BIOTYPE VARIATION IN *TRIBOLIUM CASTANEUM* (HERBST.) FROM MALI

Thesis submitted for the degree of
Doctor of Philosophy
at the University of Leicester

by

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September 1993

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To my family

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Professor R H Smith for his guidance, criticism and reading of the manuscript.

Thanks are due to Mr McFarlane for his advice at the initial stage of the work.

I am grateful to the laboratory technicians M Ward and S Ison for their friendly help.

The thesis was efficiently typed by Julie Cope to whom I express my best wishes for her imminent wedding.

I am most grateful to the British Council for the fellowship awarded and to the Natural Resources Institute, both for providing the insecticide resistance test kits for this particular work and also for its useful technical co-operation with the Malian Cereal Marketing Board, OPAM.

Finally, I would like to thank my colleagues of the Stock Protection Division of OPAM for their co-operation while I was working in the OPAM laboratory.

Title: **Biotype variation in Tribolium castaneum (Herbst.) from Mali**

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ABSTRACT

Geographical strains of Tribolium castaneum (Herbst.) (Tenebrionidae) and Rhyzopertha dominica (Fab.) (Bostrychidae) were collected in different geographical locations in Mali and were tested for differences in various biological parameters.

In the preliminary investigations, strains of both species were found to be significantly different in their capacity to damage grain, in their capacity to tolerate pesticides, and in some life-history traits, and these strains were designated as biotypes. There was no evidence of correlation between the fitness of strains and their resistance status, ie no evidence of a 'cost' of resistance. After the preliminary investigations, two biotypes of T. castaneum were retained for the detailed studies.

Differences between strains in their ability to damage grain were found to be related to their productivity but not to their metabolic rate, which was determined by measuring their oxygen consumption. The significant difference in body weight found between strains provided an explanation for the significant difference found in oxygen consumption.

Significant differences between strains were also found in productivity. Using the techniques of quantitative genetics, significant heritability of body weight was found within one strain of Tribolium castaneum. A selection experiment, with two selection regimes based on culture interval, was designed to identify possible genetic trade-offs that might be maintaining genetic variability in components of fitness. Selection using different culture intervals did not affect developmental period, productivity or body weight. There was no evidence of genetic trade-offs between any of these life-history traits.

CONTENTS

Chapter 1 GENERAL INTRODUCTION

Tribolium castaneum (Herbst.) is a cosmopolitan storage pest (Hinton, 1948) found throughout temperate and tropical regions. Hinton (1948) provides a synopsis of the genus Tribolium macleay with some remarks on the evolution of its species groups. There are many publications showing differences between populations of T. castaneum (eg Soliman, 1968; Soliman and Hardin, 1971, 1972; Bradshaw, 1984). T. castaneum is a common pest in Mali where it was first reported by Lesne (1924b). T. castaneum is found all over Mali in all storage conditions (State and private stores, traditional family granaries). As background to the work I will outline the climatic conditions and the food security policy in Mali, as well as the biotype concept.

CLIMATE AND AGRICULTURE IN MALI

The Republic of Mali is a land-locked, sahelian country lying between 10° and 26°N and 12°W and 4°15'E (ICRISAT, 1984). Mali has a warm environment that is modified seasonally by changes in water regimes. The hottest parts in Mali are in the drier north where the cloudless sky allows the direct rays of sun to reach the ground unimpeded and where the daily maximum air temperatures in the shade exceed 38°C during the hottest months (Reuben, 1978). Maps 1 and 2 show respectively the different locations (cities) where insects were collected and the rainfall zones in Mali. The annual rainfall is below 250mm in the north (eg Gao) and is above 1400mm in the south (eg Sikasso, Zangasso). As a consequence of the climatic conditions, the northern regions are zones of agricultural deficit and the southern regions are areas of agricultural surplus. Mali was hit by a severe drought which began in the early 1970s and caused frequent food shortage. For example the total production of coarse grain (sorghum and millet) in 1979 in Mali was 461000 MT (FAO, 1992) which is very low compared to a production of 1 million MT in any normal year. For the food security of the country, policies are made which have to be implemented by the National Cereal marketing Board

(Office des Produits Agricoles du Mali; OPAM). Under the current food security policy the tasks of OPAM are:

- management of security stocks;
- management of food aids;
- supply of deficit areas.

