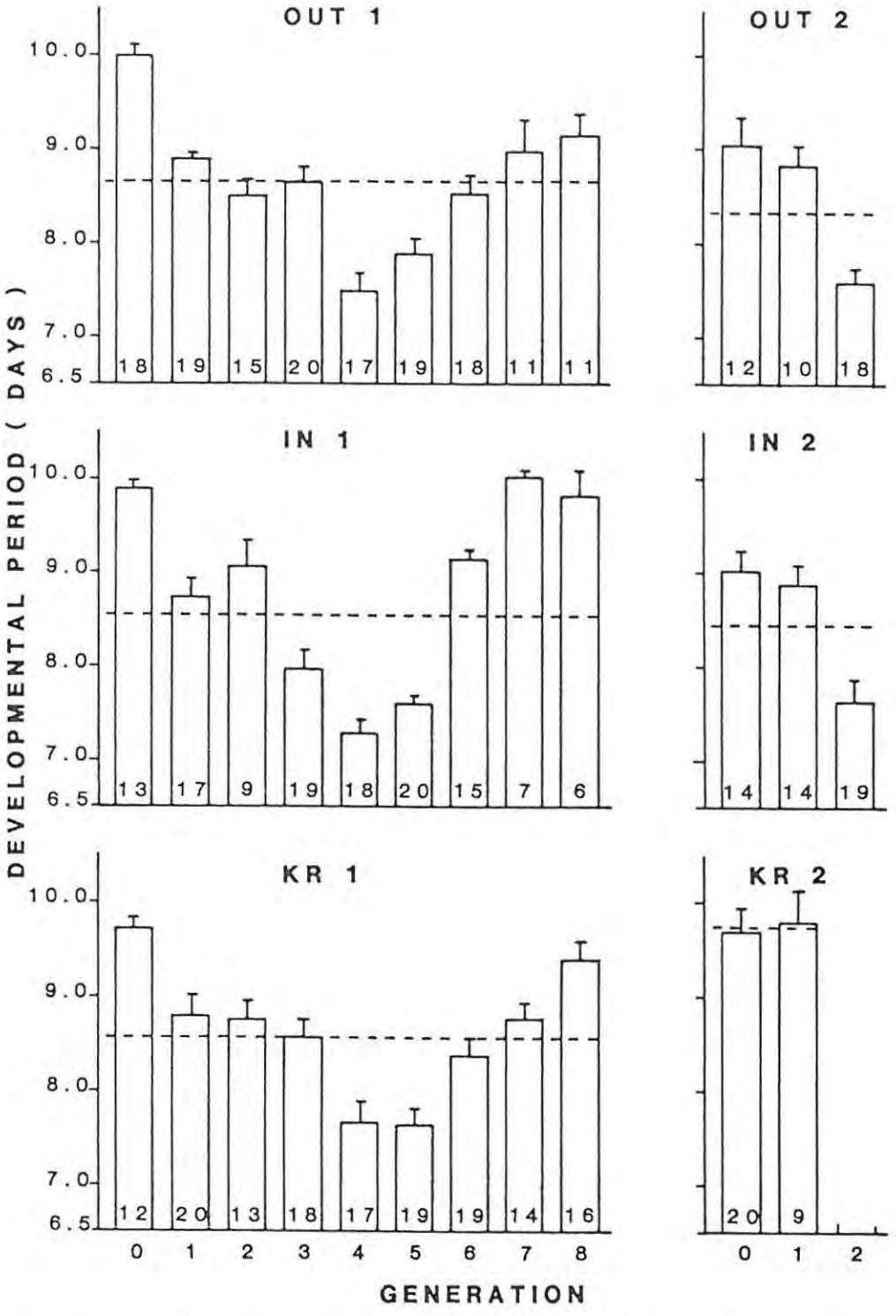


Fig 11. The mean female egg developmental period (days) in each generation (see Table 1 for calculation) in the various treatments of M. pulchellalis. The mean for all generations combined (----), the number of lines in a generation from which the mean value was derived, and standard error (X1, vertical line) are also shown.

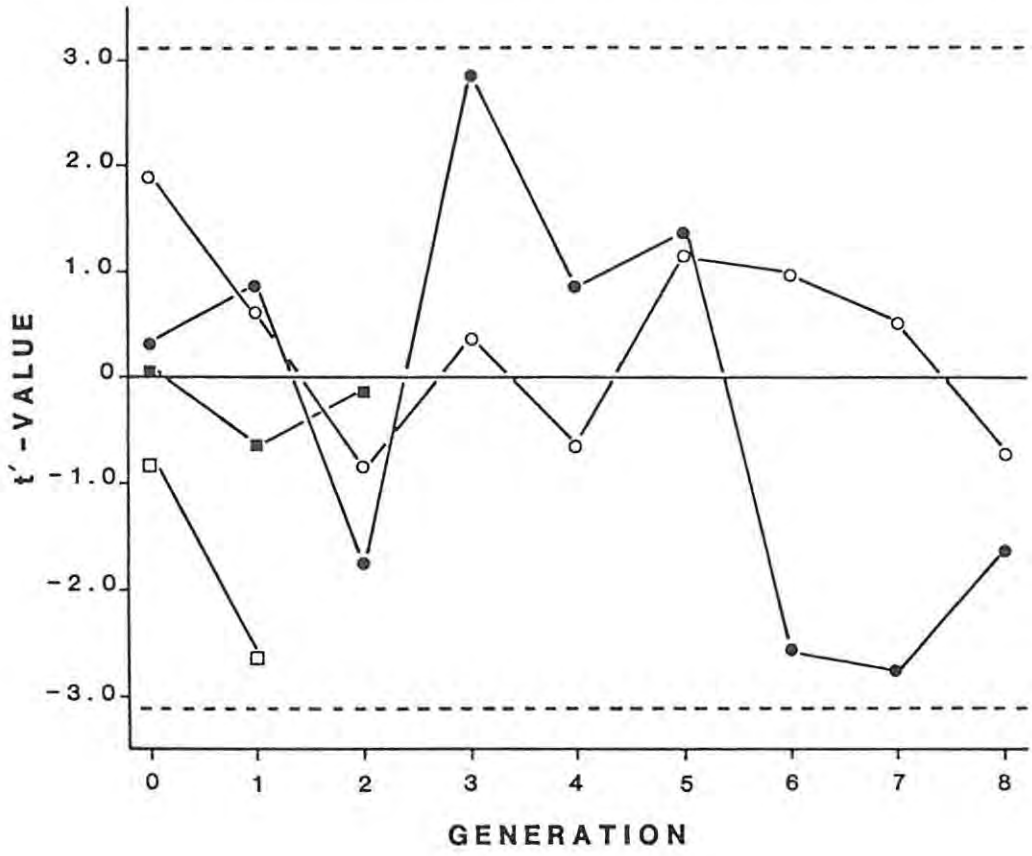


Fig 12. The differences in the mean female egg developmental period (days) in each generation were determined between treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 (● — ●) and KR 1 (O — O) and OUT 2 was compared with IN 2 (■ — ■) and KR 2 (□ — □). The t'-values are depicted and the level of probability ($t'_{0.005(2),20} = \pm 3.153$) is shown as two horizontal dotted lines.

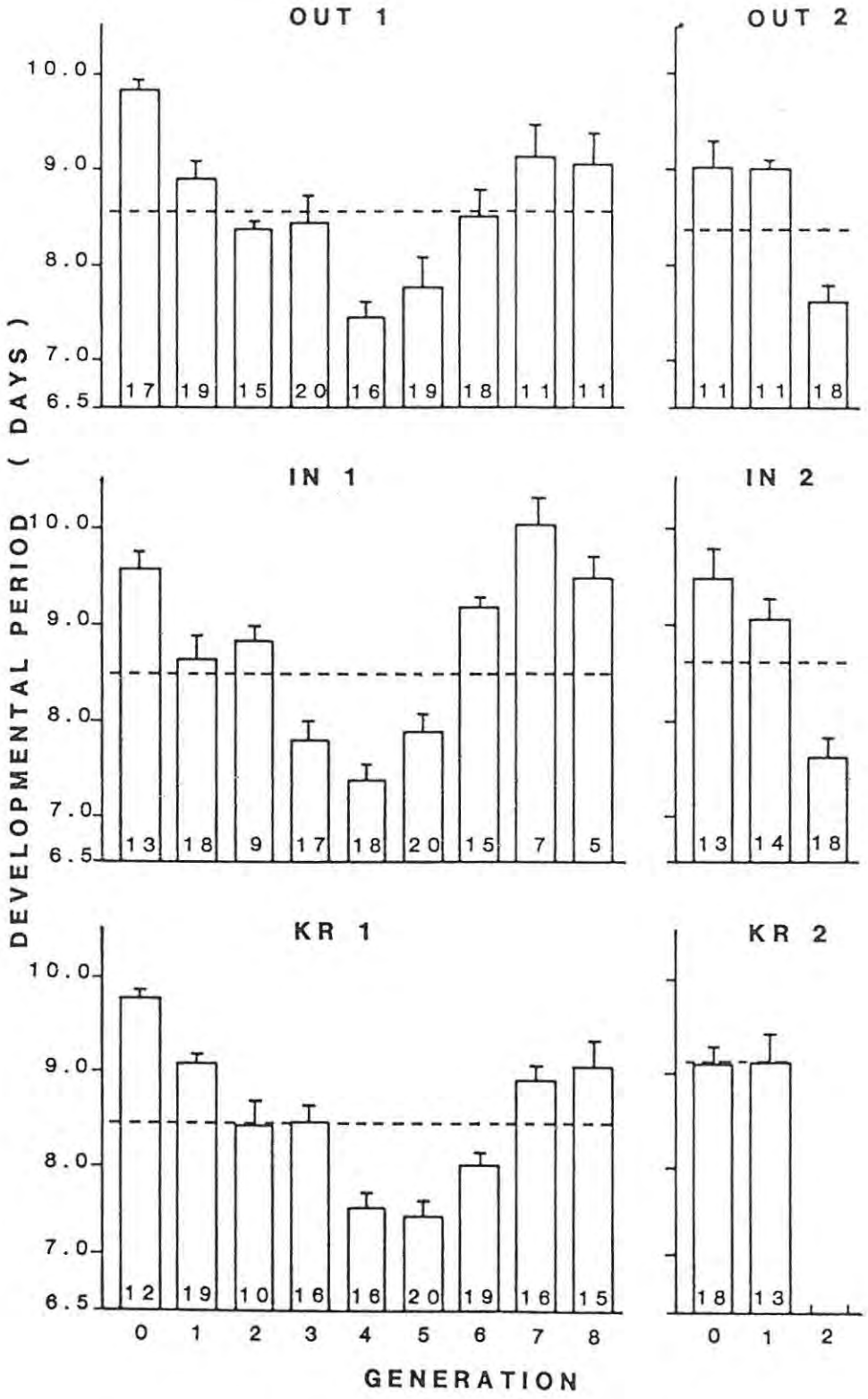


Fig 13. The mean male egg developmental period (days) in each generation (see Table 1 for calculation) in the various treatments of M. pulchellalis. The mean for all generations combined (- - -), the number of lines in a generation from which the mean value was derived, and standard error (X1, vertical line) are also shown.

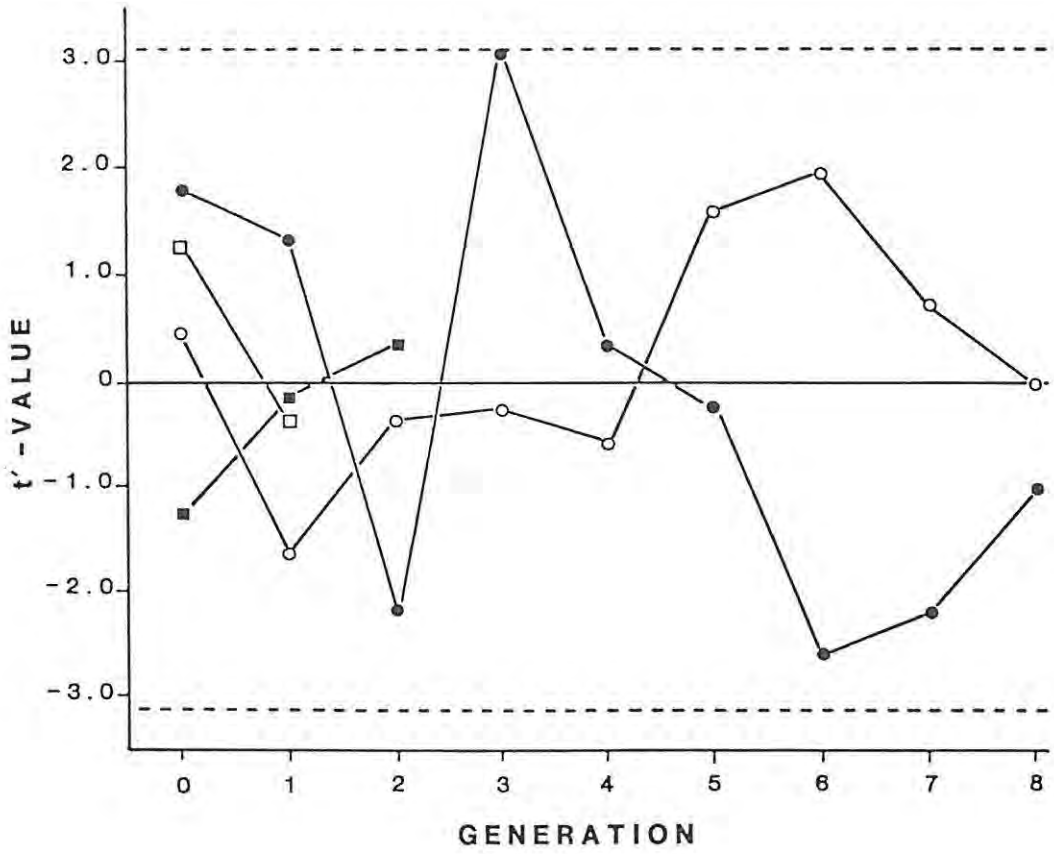


Fig 14. The differences in the mean male egg developmental period (days) in each generation were determined between treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 (●——●) and KR 1 (○——○) and OUT 2 was compared with IN 2 (■——■) and KR 2 (□——□). The t'-values are depicted and the level of probability ($t'_{0.005(2),20} = \pm 3.153$) is shown as two horizontal dotted lines.

(vii) Larval penetration

On no occasion was the percentage of larvae that penetrated the cactus lower than 84% for all generations combined in each treatment (Table 13). There were only three generations in which larval penetration was lower than 80% (F_7: IN 1, F_0: OUT 2, F_1: KR 2, Fig 15, hatched histogram bars). This emphasises the ability of the larvae to penetrate the cactus cladode. Therefore, it is not unexpected that there were no significant differences between the treatments in each generation (Fig 16) or for all generations combined (Table 14). Neither was there a linear inbreeding depression in the percentage larval penetration in IN 1 because the correlation between these values and the inbreeding coefficients was not significant ($r=0.077$, $n=9$). Clearly, inbreeding did not affect the success of larval penetration.

Table 13. The mean percentage larval penetration for all generations combined in the various treatments of M. pulchellalis. The standard error (X1) and the mean number of lines (N) are given for all generations combined.

	OUT			IN			KR		
POP	MEAN %	SE	N	MEAN %	SE	N	MEAN %	SE	N
1	90.7	0.01	168	89.3	0.01	141	89.8	0.01	155
2	85.0	0.02	63	85.6	0.02	65	84.9	0.03	34

Table 14. The differences, in the mean percentage larval penetration for all generations combined, were determined between treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 and KR 1, and OUT 2 was compared with IN 2 and KR 2. The G-statistic, degrees of freedom and the significance at the level, $\alpha = 0.005$, are given.

	OUT vs IN			OUT vs KR		
POP	G-statistic	df	sig	G-statistic	df	sig
1	0.115	2	ns	0.051	2	ns
2	0.15	2	ns	0.001	2	ns

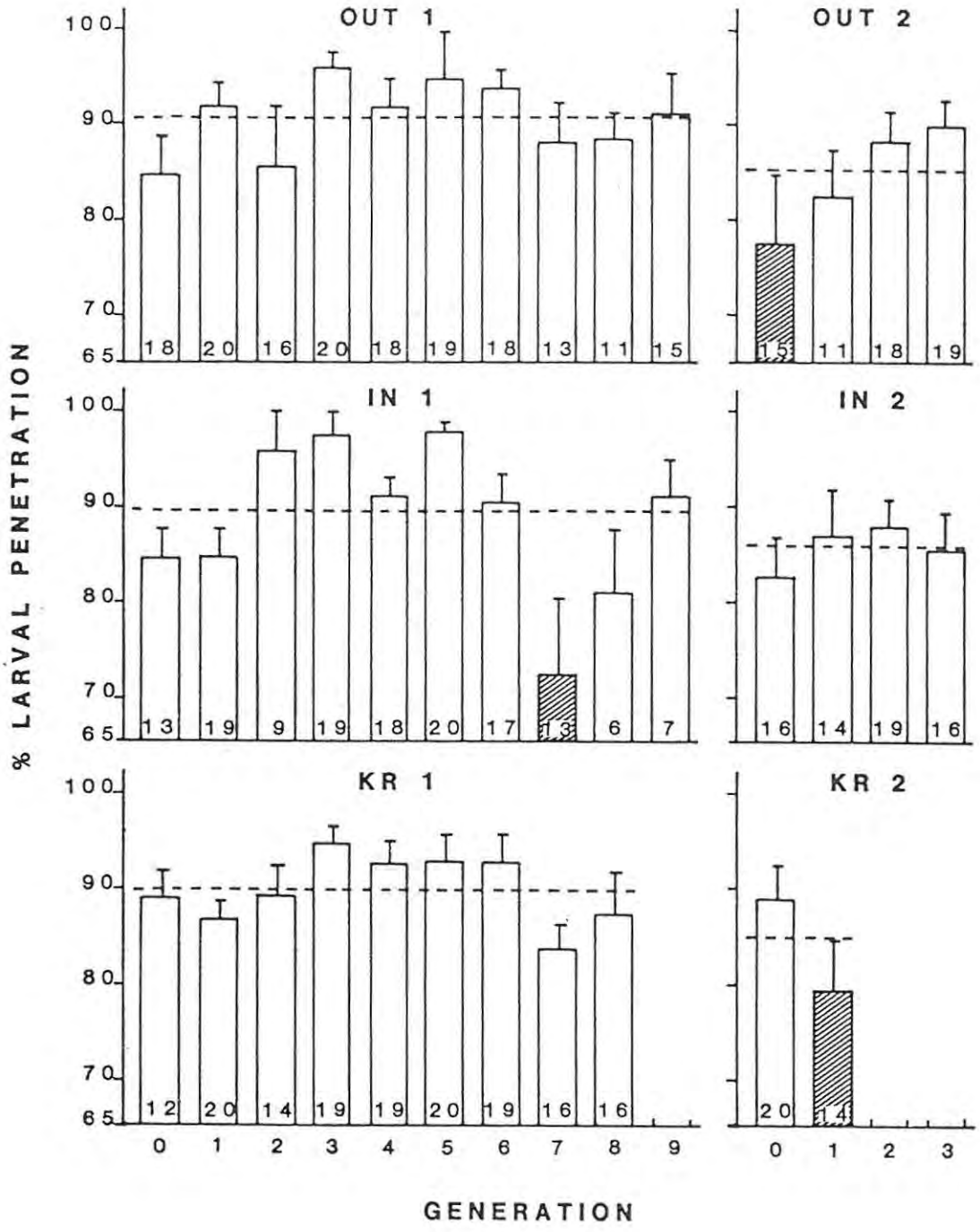


Fig 15. The mean percentage larval penetration in each generation (see Table 1 for calculation) in the various treatments of M. pulchellalis. The mean for all generations combined (– – –), the number of lines in a generation from which the mean value was derived, and standard error (X1, vertical line) are also shown.