FOOD SECURITY POLICY IN MALI

The security stocks (Stock National de Securite) consist of 52500T of grain (mainly millet and sorghum) bought locally when the national production is sufficient, or imported when the national production is not sufficient, in which case maize can also be used. Security stocks are stored in security stores located in different parts of the country. It is from these stocks or from food aids donated by various donors that grain is supplied to the deficit regions of the north.

Storage pest control was introduced to OPAM in the late 1970s under the technical assistance programme of the British Government. Advisors from the Tropical Products Institute (TPI), now the Natural Resources Institute (NRI), introduced chemical control of pests with spraying of contact insecticides and fumigation with aluminium phosphate . Details of the chemicals used are given in the chapter dealing with resistance to insecticides.

EVALUATION OF BIOTYPES

The International Dictionary of Medicine and Biology defines biotypes as a group of organisms that are genetically identical or very nearly so. For Saxena and Barrion (1987) the term biotype is an intraspecific category referring to insect populations of similar genetic composition for biological attributes. The same authors state that biotypes are usually identified based on host and food preference, behaviour, genetic composition and variation, physiological responses to physical changes and stresses in the environment (light, temperature, relative humidity, insecticides and host plants). Based on that statement, populations of

the same species (intraspecific categories) living in contrasting or different climatic conditions could be expected to be different.

The conditions described above (climatic conditions together with food security and pest management policy) are characterized by:

- contrasting climatic conditions in different parts of Mali;
- transfer of grain stocks from surplus areas (cooler regions in the south) to deficit areas (hotter regions in the north), resulting in frequent introduction of insect strains always from production regions to deficit regions;
- possible introduction of new insect strains from countries of origin of grain imported or donated as food aid;
- use of the same chemicals over long periods of time, leading to the risk of insects developing resistance to the pesticides used.

In the conditions described above and as suggested by the biotype concept, could strains of a given insect species of the southern regions be expected to be different from strains of the same species of the northern regions?

PROPOSED RESEARCH

The present work was carried out to bring some answer to the above question. The research was originally based at the University of Reading, with fieldwork carried out in Mali. The intention was to achieve some of the following:

- biotype characterisation and fitness estimation;
- estimation of genetical and environmental correlations between life-history characters;
- estimation of direct and correlated responses to selection eg for fast/slow development;
- relationship of insecticide resistance to biotype characteristics, and possible genetic linkage;
- prediction of effect of insect migration on biotypes and fitness;

- development of model of pests in stored grain, based on areas in Mali which are good producers (always export) or poor producers (always import).

The programme was initially divided into three periods.

a) Initial period of three months (October to December 1990) at Reading University to:

- gain familiarity with laboratory techniques;
- attend appropriate courses: statistics and population biology;
- review the literature.

b) Second period of 21 months (January 1991 to September 1992) in Mali to:

- collect strains from different parts of the country;
- carry out weight loss experiment to identify major differences in the abilities of different strains to damage grain;
- examine the life-history of three or four strains (including resistance status);
- carry out a transplant experiment whereby some strains collected at different latitudes would be compared in relation to fitness at the sites of collection;
- do some quantitative genetics and selection experiments.

c) Final period of 12 months (October 1992 to September 1993) at the University of Reading to complete the work using controlled environment and computing facilities available.

Two species, Tribolium castaneum (Herbst.) and Rhyzopertha dominica (Fabricius) were to be used in a preliminary investigation of weight loss, resistance and life-history. Weight loss in large culture was chosen as a rapid means of identifying major differences in ability to damage grain (McFarlane et al., 1990). These aims and approaches are summarized in the minutes of a meeting held at Reading on 15 October 1990 (Appendix 1).

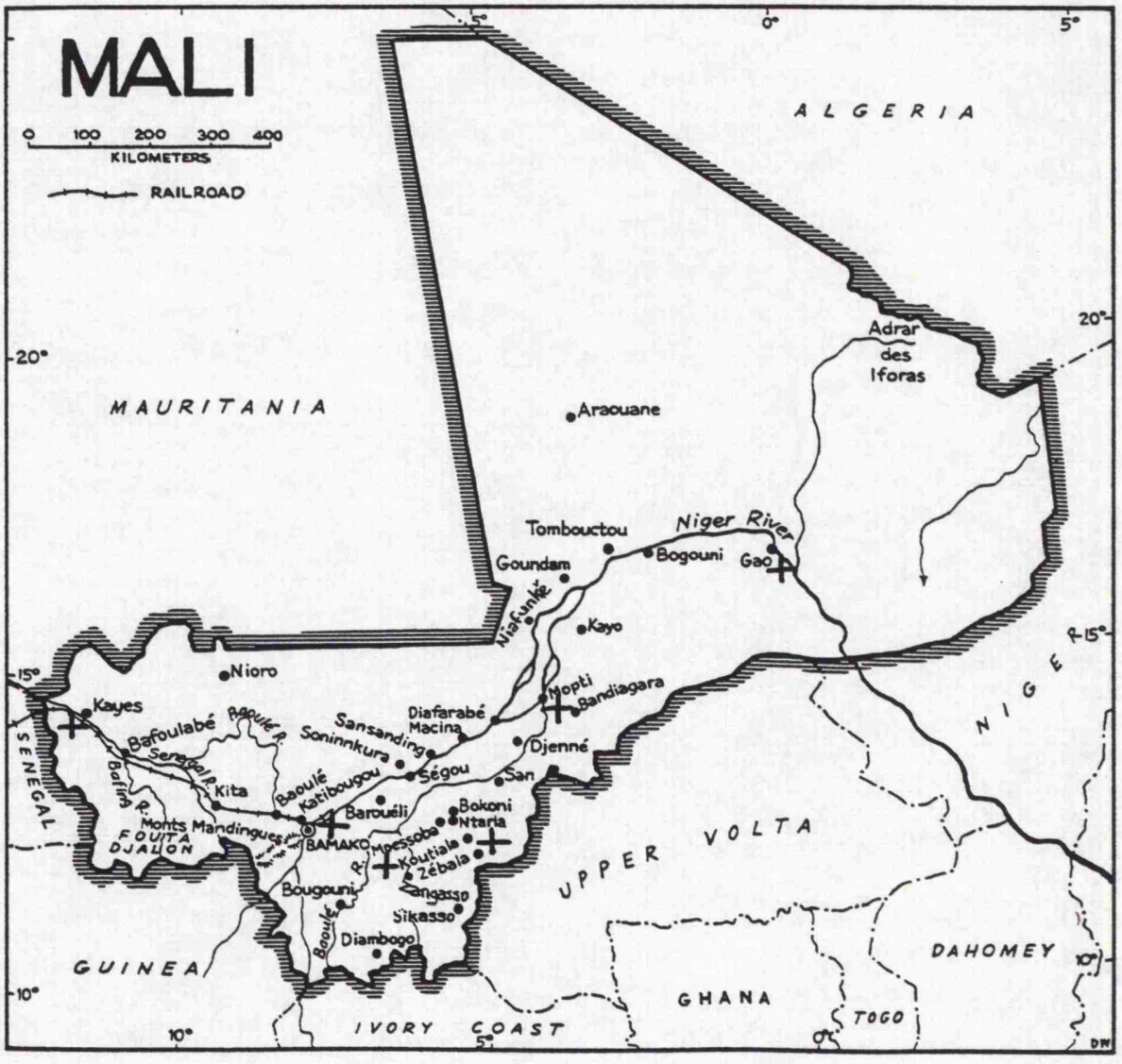
ACTUAL PROGRAMME

The original programme had to be modified substantially for two reasons.