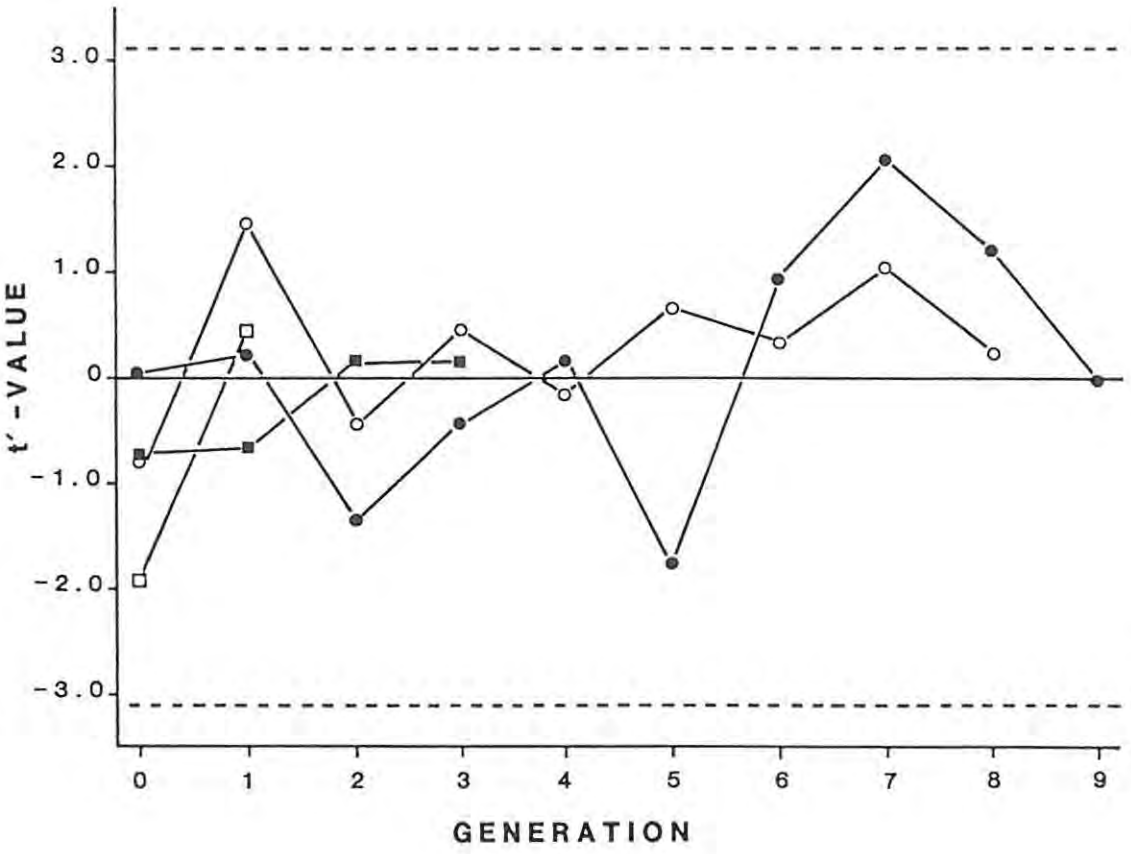


Fig 16. The differences in the mean larval penetration in each generation were determined between treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 (●——●) and KR 1 (○——○) and OUT 2 was compared with IN 2 (■——■) and KR 2 (□——□). The t'-values are depicted and the level of probability ($t'_{0.005(2),20} = \pm 3.153$) is shown as two horizontal dotted lines.

(viii) Larval survival

The larval survival in each population for all generations combined ranged from 82.4% to 94% (Table 15). In three generations the percentage dropped below 80% (F_7:OUT 1, F_7:IN 1, F_2: KR 2, Fig 17, hatched histogram bars). However, there were no significant differences between treatments in these or any other generation (Fig 18). Similarly, there were no significant differences in the larval survival for all the generations combined (Table 16). In addition an inbreeding depression did not occur in the mean values of IN 1. The relationship between the percentage of larvae that survived and the inbreeding coefficients was not significant ($r=0.192$, $n=9$). Therefore, inbreeding did not produce a linear reduction in larval survival.

Table 15. The mean percentage larval survival for all generations combined in the various treatments of M. pulchellalis. The standard error (X1) and the number of lines (N) are given for all generations combined.

	OUT			IN			KR		
POP	% MEAN	SE	N	% MEAN	SE	N	% MEAN	SE	N
1	94.0	0.01	150	89.5	0.02	133	92.0	0.01	153
2	90.0	0.03	42	89.8	0.03	49	82.4	0.03	34

Table 16. The differences, in the mean percentage larval survival for all generations combined, were determined between the treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 and KR 1, and OUT 2 was compared with IN 2 and KR 2. The G-statistic, degrees of freedom, and the significance at the probability level, $\alpha = 0.005$, are given.

	OUT vs IN			OUT vs KR		
POP	G-statistic	df	sig	G-statistic	df	sig
1	1.354	2	ns	0.295	2	ns
2	0.003	2	ns	2.261	2	ns

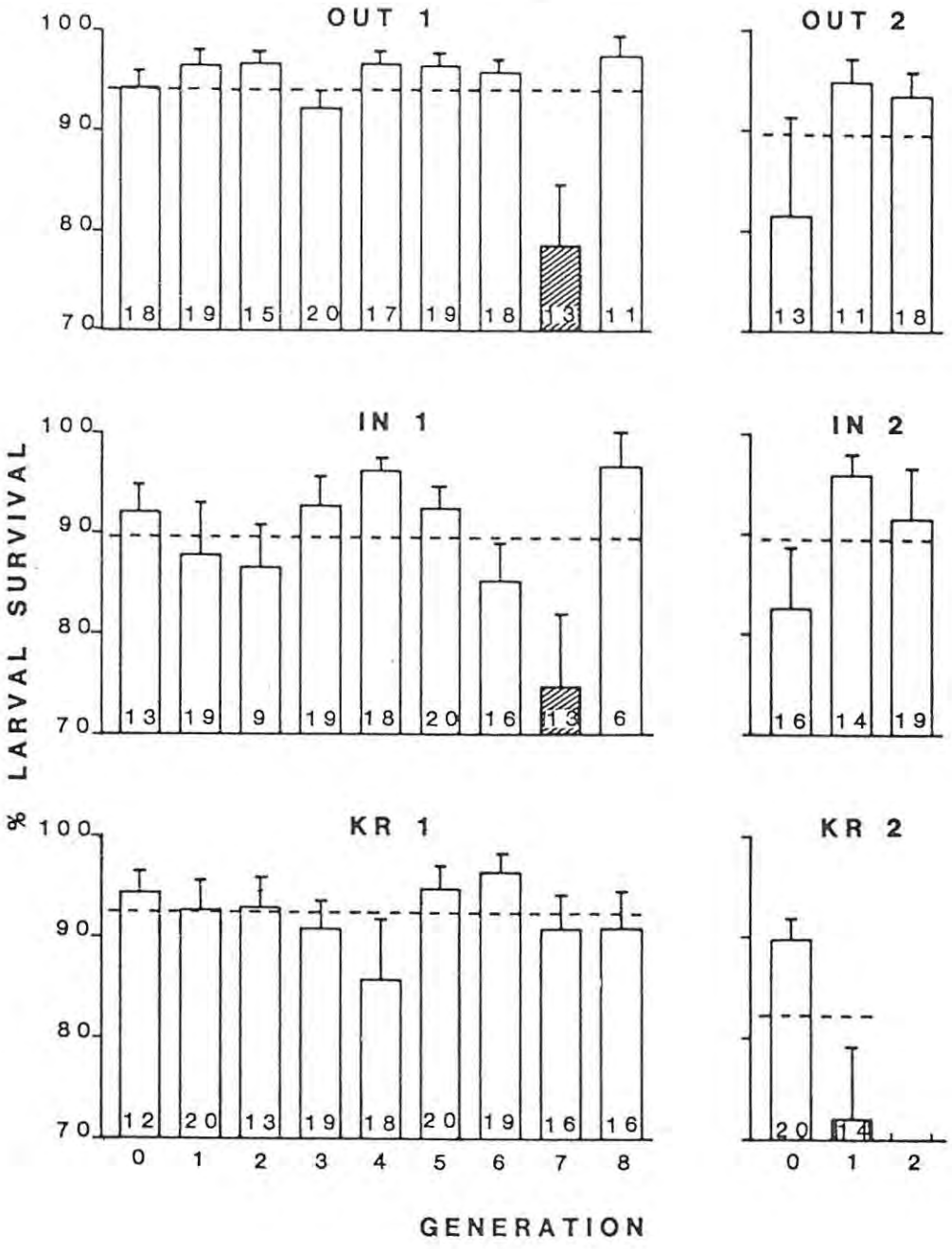


Fig 17. The mean percentage larval survival in each generation (see Table 1 for calculation) in the various treatments of M. pulchellalis. The mean for all generations combined (- - -), the number of lines in a generation form which the mean value was derived, and standard error (X1, vertical line) are also shown. Hatched bars indicate mean values lower than 80%.

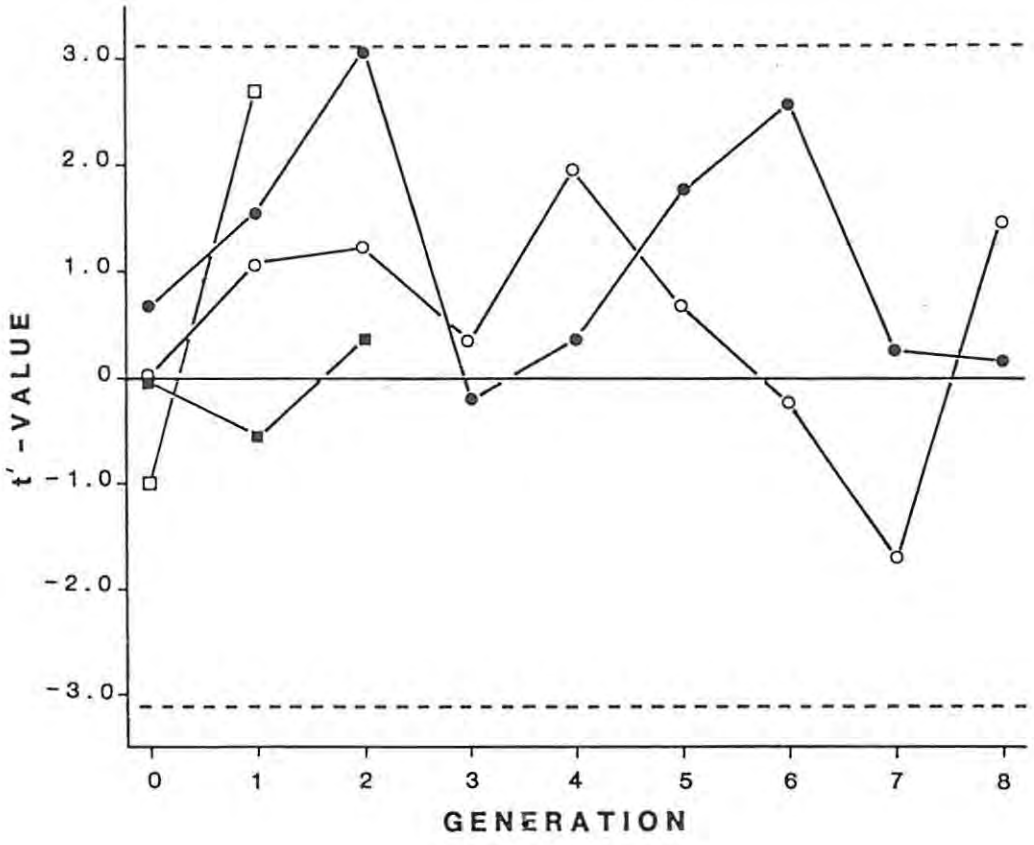


Fig 18. The differences in the mean percentage larval survival in each generation were determined between treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 (● — ●) and KR 1 (○ — ○) and OUT 2 was compared with IN 2 (■ — ■) and OUT 2 (□ — □). The t'-values are depicted and the level of probability ($t'_{0.005(2),20} = \pm 3.153$) is shown as two horizontal dotted lines.

(ix) Pupal survival

The percentage of pupae that survived in the populations for all generations combined ranged from 86% to 93.4% (Table 17). Only in the F_7 of IN 1 did the pupal survival drop below 80% (Fig 19, hatched histogram bars). In no generation were the differences between treatments significant (Fig 20) and neither were those differences between treatments for all generations combined significant (Table 18). The correlation between the percentage of pupae that survived in IN 1 and the inbreeding coefficients was not significant ($r=0.557$, $n=9$). It is clear from all these results that inbreeding did not cause a major effect on this character.

Table 17. The mean percentage pupal survival for all generations combined in the various treatments of M. pulchellalis. The standard error (X1) and the number of lines (N) for all generations combined are given.

	OUT			IN			KR		
POP	% MEAN	SE	N	% MEAN	SE	N	% MEAN	SE	N
1	93.4	0.06	150	90.9	0.01	129	90.8	0.01	152
2	89.2	0.03	42	89.0	0.02	47	86.0	0.03	33

Table 18. The differences, in the mean percentage pupal survival for all generations combined, were determined between the treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 and KR 1, and OUT 2 was compared with IN 2 and KR 2. The G-statistic, degrees of freedom, and the significance at the probability level, α = 0.005, are given.

	OUT vs IN			OUT vs KR		
POP	G-Statistic	df	sig	G-statistic	df	sig
1	0.417	2	ns	0.452	2	ns
2	0.002	2	ns	0.493	2	ns

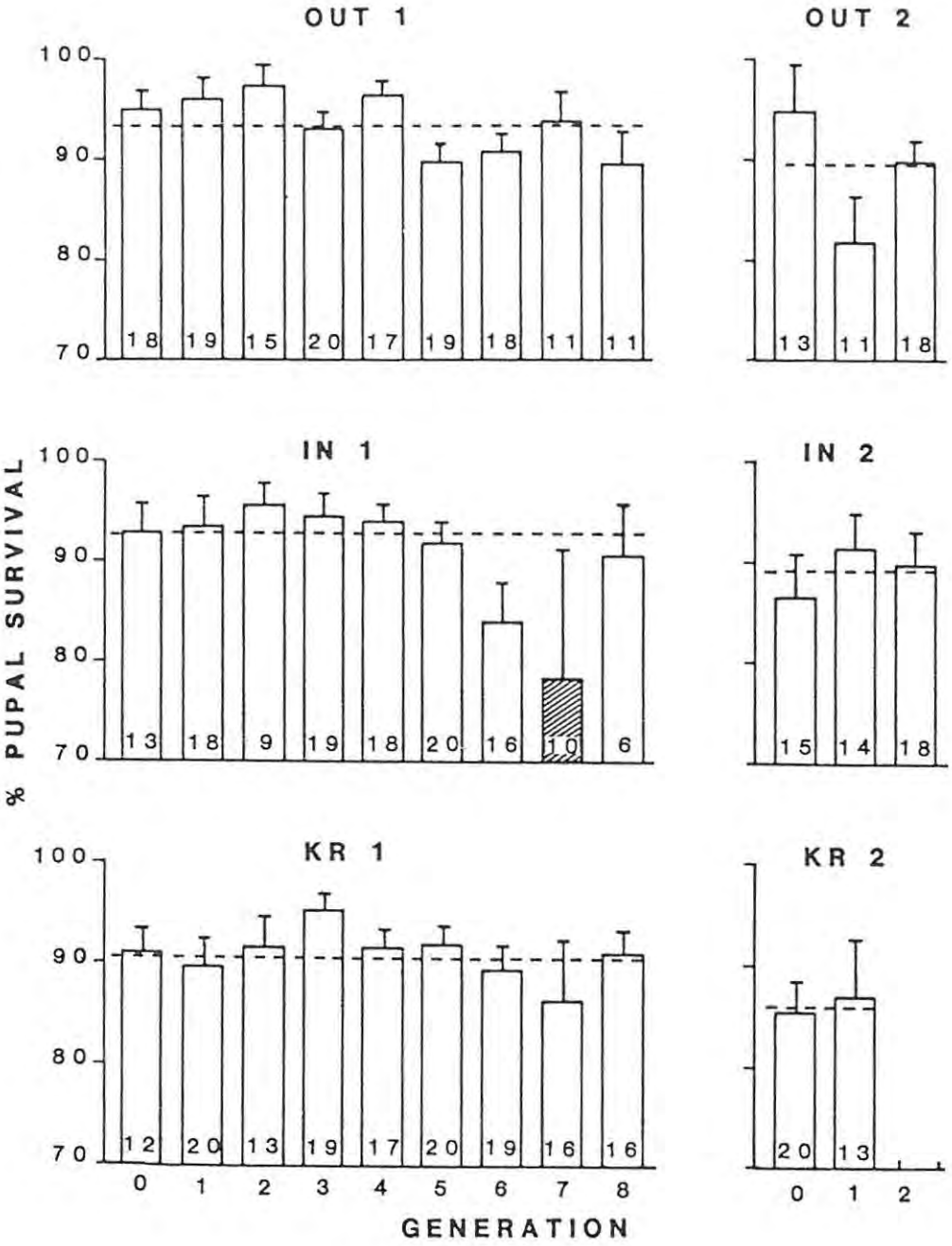


Fig 19. The mean percentage pupal survival in each generation (see Table 1 for calculation) in the various treatments of M. pulchellalis. The mean for all generations combined (---), the number of lines in a generation from which the mean value was derived, and standard error (X1, vertical line) are also shown. Hatched bars indicate value lower than 80%.

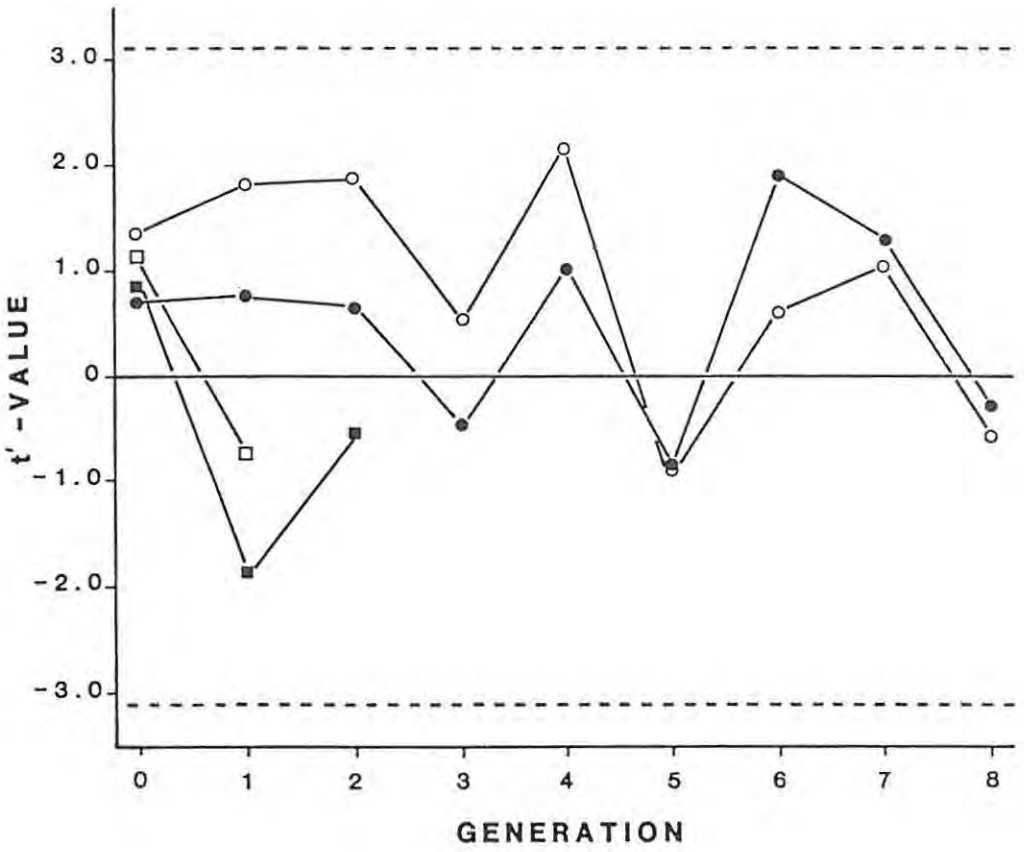


Fig 20. The differences in the mean percentage pupal survival in each generation were determined between the treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 (●——●) and KR 1 (○——○), and OUT 2 was compared with IN 2 (■——■) and KR 2 (□——□). The t'-values are depicted and the level of probability ($t'_{0.005(2),20} = \pm 3.153$) is shown as two horizontal dotted lines.

(x) Female larval to adult and (xi) Male larval to adult developmental periods

The female larval to adult developmental period for all generations combined ranged from 39.4 to 47.2 days, while that of the male larval to adult developmental period was shorter and ranged from 37.2 to 42.8 days (Table 19). The pattern was similar to that already described for the egg developmental periods and therefore could be due to the same influence, independent of the treatments, described in Chapter 2. The duration of development decreased from the base population to the F_4 and F_5, after which it increased (Figs 21 and 23). The trend also occurred amongst all of the treatments except KR 2.

There were five occasions on which the larval to adult developmental periods were significantly different between treatments (Figs 22 and 24). Two significant differences occurred in the female larval to adult developmental periods (F_1:OUT 2 vs KR 2, F_6:OUT 1 vs IN 1) and three significant differences in the male larval to adult developmental periods (F_1:OUT 2 vs KR 2, F_6 and F_8:OUT 1 vs IN 1). These differences were not always in the same generations or between the same treatments. There were no significant differences between the populations, in the values for all generations combined (Table 20). In neither the female larval to adult developmental periods ($r=0.063$, $n=9$) or the male larval to adult developmental periods ($r=0.1$, $n=9$) was there a significant linear inbreeding depression in IN 1 with respect to the inbreeding coefficients.