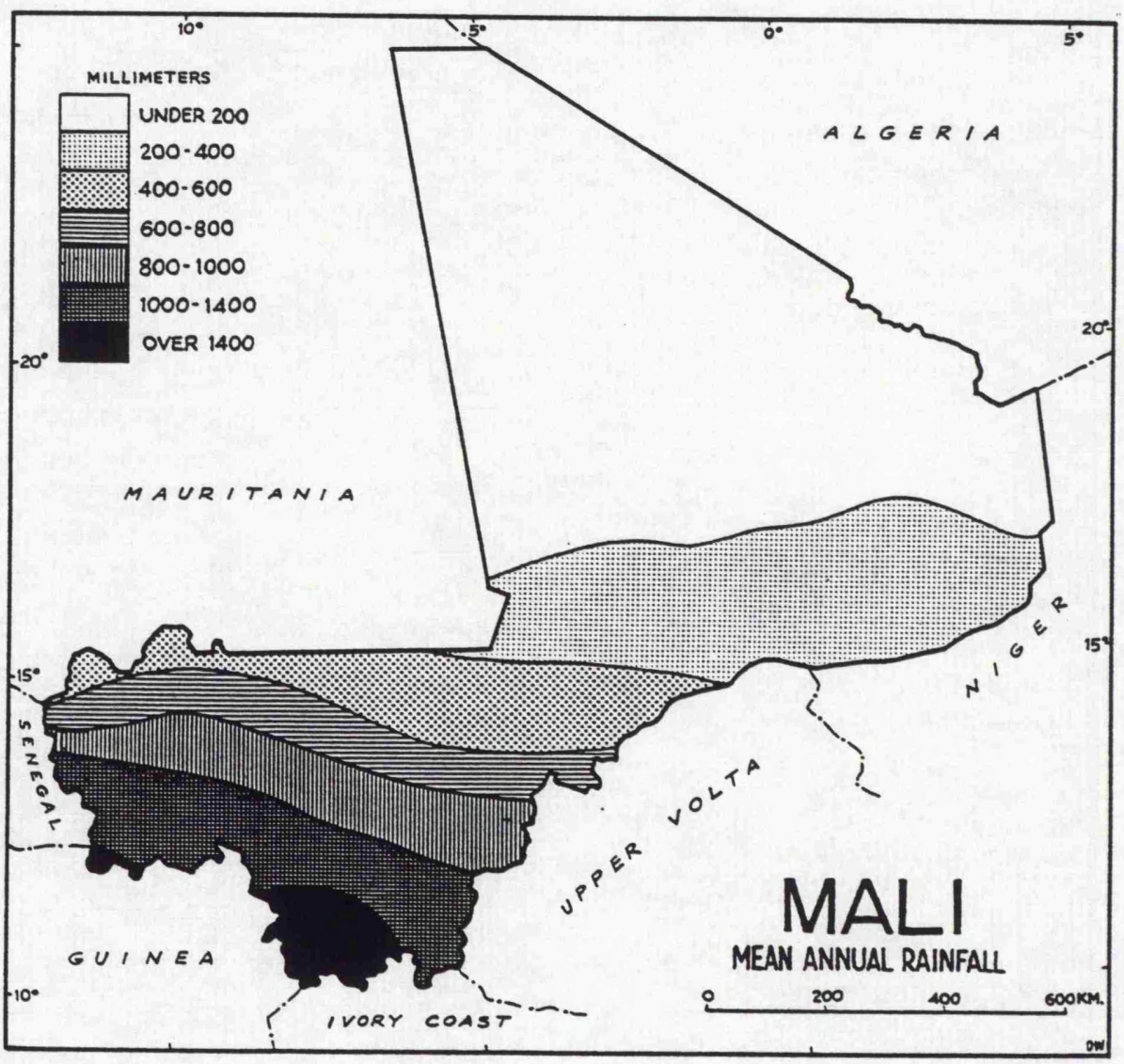
Firstly, there was a period of political instability and rioting in Mali which led to a Coup d'Etat in March 1991. It was not possible to make trips to collect insects during the period of instability. OPAM stores were attacked by demonstrators in many places, and more than 3000 tonnes of grain was substracted from them. I was involved in the supervision of the urgent programme launched to purchase local and imported grain. When some stability was restored it was still dangerous to travel north even as far as Timbuctu because of the continuing attacks of rebel Touareg in the north. Thus the transplant experiment and further collection of insect strains became impossible.

Secondly, laboratory facilities at OPAM were very limited and there was no financial support. Thus no more equipment or materials could be obtained after the shipment of glassware and plasticware from Reading in January 1991 and the Resistance Test Kits from Natural Resources Institute (Chatham). In particular, an outbreak of predatory mites in several insect cultures could not be eradicated because acaricide for egg-washing was not available in time. Many insect strains were lost and could not be replaced. Therefore it was necessary to modify the original programme to take account of materials available, and the research programme was revised in discussion with Professor R H Smith during the supervisory visit that he made in January 1992. Following the appointment of Professor Smith to a new chair at Leicester, my registration was transferred to the University of Leicester when I returned to the UK in October 1992.

Map 1: Showing the locations (+) where strains were collected (from May 1968)



Map 2: Showing the mean annual rainfall in the different parts of Mali (from May 1968)



Chapter 2 GENERAL MATERIALS AND METHODS

2.1 THE GRAIN

Sorghum flour was used in all experiments. The specific name is Sorghum bicolor (Linn.). In English publications sorghum is sometimes referred to as great millet. Grown in the tropics and subtropics, sorghum is known in West Africa as guinea-corn. The origin and the classification of sorghum are well documented (eg Garber, 1950; Mauny, 1953; Clayton, 1961; Doggett, 1970). Sorghum and millet constitute the main staple foods in Mali and many other countries in Africa and Asia. Sorghum is fourth in importance among the world's cereals (Doggett, 1970). In 1991 the world production in sorghum was 54,949,000 MT (FAO, 1992).

Sorghum plant is a typical grass varying greatly in height (45cm to more than 2 metres). The grain varies considerably in size and colour. The varieties present in Mali are designated by local names: eg kendé, binbiri, gnarima (Touré, 1975).

Chemical analysis of sorghum grain resembles that of maize (Doggett, 1970). The differences between varieties of sorghum are so great that authors usually give a general picture of the chemical composition. Bredon (1961; cited in Doggett, 1970), for example, supplied data concerning 31 local Uganda sorghums; the average results were as follows:

Dry Matter	Crude Protein	Crude Fat	Crude Fibre	Total Ash
90.1%	10.1%	2.8%	2.5%	2.6%

Sorghum grain also contains vitamins and minerals.

Sorghum grain has several uses as food, in industry and as stockfeed. The use of sorghum as stockfeed was the reason for its development in the USA where its value as human food has been ignored, although large amounts are used as food

by people of many countries (Doggett, 1970). In the food industry, sorghum grain is, for example, used for the production of starch.

In Mali sorghum grain is used basically as food. Several dishes are made with sorghum flour. A local beer called dolo in the bamanan language is also made with sorghum. In 1991 the total production of coarse grain (sorghum and millet) in Mali was 792,000 MT (FAO, 1992).

For the experiments described here, about 100kg of sorghum from the 1990 campaign was purchased on the market in Bamako (Mali, capital city). The grain was passed through sieves of different sizes (1.5-5mm) to remove grass, fine foreign matters and immature grains. A fumigation was then carried out in a 200l plastic drum with a 3g Detia tablet for 7 days. This fumigation eliminated a light infestation of Rhyzopertha dominica. Prior to each use the fumigated grain was sieved again to remove any dead insects.

The moisture content was measured using the oven method according to ISO's (International Standards Organization) recommendations. Ground samples were exposed to 130°C for 1 hour 30 minutes. The moisture content was found to be 7.20%.

Whole grain was used for R. dominica and flour was used for T. castaneum .To obtain flour the grain was ground using a local mill. Finally, the flour was mixed with dry yeast at the ratio of 12 parts of flour and 1 part of yeast (Dobie et al., 1991).

Before use, the required amount of diet was kept in the cooled incubator with an accuracy of 1°C at about -10°C for at least 3 days to kill any possible infestation and equilibrated to experimental conditions for 3 days. The grain to be used in the UK was brought to Reading by Professor Smith following his supervisory visit in February 1992. It was stored in a freezer until required for use.

2.2 THE INSECTS

The insect species studied were Tribolium castaneum (Herbst.) and Rhyzopertha dominica (Fab.).

2.2.1 Tribolium castaneum (Herbst.)

Tribolium castaneum is a cosmopolitan insect of the family of the Tenebrionidae found throughout temperate and tropical regions (Sokoloff, 1972).

The origin of the genus is a matter of speculation but the country of origin of each species can be determined (Sokoloff, 1974). According to Hinton (1948) many species are from Africa but T. castaneum is cosmopolitan. It is interesting to note that two species are said to have originated in Timbuctu (a city in Northern Mali): T. dowsi and T. semele (Hinton, 1948).