The erratic occurrences of the significant differences make it seem unlikely that they could be attributable to any effect of inbreeding. In addition there were no significant differences in the mean values for all generations combined and no linear inbreeding depression could be demonstrated in IN 1.

Table 19. The mean female larval to adult and male larval to adult developmental periods (days) for all generations combined in the various treatments of M. pulchellalis. The standard error (X1) and the number of lines (N) are given for all generations.

		OUT			IN			KR		
	POP	MEAN	SE	N	MEAN	SE	N	MEAN	SE	N
larval	1	39.4	0.43	148	39.7	0.46	124	39.7	0.47	148
to adult dev	2	42.8	1.24	40	42.6	0.93	47	47.2	0.90	29
larval	1	37.2	0.37	146	37.4	0.40	122	37.7	0.37	143
to adult dev	2	38.8	0.90	40	39.8	0.84	45	42.8	0.75	31

Table 20. The differences, in the mean female larval to adult and male larval to adult developmental periods (days) for all generations combined, were determined between treatments of M. pulchellalis. Four comparisons (for each component) were made; OUT 1 was compared with IN 1 and KR 1, and OUT 2 was compared with IN 2 and KR 2. The t-value, degrees of freedom, and the significance at the probability level, α = 0.005, are given.

		OUT vs IN			OUT vs KR		
	Pop	t-value	df	sig	t-value	df	sig
larval to	1	-0.47	270	ns	0.05	294	ns
adult dev	2	0.12	85	ns	-1.49	67	ns
larval to	1	-0.35	266	ns	-0.76	287	ns
adult dev	2	-0.82	83	ns	-3.33	69	sig

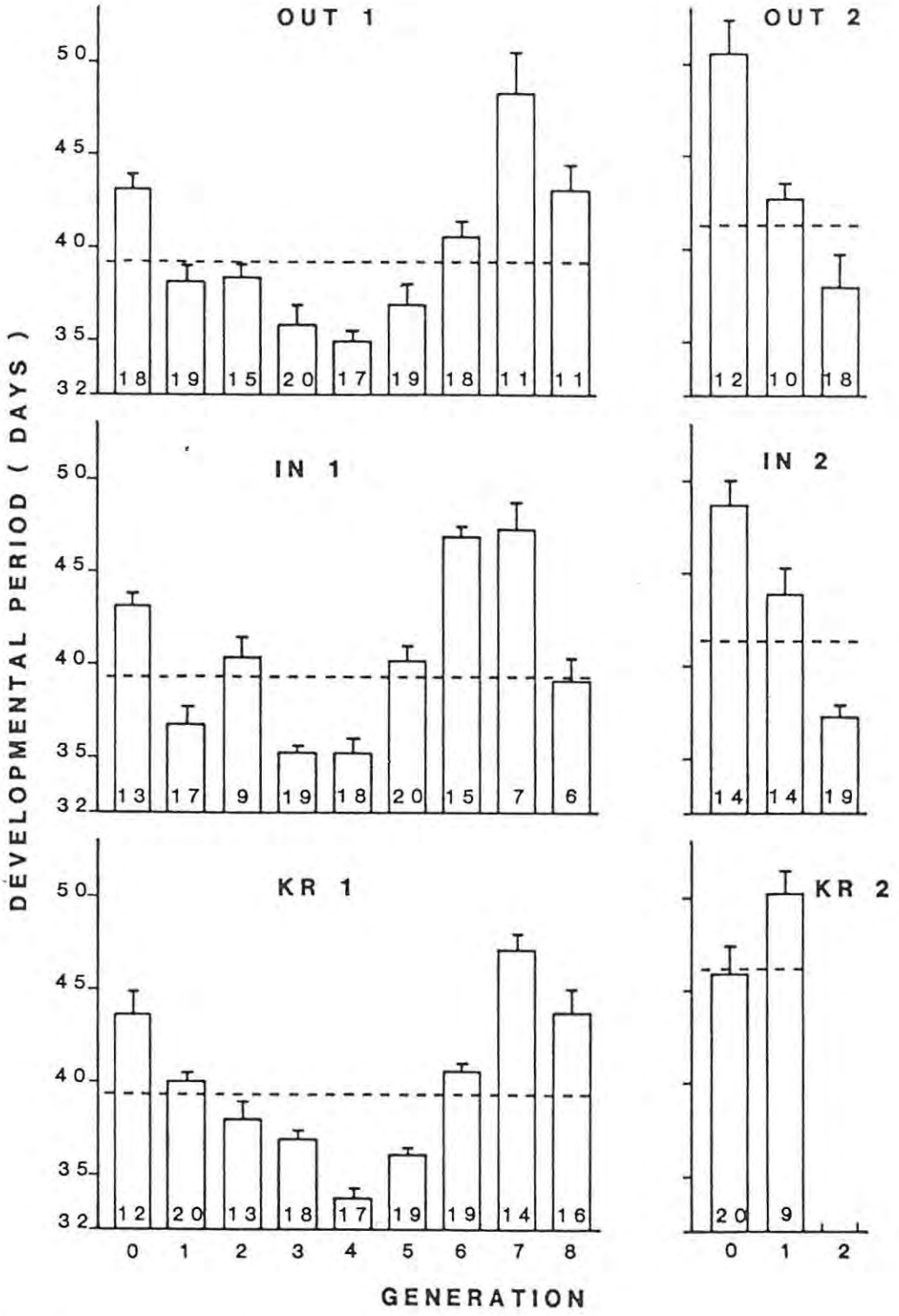


Fig 21. The mean female larval to adult developmental period (days) in each generation (see Table 1 for calculation) in the various treatments of M. pulchellalis. The mean for all generations combined (- - -), the number of lines in a generation from which the mean value was derived, and standard error (X1, vertical line) are also shown.

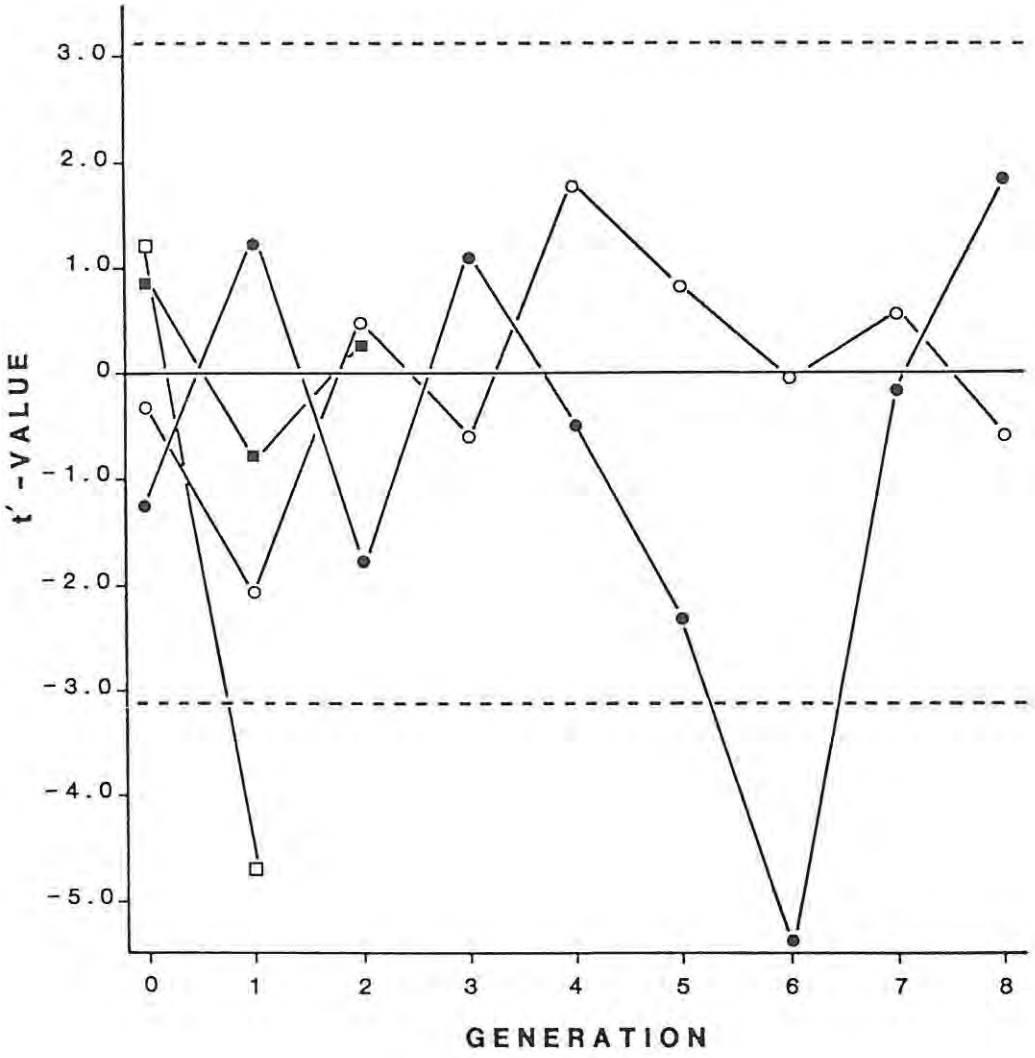


Fig 22. The differences in the mean female larval to adult developmental period (days) in each generation were determined between treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 (●——●) and KR 1 (○——○) and OUT 2 was compared with IN 2 (■——■) and KR 2 (□——□). The t'-values are depicted and the level of probability ($t'_{0.005(2),20} = \pm 3.153$) is shown as two horizontal dotted lines.

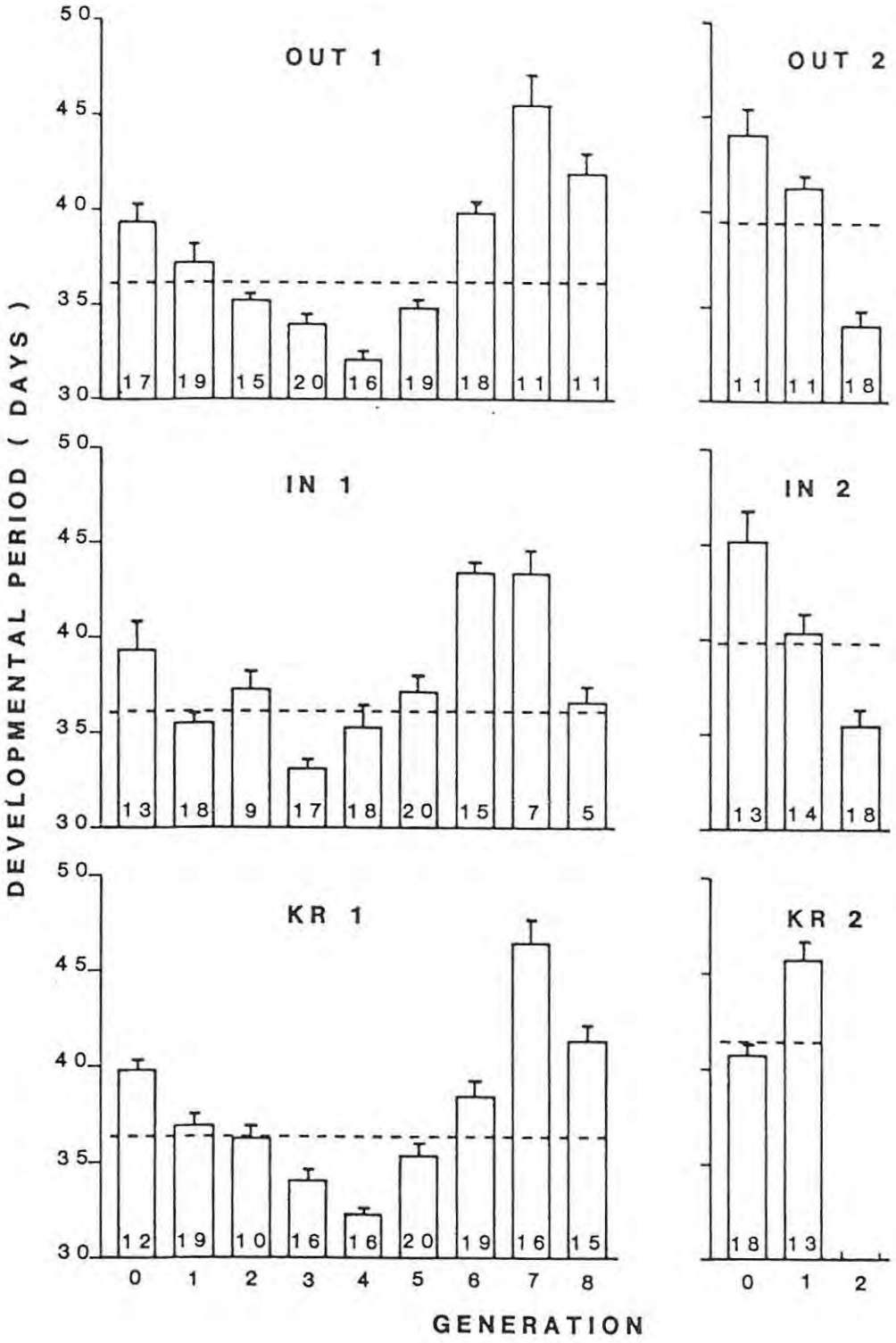


Fig 23. The mean male larval to adult developmental period (days) in each generation (see Table 1 for calculation) in the various treatments of M. pulchellalis. The mean for all generations combined (– – –), the number of lines in a generation from which the mean value was derived, and standard error (X1, vertical line) are shown.

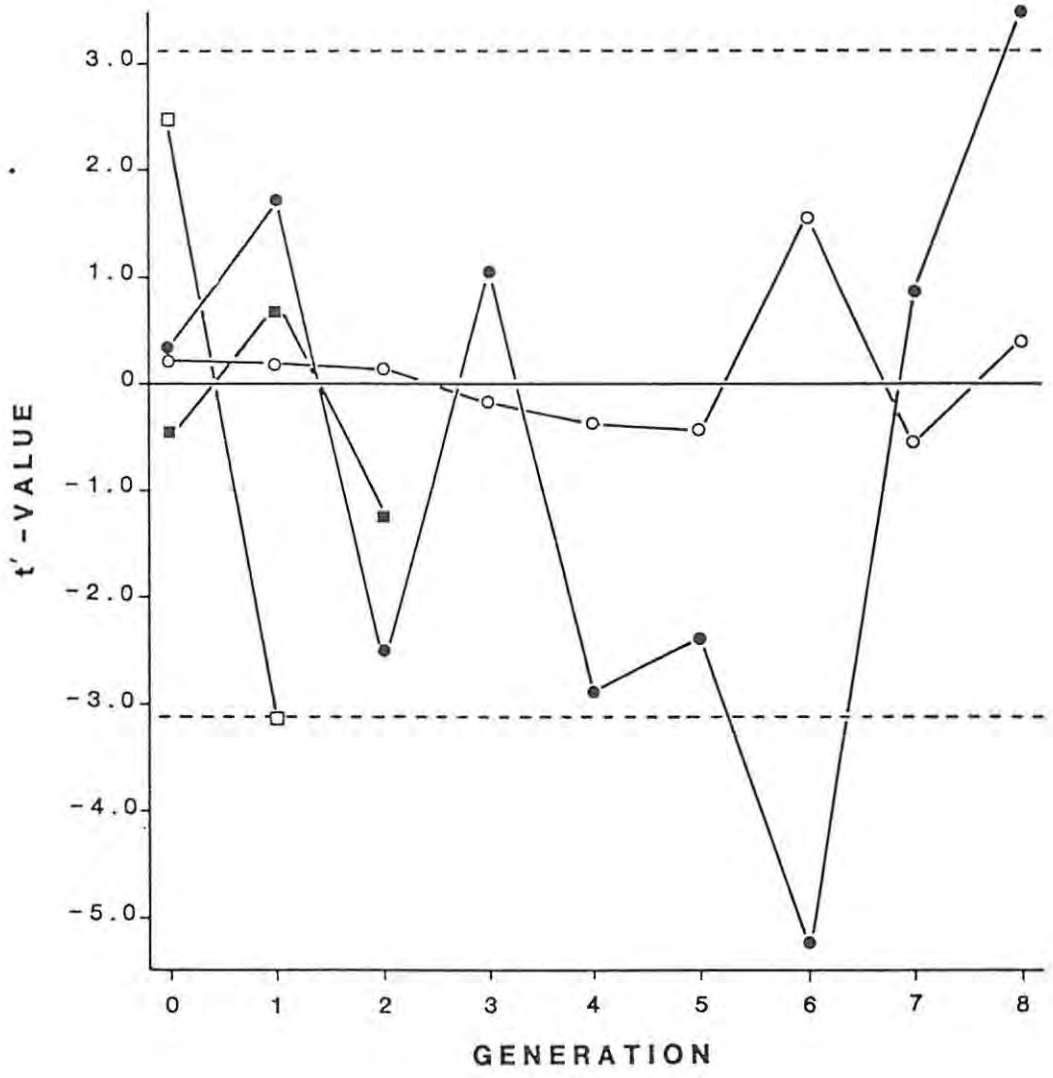


Fig 24. The differences in the mean male larval to adult developmental period (days) in each generation were determined between treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 (●——●) and KR 1 (○——○) and OUT 2 was compared with IN 2 (■——■) and KR 2 (□——□). The t'-values are depicted and the level of probability ($t'_{0.005(2),20} = \pm 3.153$) is shown as two horizontal dotted lines.

(xii) Virgin female longevity

The virgin female longevity ranged from 7.8 to 9.6 days, for all generations combined (Table 21). There was little variation amongst the mean values for each generation in each treatment (Fig 25). Two significant differences occurred in the mean values for each generation between OUT 1 and KR 1 (Fig 26) while none occurred between treatments for all generations combined (Table 22). Neither was an inbreeding depression evident in the longevity of the virgin females in IN 1 since there was no significant linear correlation ($r=0.525$, $n=10$) between these values and the inbreeding coefficients. These results show that inbreeding did not affect the virgin female longevity.

Table 21. The mean virgin female longevity (days) for all generations combined in the various treatments of M. pulchellalis. The standard error (X1) and the number of lines (N) are given for all generations combined.

	OUT			IN			KR		
POP	MEAN	SE	N	MEAN	SE	N	MEAN	SE	N
1	8.9	0.12	142	9.0	0.15	115	9.6	0.12	145
2	8.5	0.27	40	8.8	0.23	45	7.8	0.32	24

Table 22. The differences, in the mean virgin female longevity (days) for all generations combined, were determined between treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 and KR 1, and OUT 2 was compared with IN 2 and KR 2. The t-value, degrees of freedom and the significance at the probability level, α = 0.005, are given.

	OUT vs IN			OUT vs KR		
POP	t-value	df	sig	t-value	df	sig
1	-0.18	255	ns	-3.92	285	sig
2	-1.01	83	ns	1.56	62	ns

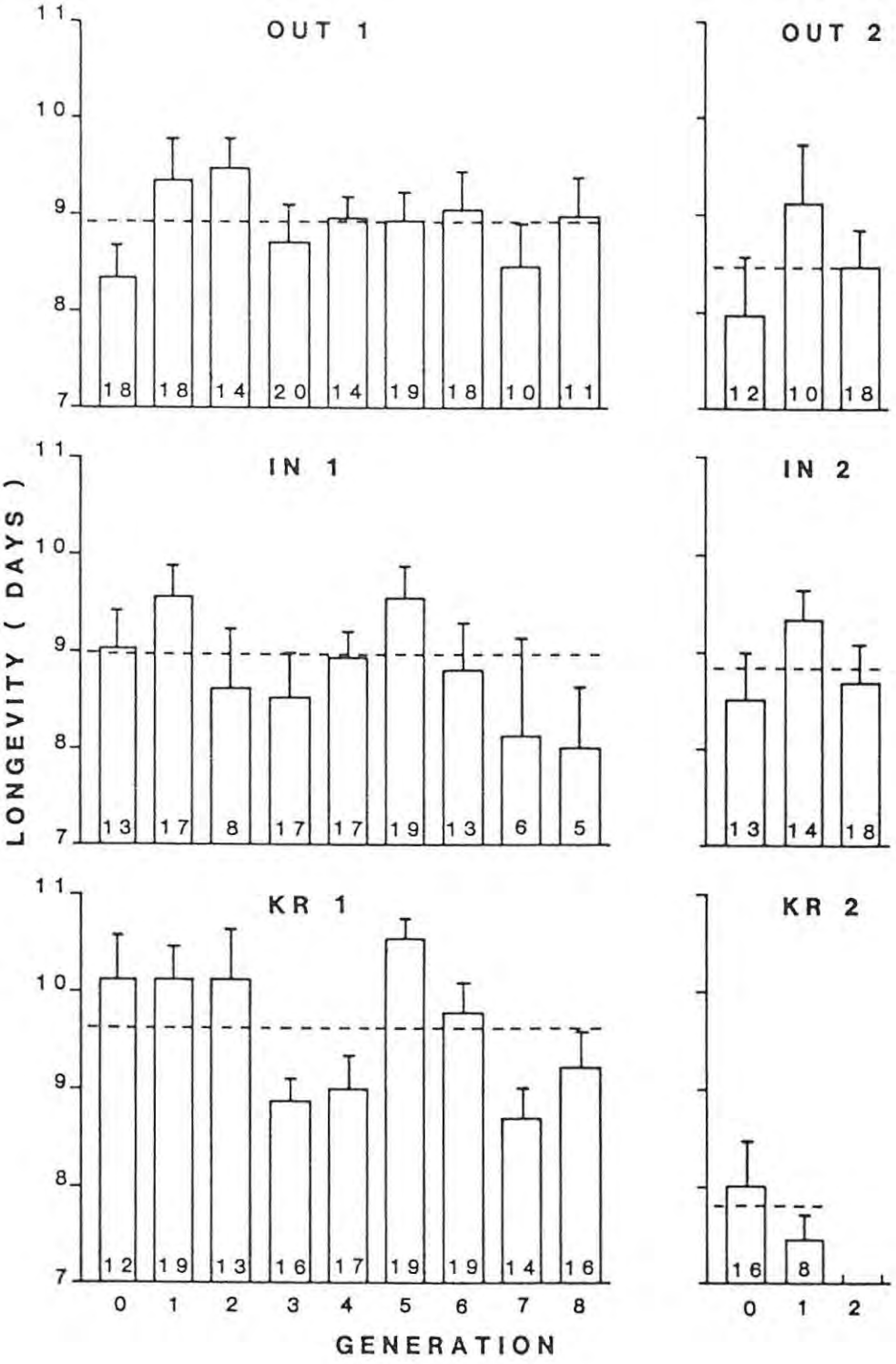


Fig 25. The mean virgin female longevity (days) in each generation (see Table 1 for calculation) in the various treatments of M. pulchellalis. The mean for all generations combined (---), the number of lines in a generation from which the mean value was derived, and standard error (X1, vertical line) are also shown.

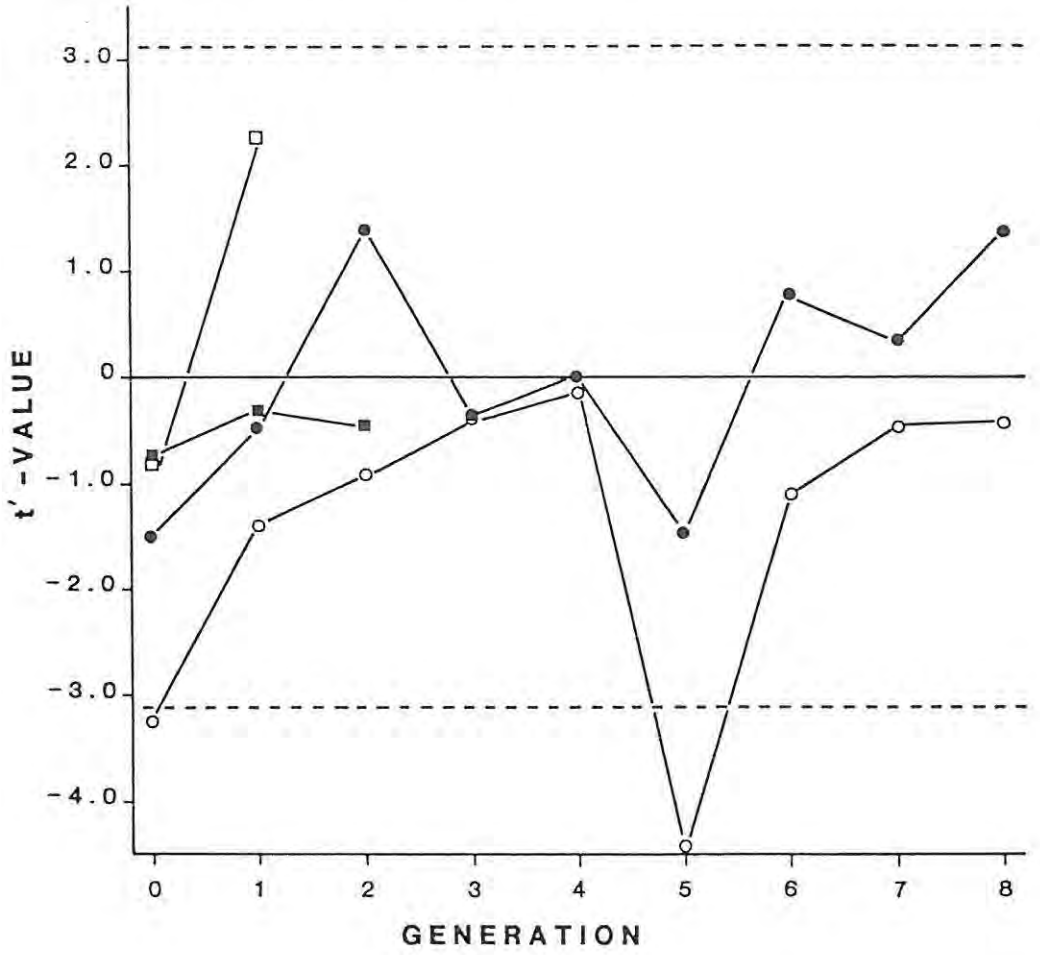


Fig 26. The differences in the mean virgin female longevity (days) in each generation were determined between the treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 (● — ●) and KR 1 (○ — ○) and OUT 2 was compared with IN 2 (■ — ■) and KR 2 (□ — □). The t'-values are depicted and the level of probability ($t'_{0.005(2),20} = \pm 3.153$) is shown as two horizontal dotted lines.

(xiii) Mated female longevity

The longevity of the mated females ranged from 6.1 to 7.0 days for all generations combined (Table 23). The mean values in each generation of this component did not vary greatly (Fig 27). There were no significant differences between treatments in each generation (Fig 28) or for all generations combined (Table 24). In addition, there was no significant linear correlation between the mean values of IN 1 and the inbreeding coefficients ($r=0.234$, $n=9$). Therefore, the mated female longevity did not change as a consequence of inbreeding.

Table 23. The mean mated female longevity (days) for all generations combined in the various treatments of M. pulchellalis. The standard error (X1) and the number of lines (N) are given for all generations combined.

	OUT			IN			KR		
POP	MEAN	SE	N	MEAN	SE	N	MEAN	SE	N
1	6.9	0.15	109	6.8	0.16	110	7.0	0.16	108
2	6.1	0.28	26	6.8	0.29	36	6.2	0.33	14

Table 24. The differences, in the mean mated female longevity (days) for all generations combined, were determined between treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 and KR 1, and OUT 2 was compared with IN 2 and KR 2. The t-value, degrees of freedom, and the significance at the probability level, α = 0.005, are given.

	OUT vs IN			OUT vs KR		
POP	t-value	df	sig	t-value	df	sig
1	0.85	217	ns	-0.23	215	ns
2	-0.64	60	ns	-0.22	38	ns

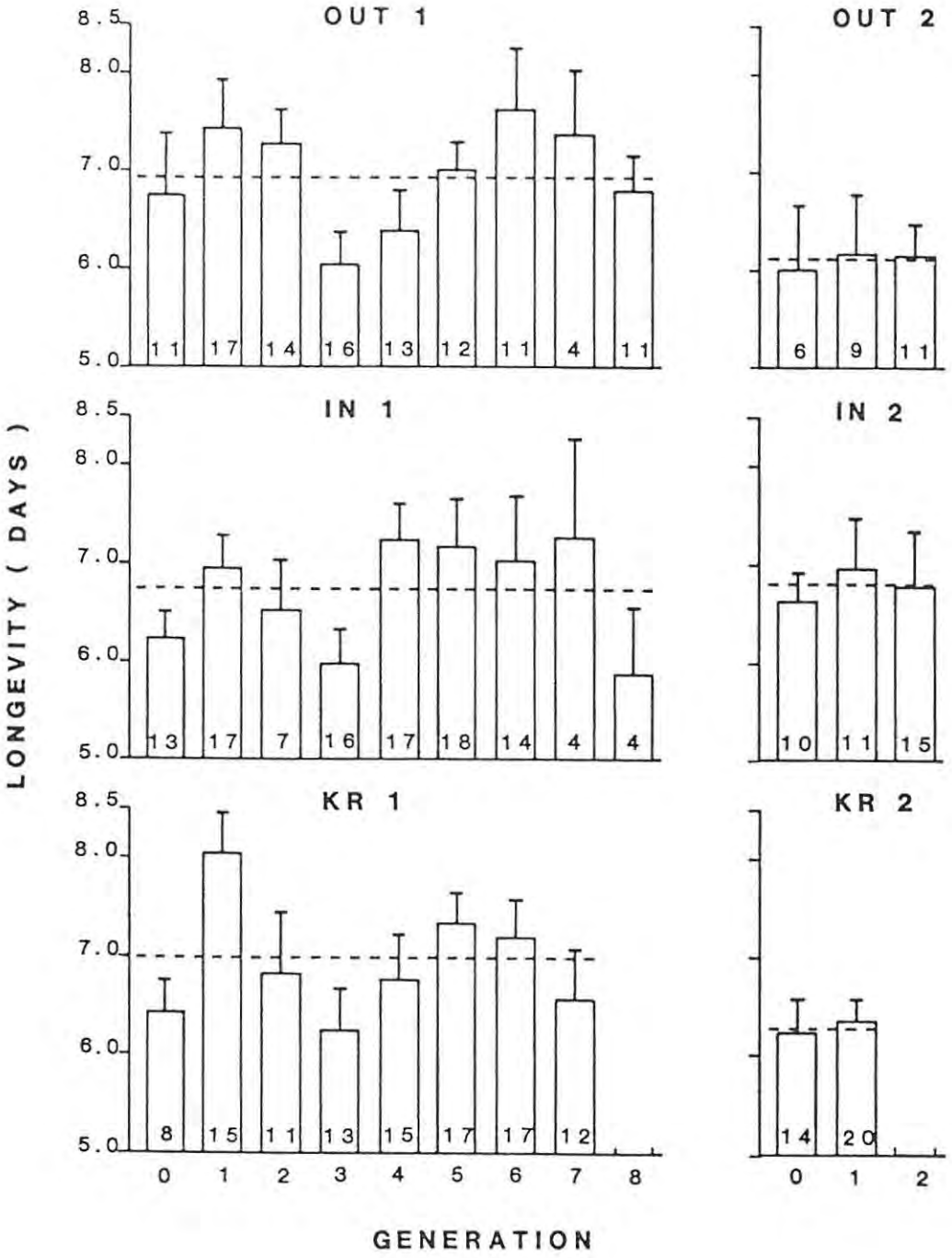


Fig 27. The mean mated female longevity (days) in each generation (see Table 1 for calculation) in the various treatments of M. pulchellalis. The mean for all generations combined (- - -), the number of lines in a generation from which the mean value was derived, and standard error (X1, vertical line) are also shown.

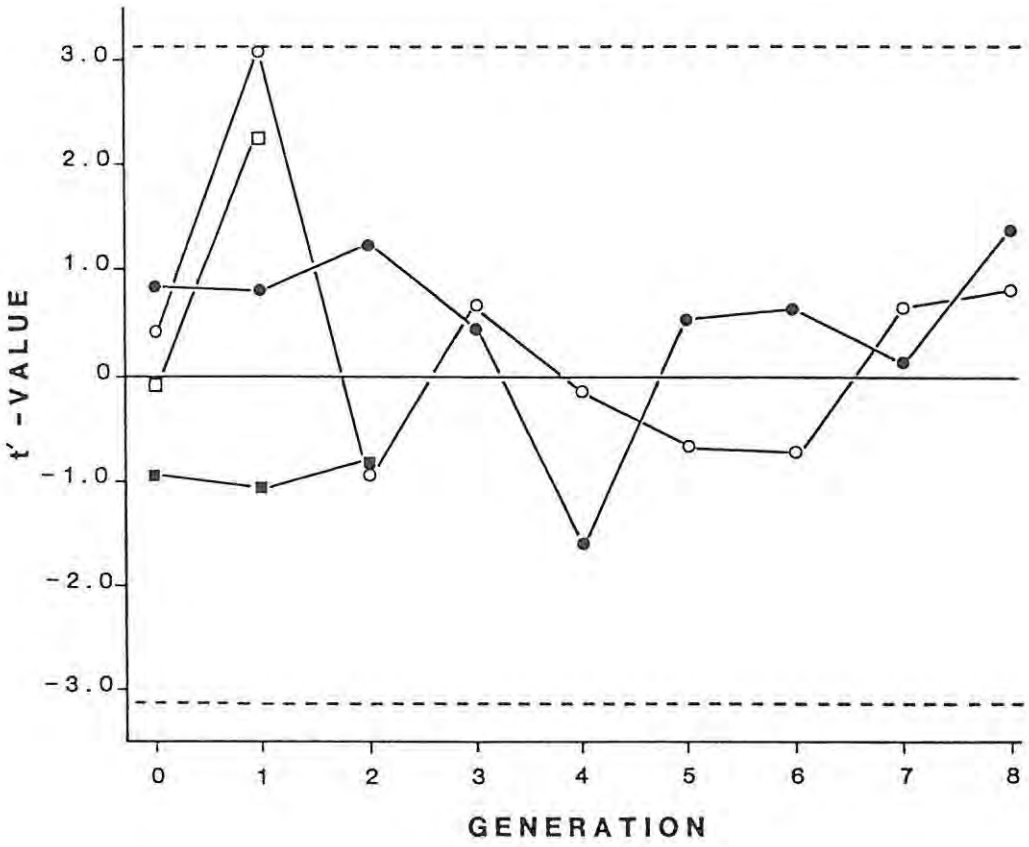


Fig 28. The differences in the mean mated female longevity (days) in each generation were determined between the treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 (●—●) and KR 1 (○—○) and OUT 2 was compared with IN 2 (■—■) and KR 2 (□—□). The t'-values are depicted and the level of probability ($t'_{0.005(2),20} = \pm 3.153$) is shown as two horizontal dotted lines.

(xiv) Wing length

The wing length of the females ranged from 8.8 to 9.3 mm for all generations combined (Table 25). The mean values for each generation are shown in Fig 29. There were no significant differences between treatments in each generation (Fig 30) or for all generations combined (Table 26). Neither was there a linear inbreeding depression in the wing length of the females in IN 1 since there was no significant correlation ($r=0.391$, $n=8$) between these values and the inbreeding coefficients. Clearly, inbreeding did not cause demonstrable changes in the wing length.

Table 25. The mean wing length (mm) for all generations combined in the various treatments of M. pulchellalis. The standard error (X1) and the number of lines (N) are given for all generations combined.

	OUT			IN			KR		
POP	MEAN	SE	N	MEAN	SE	N	MEAN	SE	N
1	9.3	0.005	189	9.3	0.005	159	9.1	0.005	168
2	9.0	0.009	59	9.1	0.008	68	8.8	0.009	39

Table 26. The differences, in the mean wing length (mm) for all generations combined, were determined between treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 and KR 1, and OUT 2 was compared with IN 2 and KR 2. The t-value, degrees of freedom and the significance at the probability level, α = 0.005, are given.

	OUT vs IN			OUT vs KR		
POP	t-value	df	sig	t-value	df	sig
1	-0.75	331	ns	-2.76	347	ns
2	-0.93	124	ns	0.14	93	ns

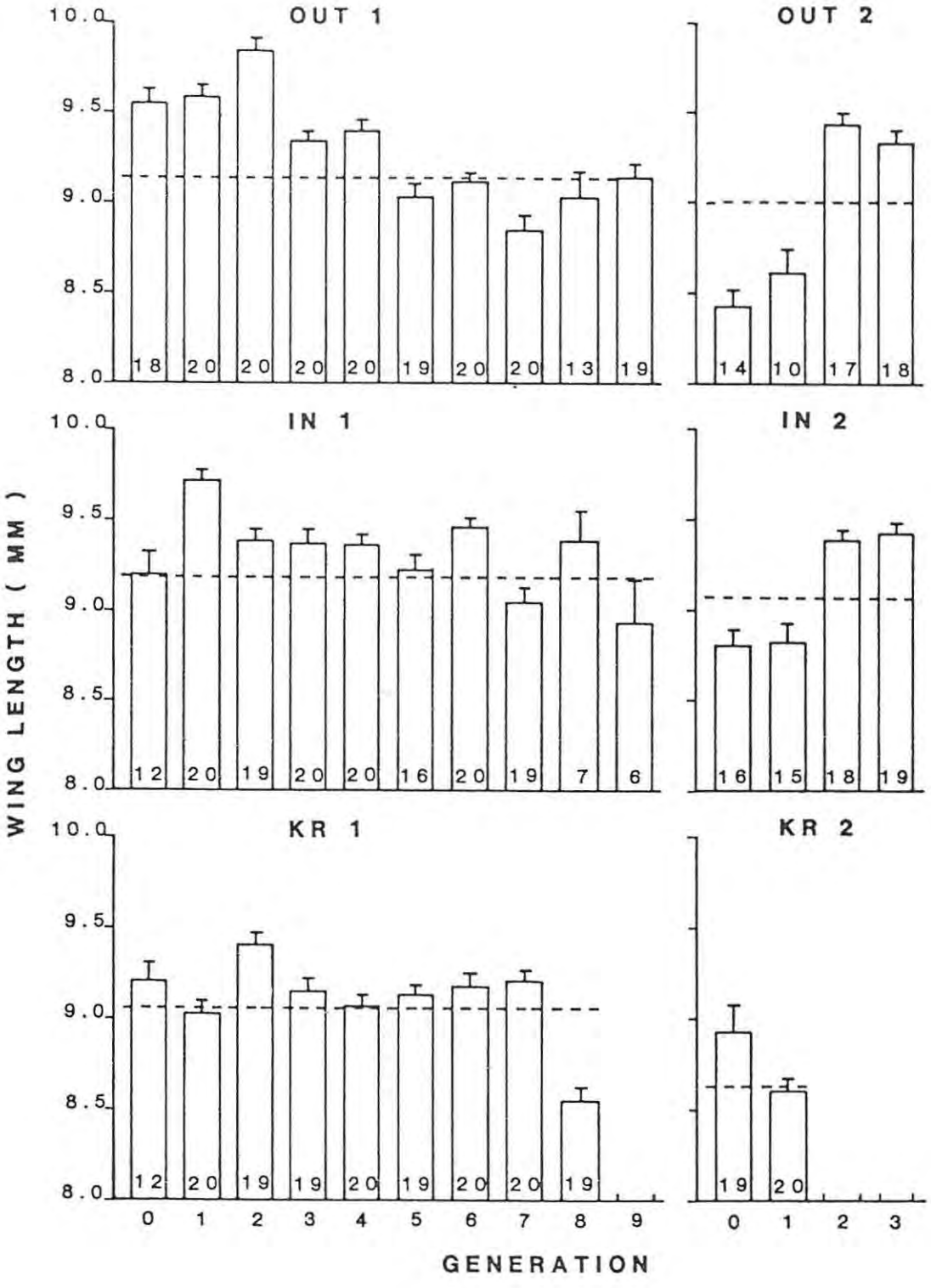


Fig 29. The mean wing length in each generation (see Table 1 for calculation) in the various treatments of M. pulchellalis. The mean for all generations combined (– – –), the number of lines in a generation from which the mean value was derived and standard error (X1, vertical line) are also shown.

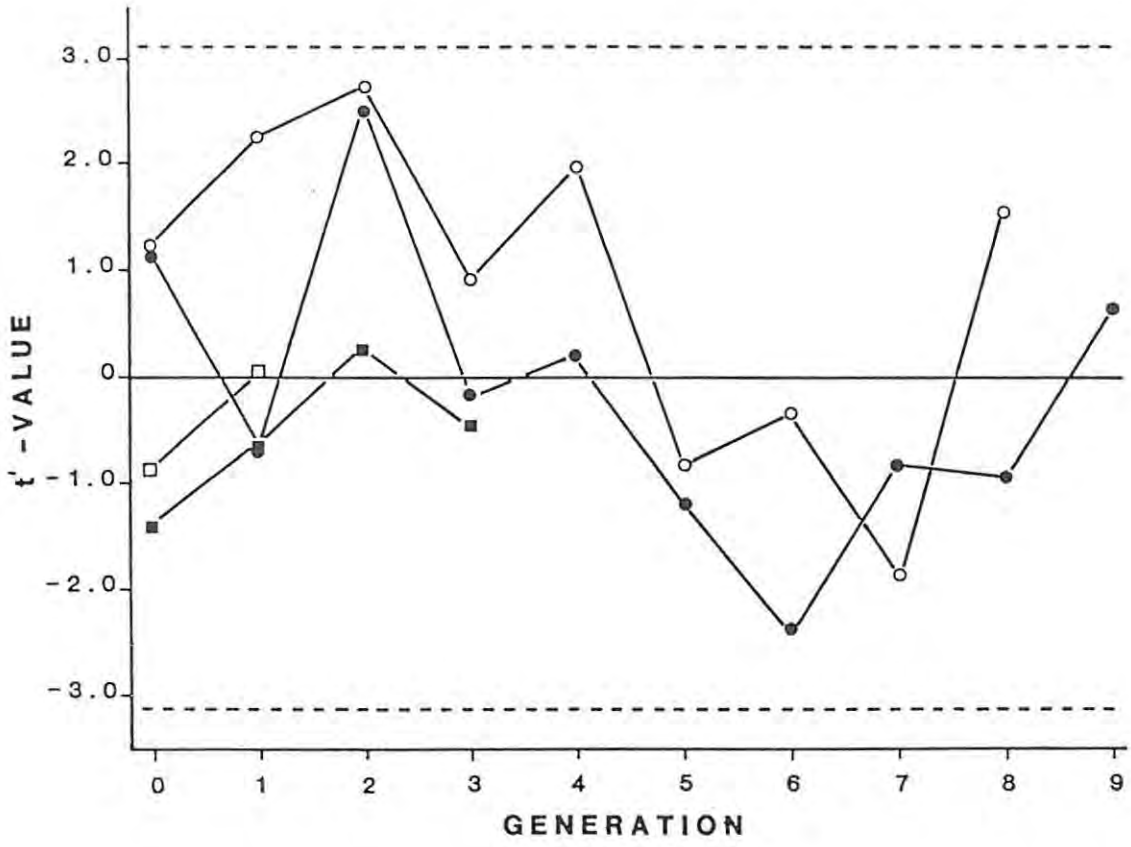


Fig 30. The differences in the mean wing length (mm) in each generation were determined between the treatments of M. pulchellalis. Four comparisons were OUT 1 was compared with IN 1 (● — ●) and KR 1 (O — O) and OUT 2 was compared with IN 2 (■ — ■) and KR 2 (□ — □). The t'-values are depicted and the level of probability ($t'_{0.005(2),20} = \pm 3.153$) is shown as two horizontal dotted lines.

(xv) Sex ratio

The sex ratio ranged from 0.7 to 1.2 for all generations combined (Table 27). Usually more females emerged than males but in at least one generation in each treatment there was a male bias (Table 29). There were no significant differences between treatments for each generation (Fig 31) or for all generations combined (Table 28). The differences for each generation are very small as the t'-values centre around zero. Neither was there a significant linear correlation between the mean values in IN 1 ($r=0.289$, $n=8$) and the inbreeding coefficients. All these results show that inbreeding did not affect the adult sex ratio.

Table 27. The mean sex ratio for all generations combined in the various treatments of M. pulchellalis. The standard error (X1) and the number of lines (N) are given for all generations combined.

	OUT			IN			KR		
POP	MEAN	SE	N	MEAN	SE	N	MEAN	SE	N
1	1.2	0.08	146	1.3	0.09	122	1.1	0.06	143
2	1.0	0.18	40	1.1	0.13	45	0.7	0.10	31

Table 28. The differences, in the mean sex ratio for all generations combined, were determined between treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 and KR 1, and OUT 2 was compared with IN 2 and KR 2. The t-values, degrees of freedom, and the significance at the probability level, $\alpha = 0.005$, are given.

	OUT vs IN			OUT vs KR		
POP	t-value	df	sig	t-value	df	sig
1	-0.67	266	ns	0.40	287	ns
2	0.14	83	ns	1.79	69	ns

Table 29. The mean sex ratio (female:male adults) in each generation in the various treatments of M. pulchellalis. The standard error (X1) and the number of lines (N) are given for each generation.

POP 1	OUT			IN			KR		
GEN	MEAN:1	SE	N	MEAN:1	SE	N	MEAN:1	SE	N
F_0	1.38	0.25	17	1.41	0.29	13	1.33	0.27	12
F_1	1.30	0.25	19	1.49	0.33	18	1.10	0.13	19
F_2	0.92	0.12	15	1.42	0.37	9	0.98	0.14	10
F_3	1.14	0.18	20	1.18	0.20	17	1.19	0.20	16
F_4	1.40	0.30	16	1.33	0.24	18	1.18	0.24	16
F_5	1.03	0.11	19	1.06	0.08	20	1.17	0.15	20
F_6	1.12	0.12	18	1.08	0.23	15	1.16	0.19	19
F_7	1.25	0.51	11	1.55	0.49	7	1.23	0.23	16
F_8	1.24	0.29	11	0.99	0.29	5	1.03	0.19	15
POP 2									
F_0	1.35	0.58	11	1.19	0.25	13	0.84	0.12	18
F_1	0.98	0.23	11	0.92	0.12	14	0.56	0.15	13
F_2	1.08	0.14	18	1.17	0.25	18			

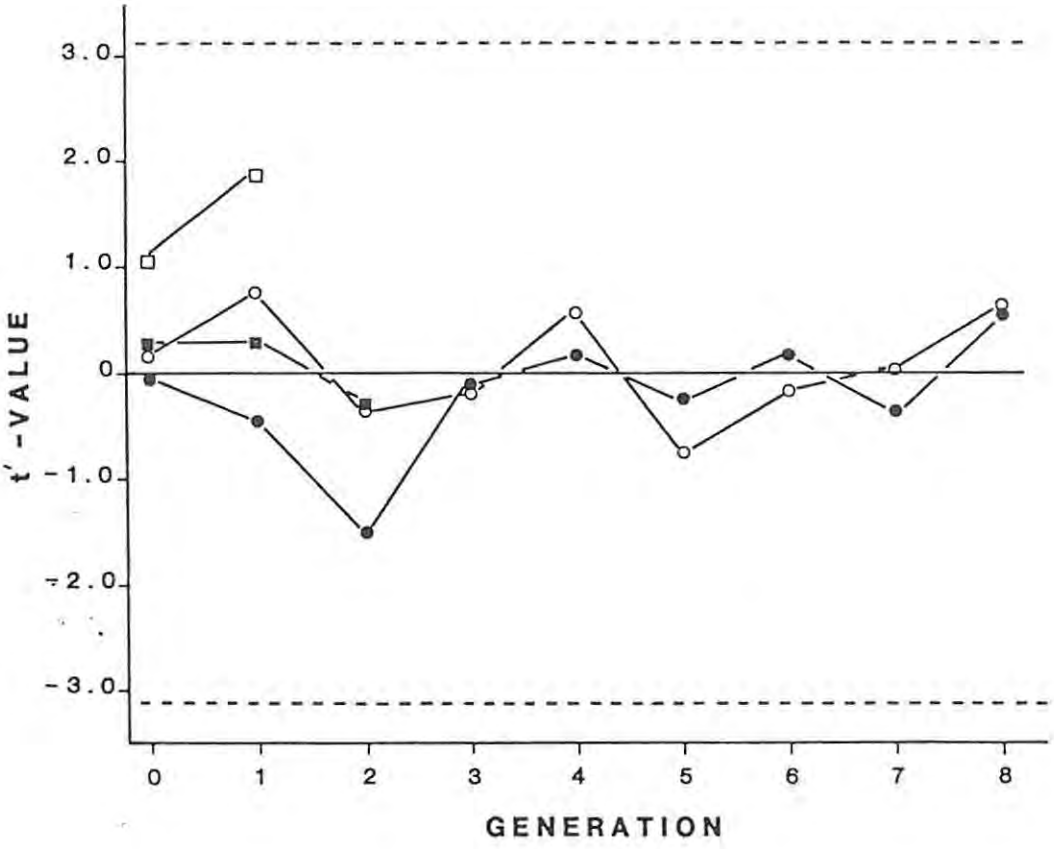


Fig 31. The differences in the mean sex ratio in each generation were determined between the treatments of M. pulchellalis. Four comparisons were made; OUT 1 was compared with IN 1 (● — ●) and KR 1 (○ — ○) and OUT 2 was compared with IN 2 (■ — ■) and KR 2 (□ — □). The t'-values are depicted and the level of probability ($t'_{0.005(2),20} = \pm 3.153$) is shown as two horizontal dotted lines.

CHAPTER 4

POPULATION FITNESS

The fitness of the OUT 1 and 2, IN 1 and 2 and KR 1 and 2 populations of M. puchellalis is discussed in this chapter. Initially, (4.1) life tables were created for each population to assess the contribution of each potential mortality factor (k) to the total generation mortality (K). From the life tables the key factor or that stage in which the highest mortality occurred could be discovered. The percentage survival obtained from the life table data enabled (4.2) a hypothetical estimate of the numbers and growth rate of each of the populations to be calculated. In each case the initial population was a thousand individuals. A ratio of the size of the control (OUT 1) population was made to that of the sibmated (IN 1) and field-introduced (KR 1) populations, in each generation. From this it could be determined whether the difference in the fitness of the respective populations increased, decreased or remained constant.

(4.1) Life tables

OUT 1 and 2

The total generation mortality was different for each of these populations (Fig 32). In OUT 1 the mortality peaked in three generations (F_2, F_4, F_7) while the mortality showed a decreasing trend in OUT 2. The reduction of egg viability was the key factor contributing to the total generation mortality. The k-values of this stage were greater than in any other stage, and the association between the changes in the total generation mortality and those of egg viability were significant ($r=0.949$, $p<0.001$). The changes in larval survival also contributed significantly to the total generation mortality ($r=0.843$, $p<0.005$), but the extent of the contribution in terms of the k-values was not as large that of egg viability.

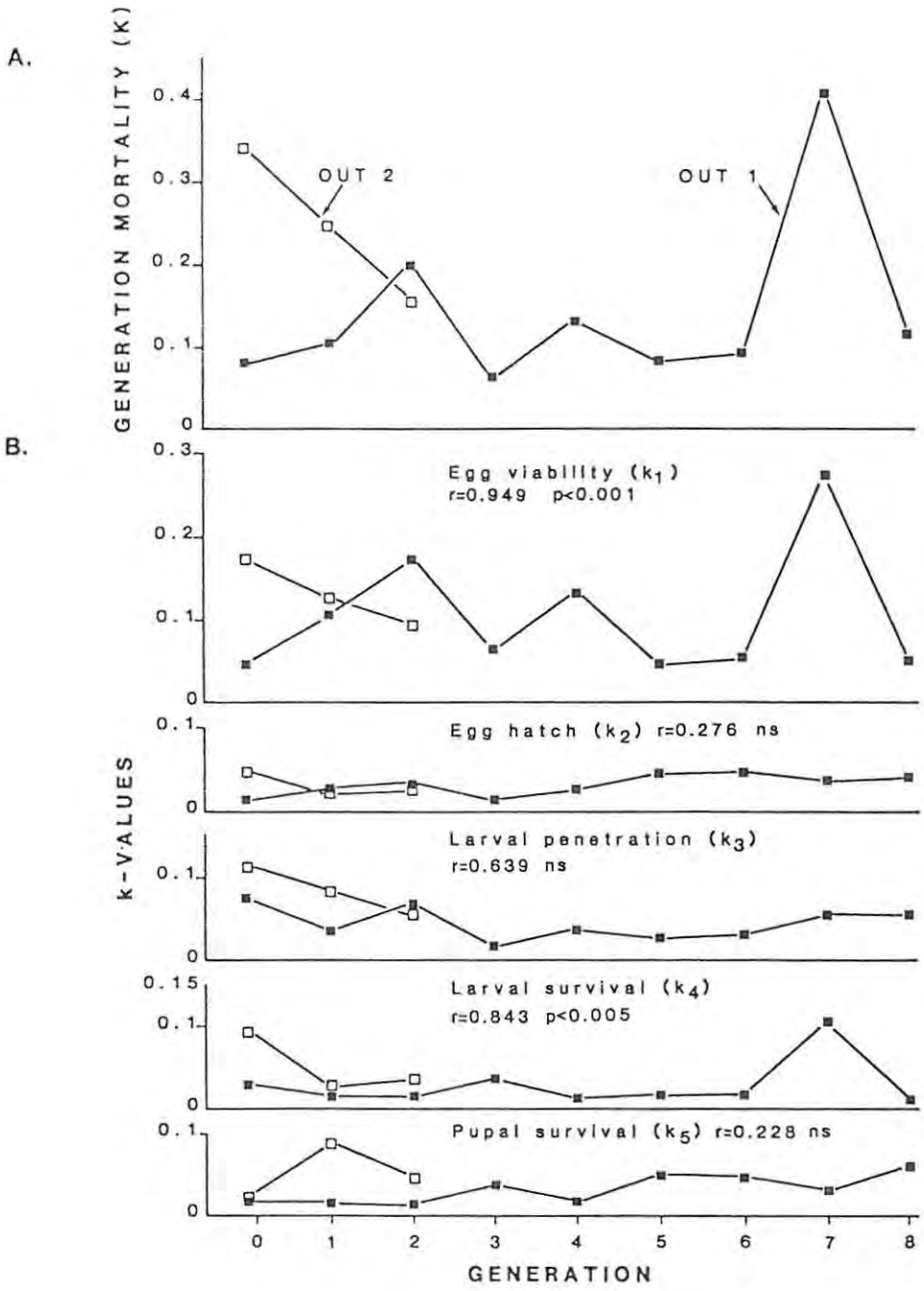


Fig 32 A. The changes in the total generation mortality (K) of the OUT 1 (■ ——■) and OUT 2 (□ —— □) populations of M. pulchellalis. The total generation mortality is expressed as the sum of the mortality factors ($k_1 + k_2 + k_3 + k_4 + k_5$).

B. The changes in the generation mortality factors affecting egg viability (k_1), egg hatch, (k_2), larval penetration (k_3), larval survival (k_4), and pupal survival (k_5) were correlated with the total generation mortality. The mortality affecting egg viability was the key factor which contributed to the total generation mortality.

Similarly, the decrease in egg hatch, larval penetration and pupal survival were not as important as the reduction of egg viability.

IN 1 and 2

The total generation mortality in IN 1 (Fig 33) was higher than OUT 1 although the changes in the mortality were significantly associated with those of OUT 1 ($r=0.761$, $p=0.005$). Increases in the mortality occurred in the F_2 and F_7 in IN 1 while in contrast that of IN 2 decreased in the F_2. Once again the reduction of egg viability was the key factor that contributed to the total generation mortality in IN 1 as the k-values were the highest of the mortality factors. The changes in this stage also contributed to those of the total generation mortality ($r=0.776$, $p=0.005$). The changes in egg hatch were also significantly associated with those of the total generation mortality ($r=0.852$, $p<0.001$), but the k-values were lower than those of egg viability. The mortality in the components larval penetration and larval and pupal survival was not as important as egg viability.

KR 1 and 2

Although increases in the total generation mortality of KR 1 (Fig 34) occurred in the F_2, F_4 and F_7, the association between the OUT 1 and KR 1 was not significant ($r=0.704$, $p=0.02$). KR 2 differed from both OUT 2 (Fig 32) and IN 2 (Fig 33) as there was an increase in mortality rather than a decrease. KR 1 resembled OUT 1 and IN 1 because the reduction in egg viability was also the key factor that contributed to the total generation mortality. The k-values of egg viability were higher than those of egg hatch, larval penetration, and larval and pupal survival. The changes in the total generation mortality were also significantly associated with those of egg viability ($r=0.976$, $p<0.001$).

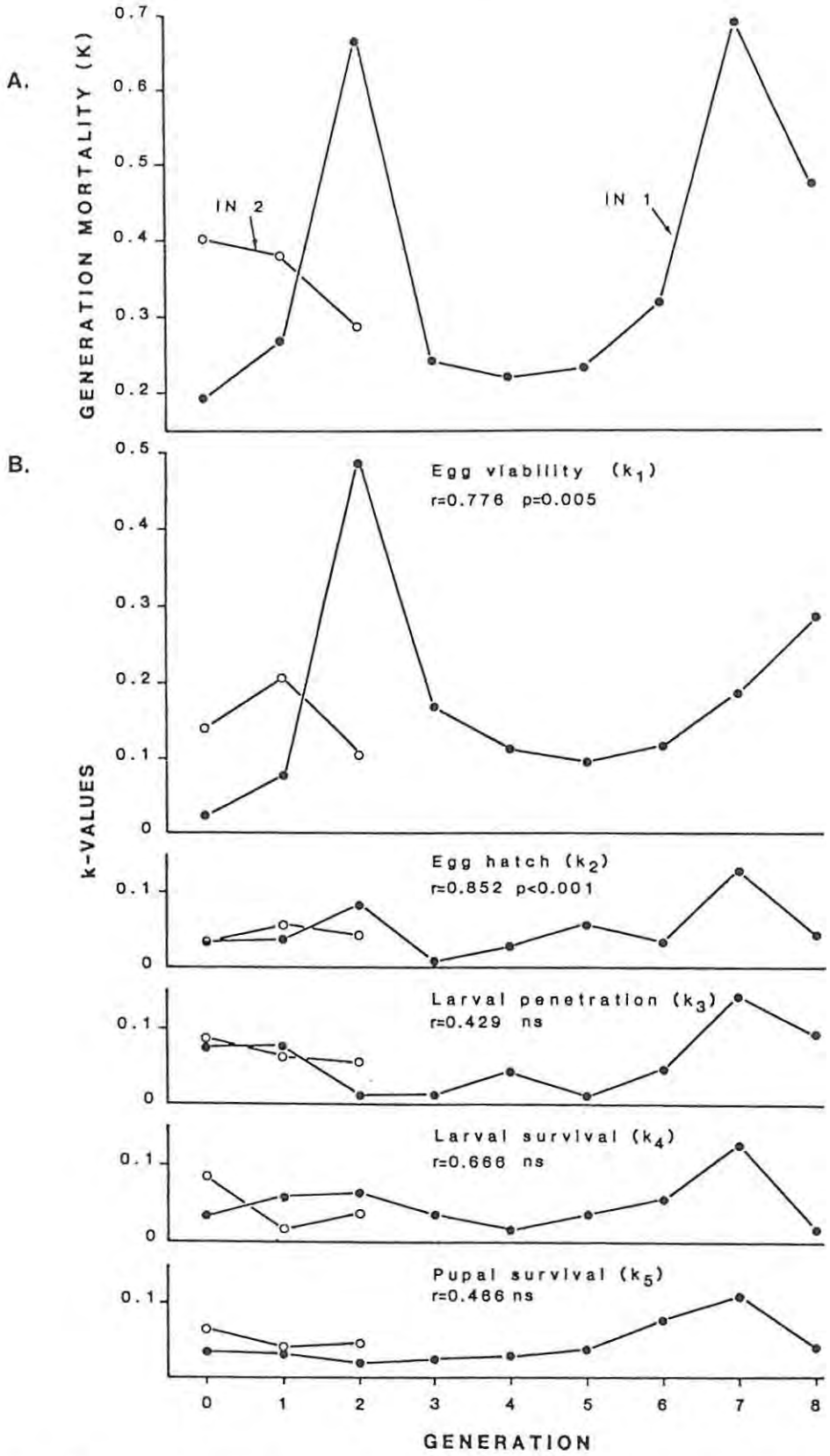


Fig 33 A. The changes in the total generation mortality (K) of the IN 1 (●——●) and IN 2 (O——O) populations of M. pulchellalis. The total generation mortality is expressed as the sum of the mortality factors ($k_1 + k_2 + k_3 + k_4 + k_5$).

B. The changes in the generation mortality factors affecting egg viability (k_1), egg hatch (k_2), larval penetration (k_3), larval survival (k_4) and pupal survival (k_5) were correlated with the total generation mortality. The mortality affecting egg viability was the key factor which contributed to the total generation mortality.

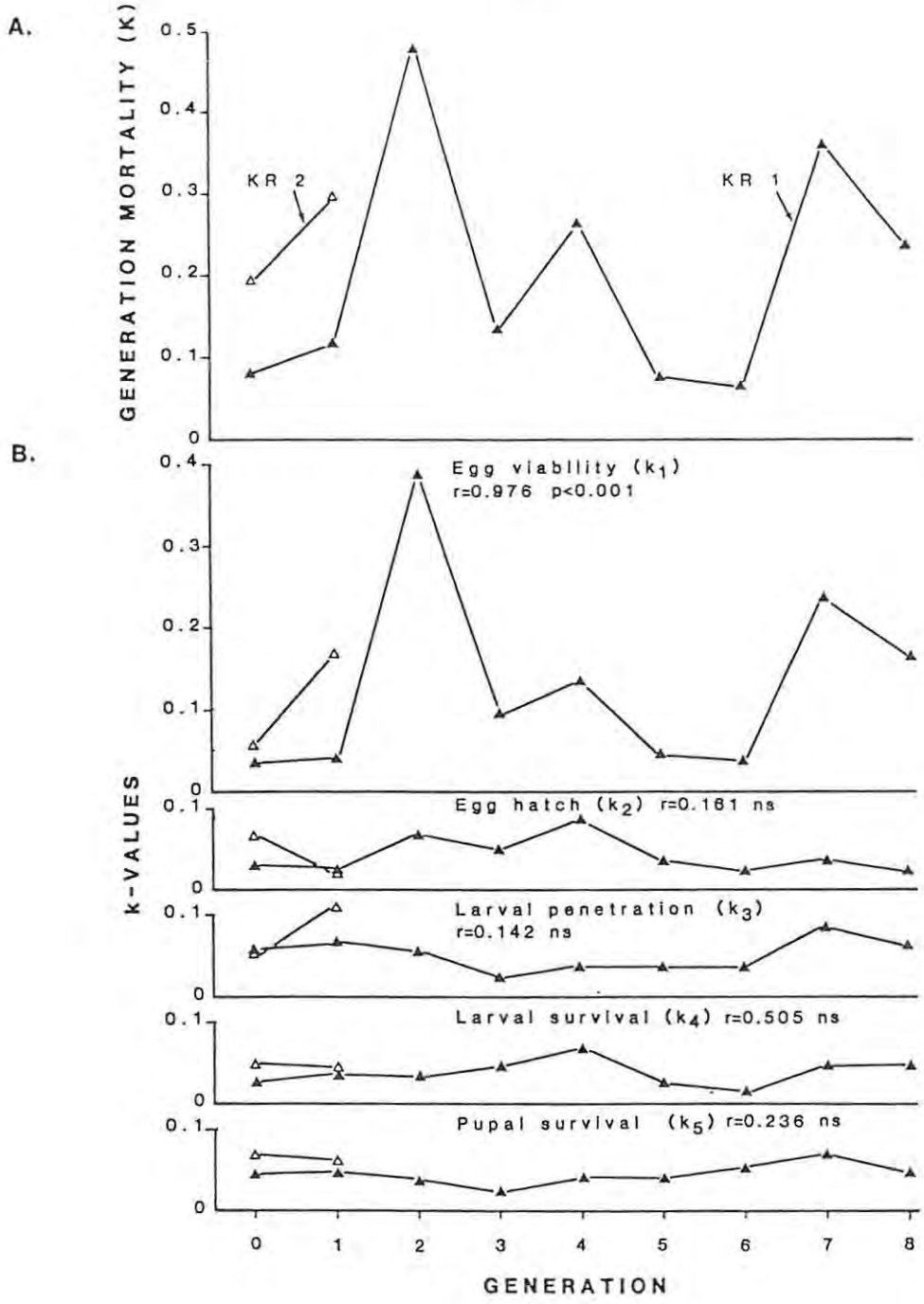


Fig 34 A. The changes in the total generation mortality (K) of the KR 1 (▲——▲) and KR 2 (△——△) populations of M. pulchellalis. The total generation mortality is expressed as the sum of the mortality factors ($k_1 + k_2 + k_3 + k_4 + k_5$).

B. The changes in the generation mortality factors of egg viability (k_1), egg hatch (k_2), larval penetration (k_3), larval survival (k_4) and pupal survival (k_5) were correlated with the total generation mortality. The mortality affecting egg viability is the key factor which contributed to the total generation mortality.

(4.2) A hypothetical estimate of population size and growth rate

There was a possibility of a cumulation of small inbreeding effects on the fitness components having a major impact on eventual population numbers (Fig 35). This proved to be the case. In the F_1, IN 1 had a larger population size than both OUT 1 and KR 1. However, this position was reversed, after the F_2 generation when OUT 1 achieved and maintained the largest number of individuals and IN 1 the least. In comparison to OUT 1, IN 1 and KR 1, OUT 2, IN 2 and KR 2 were smaller in size in equivalent generations. No clear trend had developed by the F_3 but there were slightly more individuals in OUT 2 than IN 2 in the F_3.

The rate of population growth (Fig 36) was not constant in any of the populations. For example, after the F_2 and F_7 the rate of increase declined in OUT 1, IN 1 and KR 1. These points coincide with the reduction in egg viability and the subsequent increase in the total generation mortality. In the F_2 the reduction in egg viability was not as great in OUT 1 in comparison to IN 1 and KR 1. This had a cumulative effect on the population size in subsequent generations, and is possibly responsible for the larger size of OUT 1.

OUT 2, IN 2 and KR 2 also had a slower population growth than OUT 1, IN 1 and KR 1. After the base population both IN 2 and KR 2 had a decreasing growth rate, while that of OUT 2 was positive. Therefore, in spite of KR 2 having a larger population size than OUT 2, as a result of a rapid increase in the F_1 (Fig 35), it was not increasing as fast. This was probably due to the greater generation mortality (Fig 34).

It is apparent that although the outbred populations OUT 1 and 2 were not always the larger in size their population growth rate was always greater than the sibmated IN 1 and 2 and field-introduced KR 1 and 2 populations. Not only was OUT 1 larger in size than IN 1 after the F_2 but the difference between them increased exponentially (Fig 37). In contrast the difference between OUT 1 and KR 1 remained almost constant.

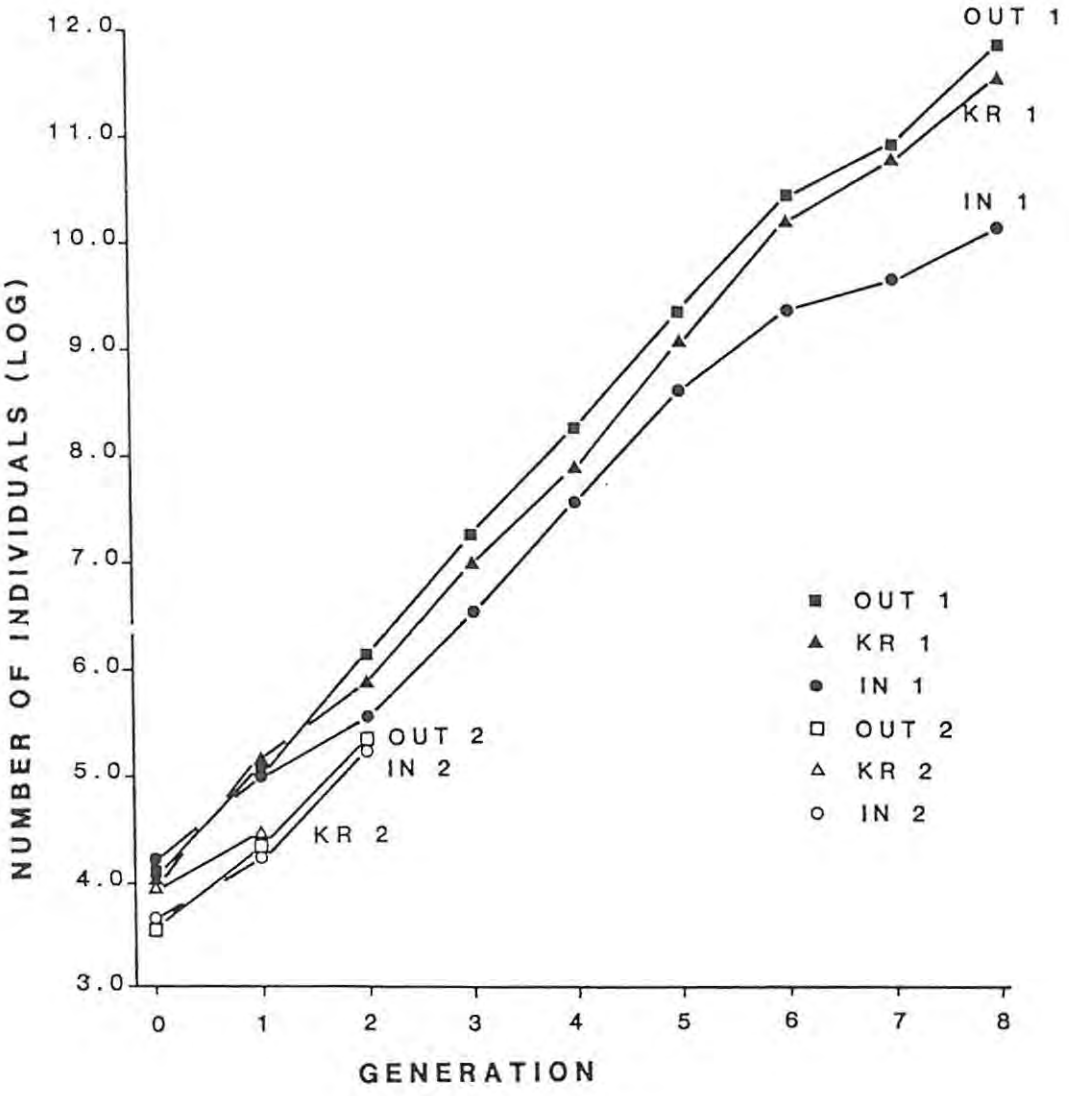


Fig 35. The cumulative increase in population numbers in each generation of M. pulchellalis. The OUT 1 (■——■), OUT 2 (□——□), IN 1 (●——●), IN 2 (○——○), KR 1 (▲——▲), and KR 2 (△——△) populations are represented.

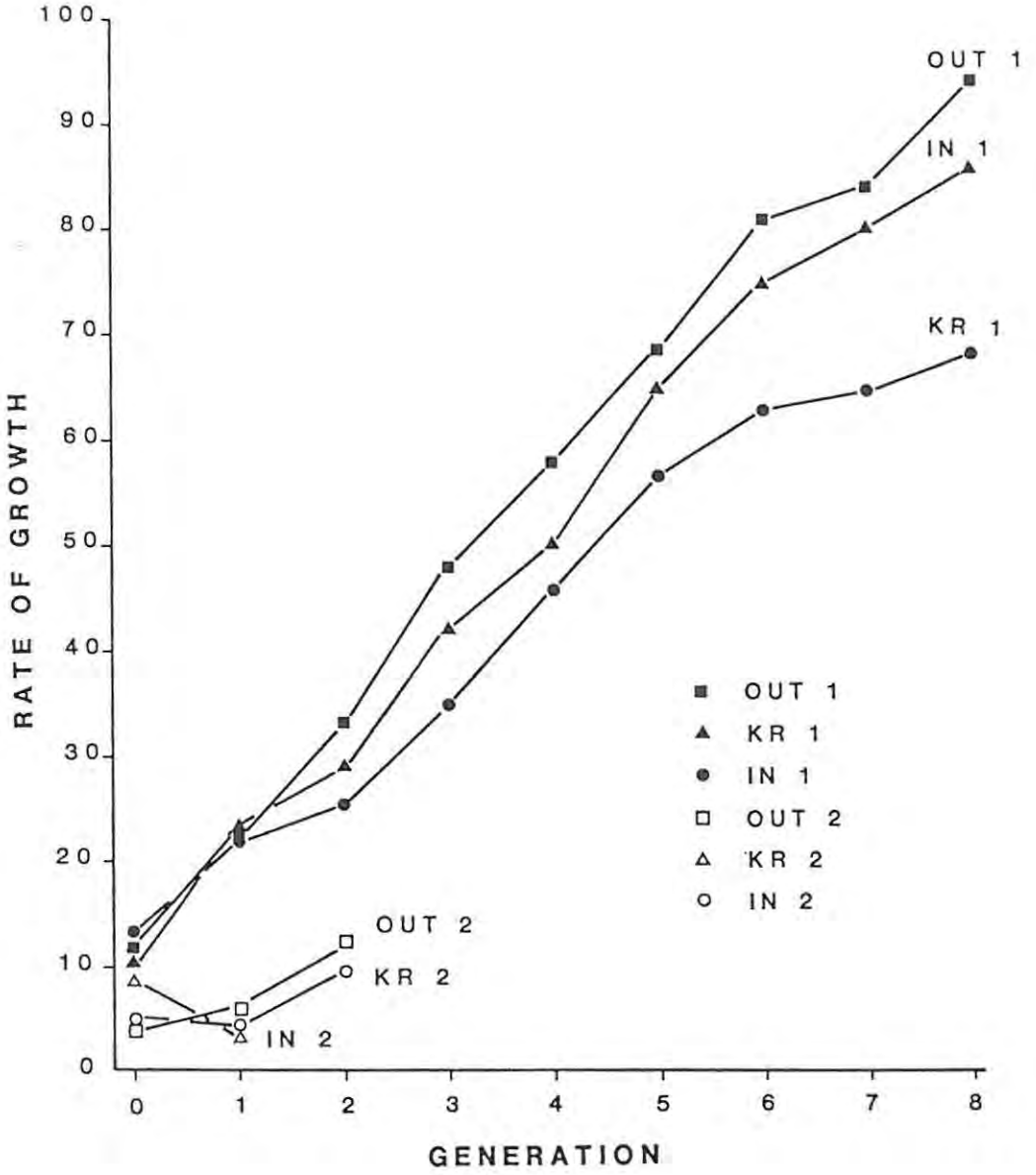


Fig 36. The rate of increase in population numbers in each generation of M. pulchellalis. The OUT 1 (■——■), OUT 2 (□——□), IN 1 (●——●), IN 2 (○——○), KR 1 (▲——▲), and KR 2 (△——△) populations are represented.

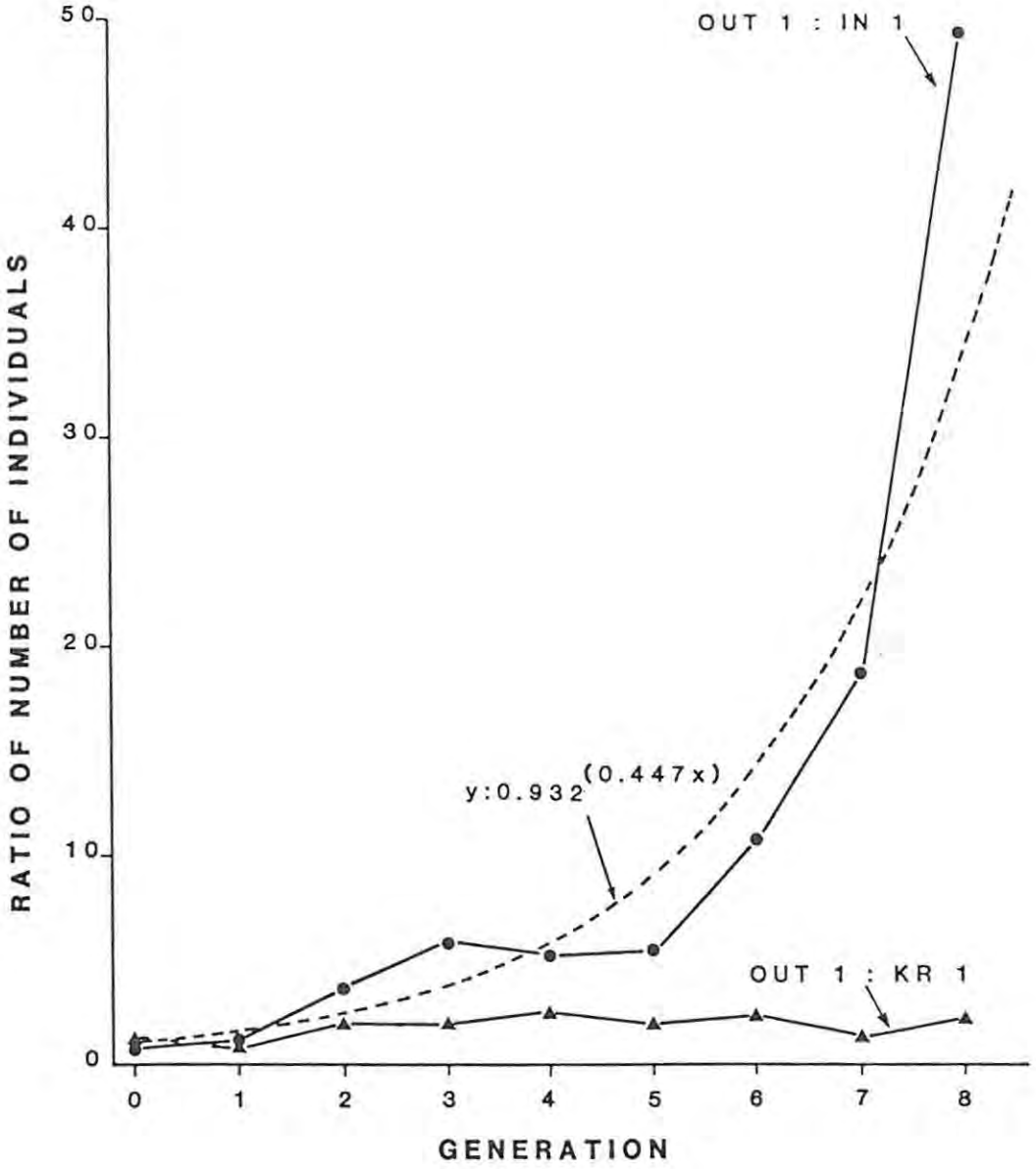


Fig 37. The ratio of the population numbers in each generation, of M. pulchellalis, between OUT 1 and IN 1 (●——●), and OUT 1 and KR 1 (▲——▲). The hypothetical curve of best fit (----) depicts the exponential increase in the difference between OUT 1 and IN 1 population numbers in each generation.

Thus it appears that there was a decrease in the population fitness of both the sibmated and field-introduced populations. The decrease in the production of the numbers of offspring resulted primarily from a reduction in egg viability (key factor). In spite of the small non-significant differences, between the treatments, in the mean values of this fitness component (see Chapter 3) it appears that the cumulation of these small differences in egg viability over generations could have a dramatic effect on a population. As mentioned earlier, in Chapter 3, a reduction in egg viability resulted from a greater number of mated females laying no viable eggs. In the following chapter, consideration of whether this was due to an inbreeding depression is made.

CHAPTER 5

DISCUSSION

Initially (5.1) the aim of this study, the way in which it was undertaken and the results obtained will be summarized. (5.2) A perspective of these results will then be given with particular emphasis on the fitness component egg viability. (5.3) Possible explanations of these results will be provided. Finally, (5.4) the relevance of this research to difficulties encountered in the laboratory-rearing of cactophagous Lepidoptera and to future biological control will be discussed.

(5.1) Small founder populations of Amalafrida leithella, Cactoblastis mundelli, Nanaia sp., Sigelgaita sp., and Sigelgaita transilis had been imported to South Africa for the biological control of jointed cactus, Opuntia aurantiaca. The laboratory cultures of these five species of cactophagous Lepidoptera declined or were lost in the second filial generation (F_2) and this was thought attributable to inbreeding. The aim of the present research was to determine the effects of inbreeding on a pyraustid moth, Mimorista pulchellalis, a successfully established biological control agent of jointed cactus.

Three populations were set up in this investigation; a randomly-mating control population (OUT 1), a sibmating experimental population (IN 1), and a randomly-mating experimental population (KR 1). The OUT 1 and IN 1 populations consisted of established laboratory stock and the KR 1 population was comprised of individuals collected in the field and introduced, in small numbers, to a laboratory. In the latter population it was hoped to recreate the conditions under which earlier unsuccessful attempts to colonize other cactophagous Lepidoptera in the laboratory had taken place. A reduction of egg viability in the F_2 of OUT 1, IN 1 and KR 1 was observed (Fig 7). At a later date duplicate treatments OUT 2, IN 2 and KR 2 were set up in order to confirm

whether this decline is a general phenomenon.

To determine inbreeding depression and also that component which contributed most to total generation mortality and subsequently, affected population fitness of M. pulchellalis, fifteen fitness components were assessed. These components were measured in each generation and for all generations combined (Fig 2). The characters were: mate success, number of eggs laid, egg viability, egg hatch, female egg and male egg developmental periods, larval penetration, larval and pupal survival, female larval to adult and male larval to adult developmental periods, virgin female and mated female longevity, wing length and sex ratio.

For all these fitness components, there were only seven isolated cases when the mean values in each generation differed between the control (OUT 1 and 2) or experimental (IN 1 and 2 and KR 1 and 2) treatments. There were only two significant differences between the treatments for all generations combined. In addition on no occasion was there a significant linear inbreeding depression of the fitness components in IN 1 in relation to the inbreeding coefficients (the probability of two genes at any locus in an individual being homozygous through ancestry).

The fitness component egg viability differed from the other fitness components because a reduction in the percentage egg viability occurred simultaneously in OUT 1, IN 1, and KR 1 in the F_2 and F_7. These reductions resulted from a greater number of mated females laying no viable eggs. This phenomenon is important in terms of population fitness in M. pulchellalis which is discussed below.

The measurement of the fitness components precluded the detection of an inbreeding depression by directly evaluating population size and growth rate. However, using life table analysis and the calculated mean values of the fitness components, a hypothetical estimate of total generation mortality was made for OUT 1 and 2, IN 1 and 2 and KR 1 and 2. Using the percentage survival in each generation, population size and growth rate could also be established. Thus the effect of inbreeding on

population fitness was determined.

With the use of life table analysis it was evident that egg viability was the key factor contributing to total generation mortality and subsequently, affecting population fitness in M. pulchellalis (Chapter 4). The sibmated (IN 1 and 2) and field-introduced (KR 1 and 2) populations incurred a larger generation mortality, primarily through a greater reduction in egg viability than the control outbred populations (OUT 1 and 2). In addition the life table analysis revealed that inbreeding affected the population fitness of IN 1 and 2 and KR 1 and 2. OUT 1 and 2 were fitter than IN 1 and 2 and KR 1 and 2 with respect to population size and growth rate, or, in the case of KR 2, only in terms of growth rate. Similarly, OUT 1, IN 1 and KR 1 had a smaller generation mortality, larger population size and more rapid growth rate in contrast to OUT 2, IN 2 and KR 2.

Therefore the two approaches, analysis of the individual fitness components and life table analysis, indicated that egg viability was important for two inter-related reasons. Firstly, a reduction in egg viability in OUT 1, IN 1 and KR 1 in the F_2 and F_7 was shown to be a consequence of mated females laying no viable eggs. Secondly, egg viability was the key factor contributing to total generation mortality and affecting population fitness in M. pulchellalis. Because inbreeding clearly affected population fitness it is likely that the small differences between treatments in egg viability resulted from inbreeding.

(5.2) Two difficulties arise in the consideration of the possible effects of inbreeding on the fitness component, egg viability, in M. pulchellalis. The first problem is related to the comparison of the results of egg viability with other investigations of inbreeding reported in the literature. In the present study, differentiation was made between egg viability and egg hatch. The number of eggs laid by mated females that were viable or in other words turned black were recorded as egg viability (those which remained yellow and

subsequently shrivelled were not viable) while those viable eggs that failed to hatch were recorded as egg hatch. However, in most available reports no distinction is made between these two components and an inbreeding depression occurring in the egg stage is usually expressed as the failure of eggs to hatch. The second problem arises because the failure of the eggs to darken in M. pulchellalis may, in fact, be the result of either (a) a poor quality of oocyte or (b) an insufficient quality and quantity of sperm (Maynard Smith 1956) or, perhaps, (c) early embryonic mortality (Oliver 1981). The possible importance of these three factors is discussed below.

(a) As far as oocyte quality is concerned, it is known that the process of maturation can be affected by nutritional factors (Engelmann 1970). Both lipids and proteins are necessary for yolk formation while vitamins and salt, for example, are needed for the hormonally controlled process of yolk formation. The latter two minerals are also incorporated into the actual oocyte. Some such deficiency in the food supply could have been an important factor in influencing egg viability.

M. pulchellalis is autogenous because females can lay eggs without ingesting any food. Consequently, it is the quality of food taken in by the larva that will affect oocyte maturation. The jointed cactus plants used for experimental purposes were standardized, but a change in plant quality may have been responsible for the simultaneous reduction of egg viability in OUT 1, IN 1 and KR 1, in the F_2 and F_7 generations. However, the reduction in egg viability was less in OUT 1 than KR 1 which was, in turn, less than IN 1 (Chapter 3, Fig 7). A similar ranking is reflected in the total generation mortality, to which the reduction in egg viability contributed greatly. This, subsequently, influenced the respective population sizes and growth rates (Chapter 4). It seems that the cause of the reduction in egg viability was genetic, rather than nutritional, because of the way in which the effect was related to the various treatments. This deduction does not preclude the possibility that the quality of the oocytes had not been affected genetically.

(b) The quality and quantity of sperm in Drosophila subobscura Collin was thought to be affected by inbreeding, and also responsible for a decrease in the percentage egg hatch (Maynard Smith 1956). In M. pulchellalis the presence or absence of a spermatophore was recorded as mate success. It was shown that those females which produced no viable eggs had nevertheless been inseminated. However, perhaps the sperm within the spermatophore may have been defective in some way causing a reduction in egg viability.

(c) The remaining possibility, embryonic mortality, was found to be important in sibmated lines of twelve species of Lepidoptera (Oliver 1981). Embryonic mortality was detected by the yellow, shrivelled eggs which had failed to turn black (Oliver 1981). Thus embryonic mortality in these Lepidoptera corresponds to egg viability measured in M. pulchellalis. Furthermore, the increase of embryonic mortality in those twelve species of Lepidoptera occurred in the generation equivalent to that in which the reduction of egg viability occurred in M. pulchellalis.

Oliver (1981) suggested that embryonic development was very sensitive to the effects of inbreeding because it was a period of rapid tissue growth. The genetic load, or that portion of genes which if expressed phenotypically are deleterious, was calculated in relation to embryonic mortality. It was found to consist of a few strongly deleterious genes which in a homozygous combination would result in an immediate inbreeding depression. A similar genetic load in M. pulchellalis may have also been responsible for the reduction of egg viability.

To summarize, if, as appears to be the case (see above), the factor causing the reduction in egg viability is genetic then it could be affecting the quality of oocyte or sperm or increasing embryonic mortality in M. pulchellalis. It is also interesting to note that when eggs failed to turn black in the twelve species of Lepidoptera embryonic mortality (studied by Oliver 1981) was incriminated in egg loss.

In research reported on other species of insects an inbreeding depression has been related both to various fitness components and to population fitness. However, often the fitness component most affected by inbreeding and subsequently influencing population fitness was not distinguished. This was the case in the following two examples. A decrease in population growth rate was shown in sibmated lines of Aedes aegypti Linnaeus (Fergusson-Laguna and Machado-Allison 1979). Similarly, a decline in growth rate occurred in small populations of Tribolium castaneum Herbst, as a result of decreased heterozygosity (McCauley and Wade 1981).

An inbreeding depression has been shown in the following fitness components. Inbreeding caused a decrease in the number of eggs laid by the milkweed bug Oncopeltus fasciatus Dallas (Turner 1960) and also in Ae. aegypti and Anopheles stephensi Liston (Rutledge and Piper 1984). In the cecropia moth, Hyalophora cecropia Linnaeus, 41% of the eggs hatched in inbred lines in comparison to 91% in outbred lines (Waldbauer and Sternburg 1979). A reduction in female longevity occurred in seven of eight inbred lines of An. stephensi and Ae. aegypti. In addition there was a shift in sex ratio towards a female bias and the duration of the egg developmental period was increased in sibmated lines (Rutledge and Piper 1984).

However, evidence of the effects of inbreeding is variable because on some occasions inbreeding depression has not been noted. Legner (1979) showed that inbreeding did not affect the rate of population increase in two pteromalid parasitoids Muscidifurax raptor Girault and Muscidifurax zaraptor Kogan and Legner. Neither was there an inbreeding depression in the fitness component mating success which, in inbred lines, ranged from 90 to 100%. In the moth H. cecropia there was no decrease in the number of eggs laid (Waldbauer and Sternburg 1979). Similarly, there was no change in the wing length, and larval and pupal developmental periods in sibmated strains of An. stephensi and Ae. aegypti (Rutledge and Piper 1984).

(5.3) Certain results of the fitness component analyses in the present study on M. pulchellalis require further explanation. (a) Although no inbreeding depression was noted in any component, life table analysis showed that the reduction in egg viability was a consequence of inbreeding. (b) All three treatments, OUT 1, IN 1 and KR 1 showed a reduction in egg viability in the F_2 and F_7. (c) There was an inbreeding depression of egg viability yet in IN 1 the reduction was not correlated with respect to the coefficients of inbreeding.

(a) The reason no inbreeding depression was observed in any fitness component may be related to the experimental techniques employed in the present study. Justification for the methods used is given in Chapter 1. However, as it turned out, a very small inbreeding depression of the fitness components was not demonstrable because of the relatively small numbers of animals which were able to be kept. The alternate method, life table analysis, used to measure inbreeding depression was sensitive to small increments and, clearly, demonstrated an inbreeding effect on egg viability. A cumulation of small differences in egg viability apparently caused an overall effect on population fitness. There was an exponential increase in the ratio of numbers of offspring between OUT 1 and IN 1 (Fig 37), indicating the decrease of population fitness through sibmating in IN 1 and also the importance of egg viability.

(b) The limited size of the populations is also important as regards the simultaneous reduction in egg viability in OUT 1, IN 1 and KR 1. Falconer (1981) describes a large population in terms of hundreds and a small population in tens. Both the size of a population and the system of mating influence the intensity of inbreeding. Consequently, in a small population the chances of mating with a close relative are increased. Thus, in this study on M. pulchellalis the control (OUT 1 and 2) populations were also, inevitably, relatively inbred. The repercussion of this was twofold. Firstly, the simultaneous reductions in egg viability can be ascribed to each treatment being inbred. Secondly, only some fitness components, namely, number of eggs laid, egg viability, egg hatch, larval penetration and survival, and pupal

survival could be used in life table analysis, allowing detection of a possible inbreeding depression. Other components such as female egg and male egg developmental periods, female larval to adult and male larval to adult developmental periods, virgin female and mated female longevity, wing length and sex ratio may have been affected by inbreeding, but because as non-mortality factors they could not be used in life table analyses the inbreeding depression was not detected.

(c) The non-linear inbreeding depression in relation to the coefficients of inbreeding may be ascribed to the effects of selection. In addition to contributing to the decline of population fitness, the reduction of egg viability through mated females laying no viable eggs led to a catastrophic loss of lines in IN 1 that is not reflected in the analysis. Twelve of the original thirteen lines were lost (Appendix 2). Since the original objective of the present research had not been to monitor the extinction of lines the number lost in the randomly-mated OUT 1 and KR 1 was unobtainable. In IN 1 three lines were lost in the F_2, one in the F_6 and two in the F_7. In addition, two lines were lost through parasitism and three because of the asynchronous emergence of the adults.

The variability in the way that different lines responded to inbreeding is related to the severity with which and rate at which deleterious traits are expressed. This in turn depends on the characteristics of the genes, the individual and the component (Wright 1977). Perhaps those lines lost through a reduction in egg viability had been the most affected by inbreeding. Randomly chosen sublines, in which the expression of those alleles which were deleterious for egg viability were not as serious as in those lines lost, were possibly used for their replacement. Eventually, the IN 1 population consisted of artificially selected sublines all of which stemmed from the same original line. Consequently, this population was not entirely representative of the base population. This contributed to the small residual inbreeding effects. A true reflection of an inbreeding depression can only be seen in the early generations of the investigation. This increases the likelihood of the reduction in egg

viability in the F_2 being attributable to inbreeding.

The characteristics of genes such as their dominance, frequency and interaction also influences the chance of survival of an inbred line. In spite of the disadvantages of inbreeding, one line of M. pulchellalis survived the rigours of sibmating in IN 1. Remarkably, in this F_9 generation, 96% of the eggs were viable in lines which all originated from the same line (Appendix 2). Thus the genetic constitution of this line permitted its survival. Adaptability of this sort can lead to the establishment of a species from one mated female (Oliver 1981) if isolated.

Briefly, in terms of the treatment as a whole, selection has opposed the effects of inbreeding by acting against the disadvantageous homozygotes, causing a loss of lines, and favouring the heterozygote or fitter homozygote in the surviving lines. This delays the rate at which all the individuals in a line eventually become homozygous for the same allele, and thereby reduces the amount of inbreeding depression.

The different responses of the duplicate treatments require further explanation. For instance, OUT 1 and IN 1 exhibited a reduction in egg viability in the F_2 whereas OUT 2 and IN 2 did not. Further, both OUT 1 and IN 1 possessed a greater number of individuals, a faster rate of growth and smaller generation mortality than OUT 2 and IN 2 in complementary generations. In addition KR 1 and 2 differed from each other in population fitness. These differences, at least in part, may be attributed to the genetic origin of the base populations.

In M. pulchellalis the OUT 1 and IN 1 base populations consisted of the progeny of Rhodes University laboratory stock crossed with Uitenhage Weeds laboratory stock (Chapter 2). These two cultures had been separate for approximately five years, therefore may have diverged considerably. The progeny resulting from such a cross would be likely to show hybrid vigour thus restoring the fitness and genetic variability lost through inbreeding (Falconer 1981). In contrast, the

base populations of OUT 1 and 2 originated from Rhodes University laboratory stock which had not been mixed with Uitenhage Weeds Laboratory stock. Consequently, this stock may already have adjusted to a degree of inbreeding.

If the reduction in egg viability was due to inbreeding the reason OUT 2 and IN 2 were not affected in the F_2 would be because individuals in these two populations were adapted to inbreeding. The originally low genetic variability of OUT 2 and IN 2 could be the reason for the difference in population fitness in comparison to OUT 1 and IN 1. McCauley and Wade (1984) using small populations of T. castaneum showed that for every 10% decrease in heterozygosity a measurable change in the mean rate of population growth occurred. The founder populations of KR 1 and 2 each consisted of randomly collected individuals which could account for their differences in population fitness. Similar differences, existing in other species of insects, have been ascribed to the origin of the founder colony. For example, differences existing between inbred strains of Tribolium confusum du Val and T. castaneum have been attributed both to inbreeding and to the genetic constitution of the founder populations (Crenshaw and Lerner 1964). Founder effects were also responsible for the differences between inbred and outbred treatments of M. zaraptor and M. raptor rather than inbreeding (Legner 1979).

Artificial laboratory conditions may have also contributed to the differences between treatments in population fitness. In comparison to the control populations (OUT 1 and 2), the field-introduced populations (KR 1 and 2) showed a greater decrease in population fitness. This suggests that immediately the base population of KR 1 was introduced to the insectary, selection operated against those individuals not suited to the laboratory conditions. As a consequence, KR 1 had a smaller effective population size, an increased likelihood of inbreeding and decreased genetic variability, which together may have caused an initial depreciation in population fitness compared to OUT 1. Furthermore, the difference in population fitness between OUT 1 and KR 1 did not increase exponentially with respect to time while that

between OUT 1 and IN 1 did. This may be because individuals in the field-introduced population eventually adapted to laboratory conditions and like OUT 1, the chances of mating with related individuals in this population was reduced.

(5.4) Knowledge derived from this investigation has relevance not only to the loss of the laboratory-reared cactophagous Lepidopterans but also to future laboratory-rearing of other biological control agents.

Cultures of A. leithella, C. mundelli, Nanaia sp., Sigelgaita sp. and S. transilis were lost in the F_2 generation after being introduced in small consignments to a laboratory. In M. pulchellalis a reduction in egg viability resulting from mated females producing no viable eggs also occurred in the F_2. The evidence from the population modelling strongly suggests that this phenomenon was a consequence of inbreeding. In addition it was also noticed that the reduction in egg viability in the sibmated IN 1 population had a catastrophic effect because it caused the loss of several lines. From this evidence, it seems probable that the effect would be even more extreme on a wild, non-inbred, non-laboratory adapted founder population.

The ability of M. pulchellalis to withstand sibmating, while the other cactophagous species were lost under less intense inbreeding may, perhaps, be related to its naturally occurring degree of inbreeding in its wild state. This may have arisen because of the originally small area of cactus (approximately $30m^2$, Moran pers. comm.) from which the one hundred and twenty eight larvae of M. pulchellalis were collected in Parana, Argentina. Oliver (1981), suggests that the smaller the area, the smaller the genetic load (the proportion of deleterious genes) of individuals in the population, owing to naturally occurring inbreeding. This, however, is purely speculative at present. Whatever way it has arisen, the genetic constitution under the influence of selection is important in determining the effects of inbreeding and the eventual success of the laboratory-rearing of biological control insects.

In the laboratory the successful performance of an insect is usually gauged by the number of offspring produced. This is important because one function of laboratory-rearing is the mass-release of the biological control agent into the field. An exponential decrease in the numbers of offspring of sibmated IN 1, in M. pulchellalis, resulted from the cumulation of the effects of reduced egg viability over nine generations. The amount of genetic variability inherent in the founder population is also considered to be important in affecting population fitness. Thus, in these circumstances, the efficacy of laboratory-rearing is lessened. In order to improve the success of laboratory-rearing, the chances of inbreeding must be reduced, genetic variability preserved and selection against individuals under artificial conditions minimized.

Consignments of biological control agents usually consist of a relatively small number of individuals because of the practical considerations mentioned in Chapter 1. Such populations are then likely to be subjected to some degree of inbreeding but the extent of inbreeding will, of course, depend upon the effective population size and the mating regime. However, the importance of inbreeding to the success of laboratory-rearing biological control insects is contentious (Flanders and Bay 1964, Mackauer 1981, Moran and Zimmermann 1984). This has arisen primarily because some biological control agents have been successfully established from a few founder individuals. For example, the biological control of Opuntia tuna (Linnaeus) Miller in Mauritius using Dactylopius opuntiae Cockerell was initiated with one founder female (Moutia and Mamet 1945). In South Africa Dactylopius austrinus De Lotto on O. aurantiaca originated from a few individuals (Moran and Annecke 1979), subsequently reported to be five (Moran and Zimmermann 1984). In addition Mackauer (1972) has listed three examples of successful insect colonization which all originated from less than twenty founder individuals. Thus the extent to which inbreeding affects each species is very variable.

As mentioned previously, the susceptibility of biological control agents to inbreeding depends upon the amount of naturally occurring

inbreeding and also the adaptability of the insect. Until more is known about the genetic nature and adaptive diversity of each species (Unruh et al. 1983) the number of individuals that ought to be collected, to ensure successful colonization, remains speculative. Mackauer (1981) suggested approximately two to five hundred individuals are required. In practice, though, as large a sample size as possible should be collected to decrease inbreeding, avoid genetic drift and increase the chances of obtaining sufficient genetic variability (Legner 1979, Mackauer 1972, 1976, 1981, Unruh et al. 1983).

Before being exported from the country of origin, the biological control agents should be reared in the laboratory until the F_2 generation, if possible. This would avoid wasting time and the expense of shipment without knowing if the particular insect is likely to suffer a catastrophic inbreeding depression. In addition, if an inbreeding depression did occur and some individuals survived, then effectively the fitter ones would be imported for biological control purposes.

Within the laboratory the following method could be implemented to lessen the possible inbreeding depression in the F_2, thus minimizing the chances of a catastrophic loss of lines or a decrease in population fitness. The mechanism requires the division of the imported stock into two separate cultures, named for example "Culture A" and "Culture B". The resulting F_1 progeny of Culture A would be randomly crossed with those of Culture B in order to prevent an inbreeding depression of each culture. To maintain two separate cultures it is suggested that the females of Culture A be mated with the males of Culture B while the males of Culture A be mated with the females of Culture B. Employment of this process, at intervals, throughout the existence of the colony would decrease the chances of inbreeding while, at the same time, preserving as much genetic variability as possible.

For successful laboratory-rearing and the eventual success of the biological control agent in the field, the laboratory conditions must simulate those of the natural environment as far as possible, which has

been previously suggested by Mackauer (1981). Dusk, dawn and seasonal changes could be implemented within the laboratory. These could be important in terms of increasing productivity as M. pulchellalis, for example, is crepuscular (Nieman 1983). Food is also an important consideration as shown by Guthrie and Carter (1972). Larvae of the European corn borer, Ostrinia nubilalis Hübner, were fed on a meridic diet for several generations and eventually larvae failed to eat a susceptible breed of corn. It is suggested that the natural host plant of the biological control agent is used for rearing purposes, as and when it is feasible.

Close vigilance, throughout the culturing process, should be kept on the performance of the insects reared. There are several ways in which this can be undertaken. The performance of the laboratory insects could be compared with that of wild insects using genetic markers with the principle of the "competitive index". The genetic markers enable the wild-type heterozygote to be distinguished from the deleterious, recessive homozygote because the two complements differ from each other phenotypically (see Latter and Robertson 1962, for details). Theoretically, gel electrophoresis could also be used to monitor any changes in genetic variation (Bush 1975) that could have resulted from inbreeding. Huettel (1976) has listed ways in which those components of fitness important for the survival of insects can be measured, to ascertain the insect quality. Similarly, Chambers (1975, 1977) has discussed tests which can be employed for the same purpose of quality analysis.

Hopefully, with the implementation of such procedures, more cactophagous Lepidoptera will be successfully reared in the laboratory and released to the field. Eventually, by increasing the numbers of species of insects and pathogens effectively attacking the plant, a point may be reached where chemical control might be abandoned.

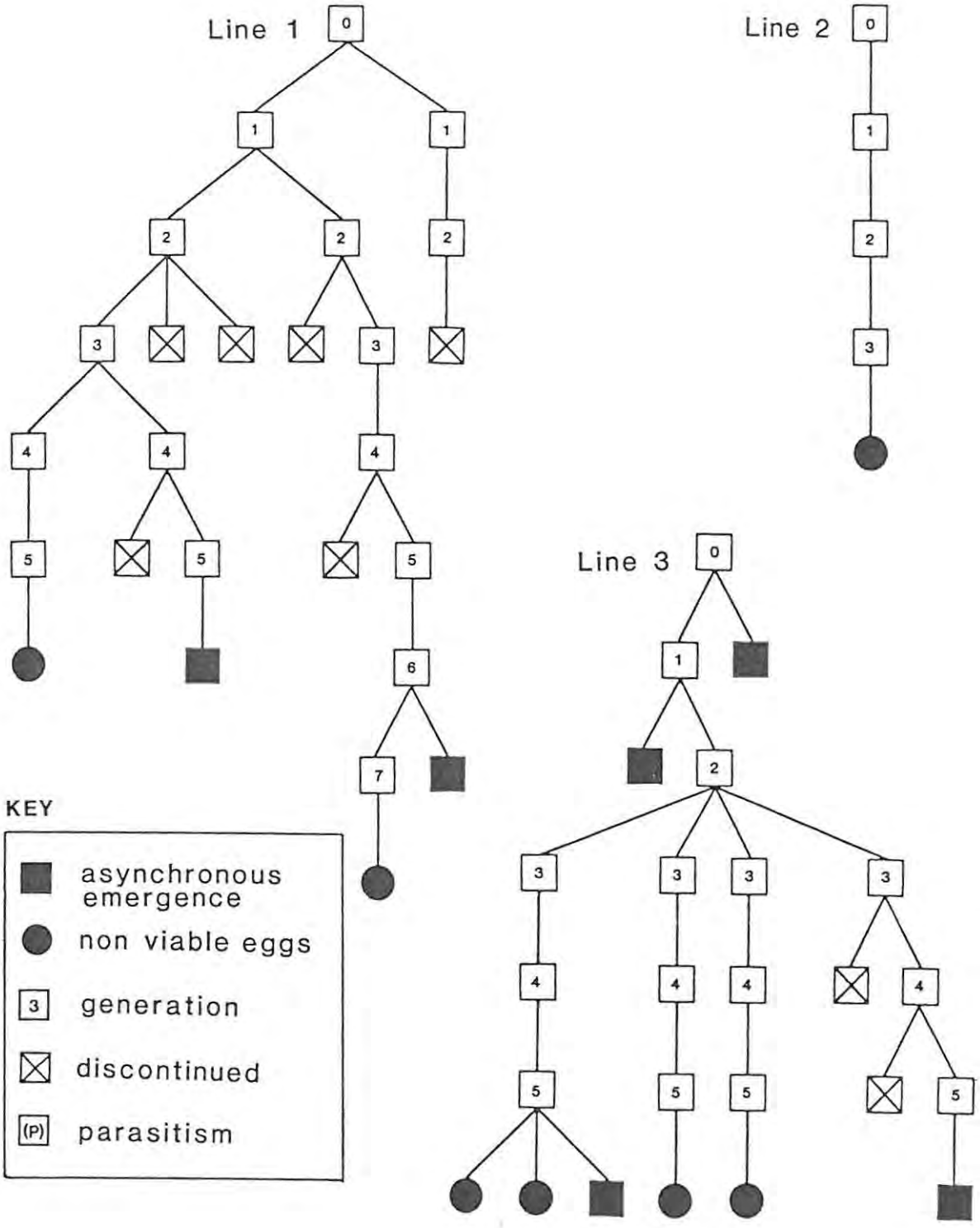
APPENDIX I

The five species, A. leithella, C. mundelli, Nanaia sp. Sigelgaita sp. and S. transilis, which were unsuccessfully reared in the laboratory. The place and country of origin, the dates on which consignments of larvae or pupae were imported, the number of adults which emerged and the generation in which the population declined or was lost are given.

Species	Collection	Date	No imported		No of adults	Generation	
			larvae	pupae	emerged	declined	lost
A. leithella	Pescadero COLUMBIA	5.10.82	74	0	no record		F_2
	Caraca VENEZUELA	5.10.82	52	0	no record		F_2
C. mundelli	Cochabamba BOLIVIA	26.6.78	31	0	31		F_2
	Parana ARGENTINA	12.6.78	12	0	no record		F_2
Nanaia sp.	Limatambo PERU	6.7.78	164	0	no record		F_2
	"	23.9.81	81	30	57	F_2	F_3
	"	11.7.82	145	0	64		F_2
	"	19.1.84	40	0	32		F_2
	"	24.4.84	16	0	16		F_2
Sigelgaita sp.	Chilete PERU	1.5.84	13	0	3		F_2
S. transilis	Huancambamba PERU	14.4.81	56	0	7		F_2
	"	1.5.84	21	14	no record		F_1
	Chicamoch COLUMBIA	1.5.81	74	14	no record		F_1

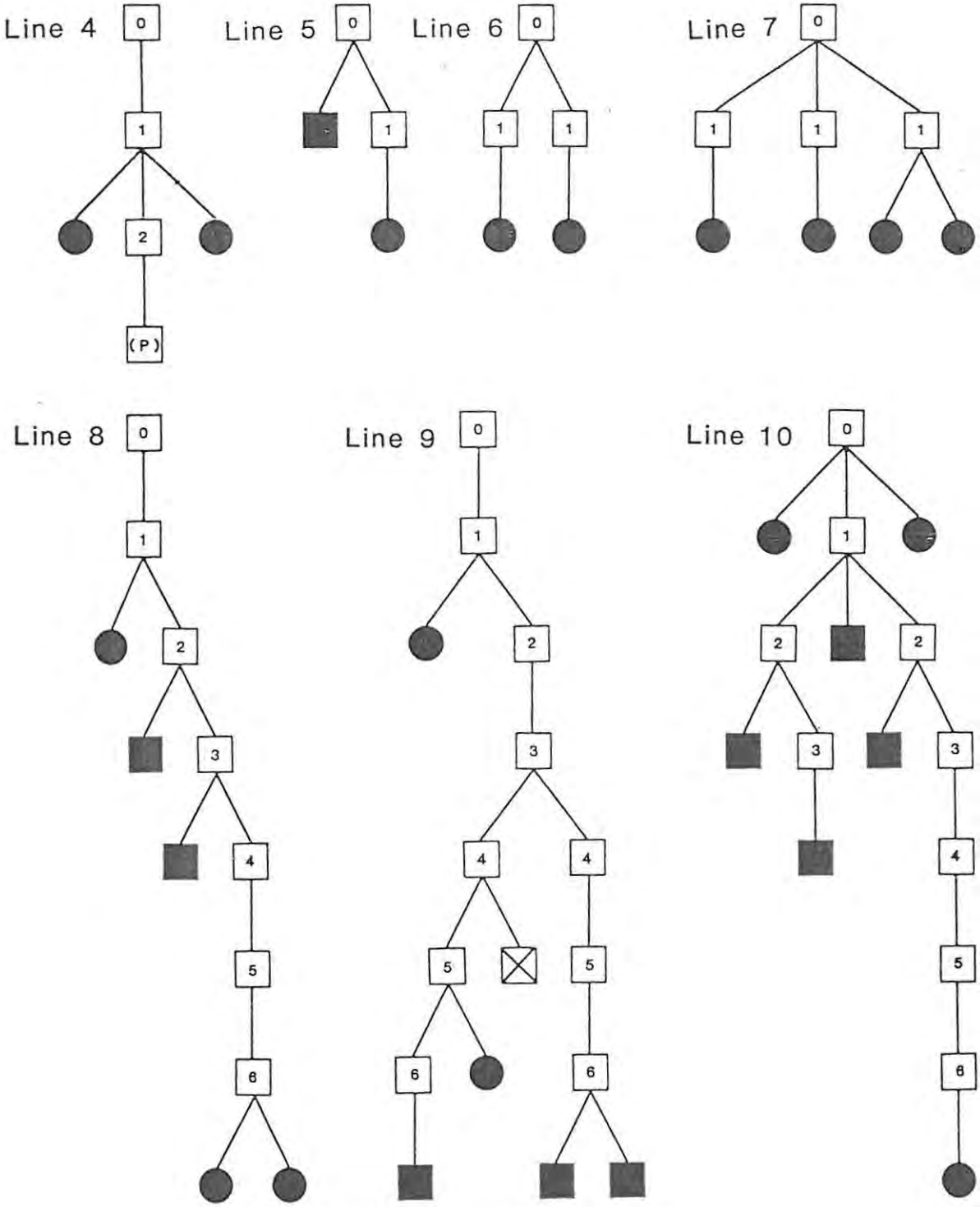
APPENDIX 2

The original thirteen lines of the IN 1 population of M. pulchellalis. The fate of these sibmated lines in each generation is shown.



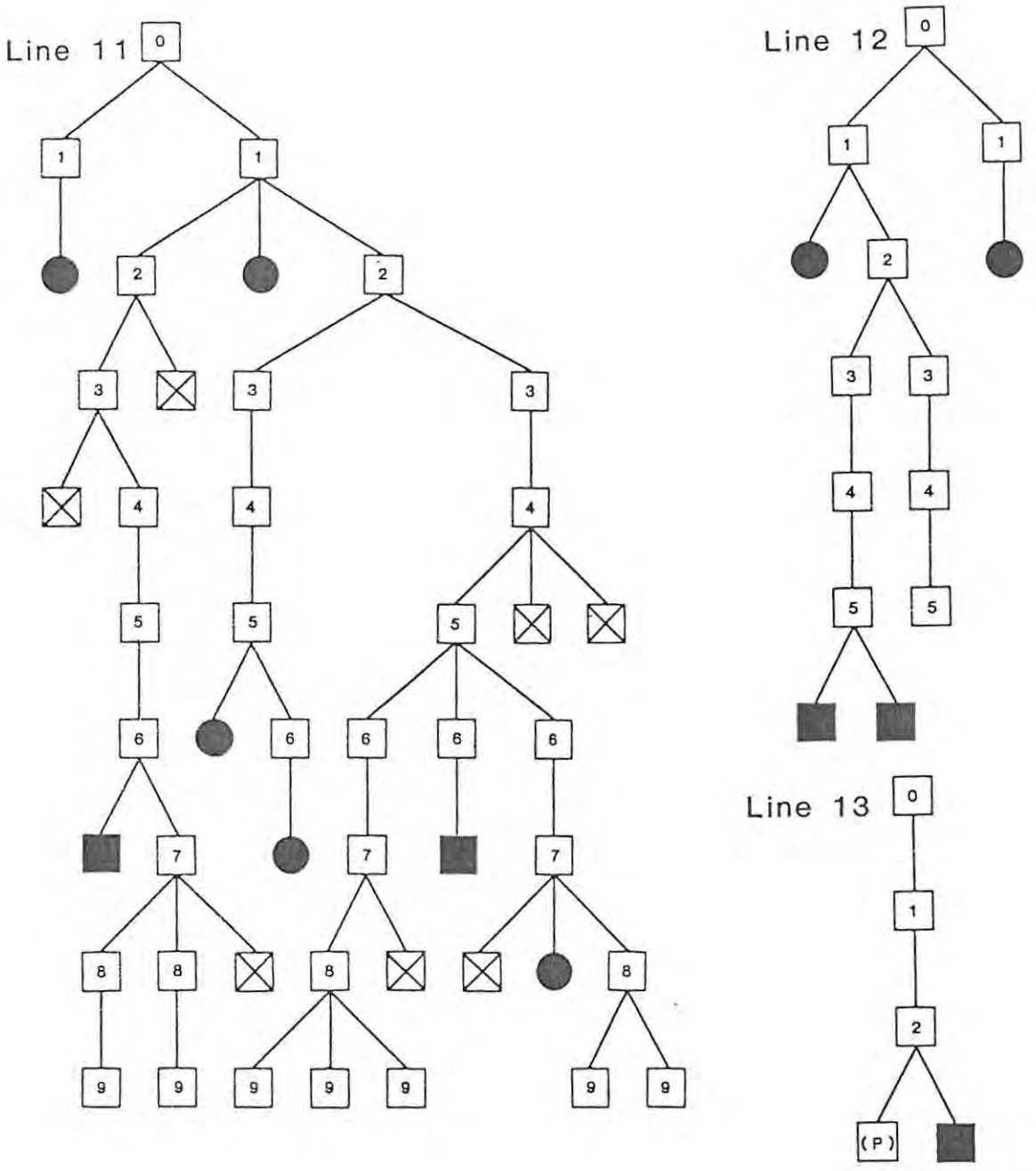
APPENDIX 2 (cont.)

The original thirteen lines of the IN 1 population of M. pulchellalis. The fate of these sibmated lines in each generation is shown.



APPENDIX 2 (cont.)

The original thirteen lines of the IN 1 population of M. pulchellalis. The fate of these sibmated lines in each generation is shown.



REFERENCES

Bush, G. L. 1975. Genetic variation in natural insect populations and its bearing on mass-rearing programs. In: Controlling fruit flies by the sterile insect technique. International Atomic Energy Agency (IAEA-PL-582/2): 9-17.

Bush, G. L. and Neck, R. W. 1976. Ecological genetics of the screwworm fly, Cochliomyia hominivorax (Diptera: Calliphoridae) and its bearing on the quality of mass-reared insects. Environmental Entomology, 5: 821-826.

Chambers, D. L. 1975. Quality in mass-produced insects. In: Controlling fruit flies by the sterile insect technique. International Atomic Energy Agency (IAEA -PL-582/2) 19-32.

Chambers, D. L. 1977. Quality control in mass rearing. Annual Review of Entomology, 22: 289-308.

Chapman, R. F. 1971. The Insects structure and function. Hodder and Stoughton, London.

Crenshaw, J. W. and Lerner, I. M. 1964. Productivity of inbred strains of Tribolium confusum and Tribolium castaneum. Ecology, 45: 697-705.

Dodd, A.P. 1940. Biological campaign against prickly-pear. Commonwealth Prickly Pear Board Bulletin, Brisbane.

Engelmann, F. 1970. The physiology of insect reproduction. Pergamon Press, Oxford.

Falconer, D. S. 1981. Introduction to quantitative genetics. Second Edition. Oliver and Boyd, London.

Fergusson-Laguna, A. and Machado-Allison, C. E. 1979. Effecto de la endogamia en sucesivas generaciones de cruces hermana-hermano en Aedes aegypti (L). Acta Cientifica Venezolana, 30: 507-510.

Ferro, D. N. and Akre, R. D. 1975. Reproductive morphology and mechanics of the mating of the codling moth, Laspeyresia pomonella. Annals of the Entomological Society of America, 68: 417-423.

Flanders, S. E. and Bay, E. C. 1964. Standardization of mass rearing procedures for entomophaga. Bulletin of the World Health Organization, 31: 505-507

Hoffmann, J. H. 1982. Evaluation of Tucumania tapiacola Dyar (Lepidoptera: Phycitidae) for biological control of jointed cactus in South Africa. Unpublished Ph.D. thesis, Rhodes University, Grahamstown.

Guthrie, W. D. and Carter, S. W. 1972. Backcrossing to increase survival of larvae of a culture of the European corn borer on field corn. Annals of the Entomological Society of America, 65: 108-109.

Huettel, M. D. 1976. Monitoring the quality of laboratory-reared insects: A biological and behavioural perspective. Environmental Entomology, 5: 807-814.

Jones, D. 1984. Use, misuse, and role of multiple-comparison procedures in ecological and agricultural entomology. Environmental Entomology, 13: 635-649.

Latter, B. D. H. and Robertson, A. 1962. The effects of inbreeding and artificial selection on reproductive fitness. Genetic Research, 3: 110-138.

Legner, E. F. 1979. Prolonged culture and inbreeding effects on reproductive rates of two pteromalid parasites of muscoid flies. Annals of the Entomological Society of America, 72: 114-120.

MacDonald, A. C. 1892. Quoted in Fisher 1892. New cactus (prickly pear). Agricultural Journal of the Cape of Good Hope, 5: 93-94.

Mackauer, M. Genetic aspects of insect production. Entomophaga, 17: 27-48.

Mackauer, M. 1976. Genetic problems in the production of biological control agents. Annual Review of Entomology, 21: 369-385.

Mackauer, M. 1981. Some aspects of quality and quality control of biological control agents during insectary propagation. Proceedings of the Fifth International Symposium on Biological Control of Weeds, Brisbane, Australia, 1980: 207-220.

McCauley, D. E. and Wade, M. J. 1981. The populational effects of inbreeding in Tribolium. Heredity, 46: 59-67.

Maynard Smith, J. 1956. Fertility, mating behaviour and sexual selection in Drosophila subobscura. Journal of Genetics, 54: 261-279.

Moran, V. C. and Annecke, D. P. 1979. Critical reviews of biological pest control in South Africa. 3. The jointed cactus, Opuntia aurantiaca Lindley. Journal of the Entomological Society of southern Africa, 42: 299-329.

Moran, V. C., Zimmermann, H. G. and Annecke, D. P. 1976. The identity and distribution of Opuntia aurantiaca Lindley. Taxon, 25: 281-287.

Moran, V. C. and Zimmermann, H. G. 1984. The biological control of cactus weeds: achievements and prospects. Biocontrol News and Information, 5: 297-320.

Myers, J. H. and Sabath, M. D. 1981. Genetic and phenotypic variability, genetic variance, and the success of establishment of insect introductions for the biological control of weeds. Proceedings of the Fifth International Symposium on Biological Control of Weeds, Brisbane, Australia, 1980: 91-102.

Moutia, L. A. and Mamet, R. 1945. A review of twenty-five years of economic entomology in the island of Mauritius. Bulletin of Entomological Research, 36: 439-47.

Nie, N. H., Hull, C., Jenkins, J. G., Steinbrenner, K., and Bent, D. H. 1975. Statistical Package for the Social Sciences. McGraw-Hill Book Company, New York.

Nieman, E. 1983. An evaluation of Mimorista pulchellalis (Dyar) (Lepidoptera: Pyraustidae) as a biocontrol agent against jointed cactus in South Africa. Unpublished M.Sc. thesis, Rhodes University, Grahamstown.

Nei, M., Marugama, T., and Chakraborty, R. 1975. The bottleneck effect and genetic variability in populations. Evolution, 29: 1-10.

Oliver, C. G. 1981. A preliminary investigation of inbreeding depression in twelve species of lepidoptera. Journal of the Lepidopterists' Society, 35: 51-60.

Richards, G. I. 1982. On the egg mortalities of Mimorista pulchellalis (Dyar) (Lepidoptera: Pyraustidae) on jointed cactus (Opuntia aurantiaca Lindley). Unpublished report on file, Weeds Laboratory, Uitenhage.

Robertson, H. G. 1985. The ecology of Cactoblastis cactorum (Berg) (Lepidoptera: Phycitidae) in relation to its effectiveness as a biological control agent of prickly pear and jointed cactus in South Africa. Unpublished Ph.D. thesis. Rhodes University, Grahamstown, South Africa.

Robertson, F. W. and Reeve, E. C. R. 1952. Heterozygosity, environmental variation and heterosis. Nature, 170: 286.

Rutledge, L. C. and Piper, G. N. 1984. Inbreeding of Aedes aegypti and Anopheles stephensi. Mosquito News. Journal of the American Mosquito Control Association, 44: 43-50.

Simmonds, F. J. 1964. Mass production of insect parasites and predators. Bulletin of the World Health Organization, 31: 511-512.

Slabber, J. D. 1964. Die doeltreffendheid van litjieskaktus bestryding in die distrikte Fort Beaufort en Viktoria-Oos. Unpublished M.Sc. thesis. University of Pretoria, Pretoria, South Africa.

Snedecor, G. W. and Cochran, W. G. 1980. Statistical methods. Seventh edition. The Iowa State University Press, Ames.

Sokal, R. R. and Rohlf, F. J. 1981. Biometry. Second Edition. W. H. Freeman and Company, San Francisco.

Turner, N. 1960. The effect of inbreeding and crossbreeding on numbers of insects. Annals of the Entomological Society of America, 53: 686-688.

Unruh, T. R., White, W., González, D., Gordh, G., and Luck, R. F. 1983. Heterozygosity and effective size in laboratory populations of Aphidius ervi (Hym.:Aphidiidae). Entomophaga, 28: 245-258.

Varley, G. C., Gradwell, G. R. and Hassell, M. P. 1973. Insect population ecology: an analytical approach. Reprinted 1975. Blackwell Scientific Publications, Oxford.

Waldbauer, G. P. and Sternburg, J. G. 1979. Inbreeding depression and a behavioural mechanism for its avoidance in Hyalophora cecropia. The American Midland Naturalist, 102: 204-208.

Wright, S. 1969. Evolution and the genetics of populations. Volume II. The theory of gene frequencies. The University of Chicago Press, Chicago.

Wright, S. 1977. Evolution and the genetics of populations. Volume III. Experimental results and evolutionary deductions. The University of Chicago Press, Chicago.

Zar, J. H. 1974. Biostatistical analysis. Prentice-Hall International, Incorporated, London.

Zimmermann, H. G. 1981. The ecology and control of Opuntia aurantiaca in South Africa in relation to the cochineal insect Dactylopius austrinus. Unpublished Ph.D. thesis. Rhodes University, Grahamstown, South Africa.

Zimmermann, H. G. and Moran, V. C. 1982. Ecology and management of cactus weeds in South Africa. South African Journal of Science, 78: 314-320.