Many works have been carried out on the genus Tribolium. Sokoloff's reviews (1972, 1974 and 1977) are undoubtedly among the most comprehensive sources of information concerning the genus Tribolium. Howe (1956, 1962 and 1965) and Dawson (1965, 1966 and 1977) have performed detailed studies of various aspects of the biology of Tribolium spp.

T. castaneum adults have the characteristic form of the Tenebrionidae. Males are distinguishable from females by the presence of a setiferous puncture on the ventral side of the anterior femur (Hinton, 1942). Because adults are very active, it is sometimes more convenient to determine sex in the pupa where females differ from males by their strongly protuberant and diverging genital appendages (Halstead, 1968).

The life history of T. castaneum has been subject to many studies. Howe (1956, 1962) in particular gives the effect of temperature and relative humidity on the development rate and oviposition of several species including T. castaneum. He

found that optimum conditions for the development of T. castaneum are 35°C and 75%RH. Under these conditions development from egg to adult may take only 20 days. T. castaneum can survive temperatures as high as 40°C or as low as 22°C.

The life history of T. castaneum starts with the eggs laid by the female. Larvae emerge from the eggs and go through seven larval instar before pupation takes place. Many workers have shown that the number of larval instars depends on conditions under which development is taking place, eg Dawson (1965).

The eggs are generally kidney-shaped and white to cream coloured (Sokoloff, 1974; Pers. obs.). The number of eggs laid by a female varies greatly; no regularity in number of eggs per female per day is observed (quoted in Sokoloff, 1974). The highest oviposition rate reported by Howe (1962) is 24 eggs per female per day. Females are very variable in fertility and fertilized females can lay eggs for a period of 5 months (Sokoloff, 1974). In general, egg hatchability is about 80% (Howe, 1962) but can be greatly affected by various factors such as temperature.

The larva is 1.18-6mm in length depending on the larval instar measured (Brindley, 1930 cited in Sokoloff, 1972). Under optimal conditions there are six larval instars (Howe, 1956); Abdelsamad et al., 1988). Howe (1962) found also that after each moult, larvae gain about twice their initial weight, the increase in size takes place before the new exoskeleton hardens.

The pupal stage is a non-feeding stage during which many morphological transformations take place in a process known as metamorphosis. The pupal stage lasts 5.5 days at 30°C (Howe, 1956) before the adults emerge.

Tribolium spp. are known as secondary storage pests because they often follow infestations of other pests; they feed on a wide range of commodities, especially stored cereals. T. castaneum is also found infesting groundnuts, nuts, spices, cocoa, dried fruits.

Cannibalism has been observed in T. castaneum; adults can feed on eggs and pupae (Lloyd, 1968).

The flying habit of T. castaneum plays an important role in its dispersal. In heavily infested stores, adults come to the surface and fly away during late afternoon (Prevett, 1964; Graham, 1970d).

In Mali, on sites where many stores known as security stores (containing security stocks meant to prevent food shortage) are built, T. castaneum has frequently been observed to fly from one store to another (Pers. obs.). This has led the stock protection division to adapt its pest control strategy by taking measures such as spraying internal as well as external surfaces of stores (the normal practice consists of spraying only the internal surfaces) or allowing stores to be open only one at a time to reduce cross infestation.

The hemipteran Xylocoris flavipes (Reuter) is one of the best known natural enemies of T. castaneum. The parasitic mite Acarophenax tribolii (Newst. and Duv.) is also a serious natural enemy of T. castaneum (Sokoloff, 1974 and Pers. obs.). During the course of the work reported here, some cultures were destroyed in Mali by a parasitic mite later identified as Acarophenax tribolii, using the MAFF Technical Bulletin N.9 (Hughes, 1976).

Tribolium castaneum is very common in Mali where it is found in State and private stores and in traditional family granaries. Although traditional control measures have been used for a long time, in certain ethnic groups (eg Bamanan), the presence of storage insect pests including Tribolium spp. was a matter of pride; it meant that the owner of the infested stock was wealthy enough to keep his stock for a period of time long enough for insect infestation to develop. Attitudes have changed nowadays. The long standing drought which started in the 1970's has made people more aware of the importance of the pest problem.

The strains used in the works reported here were collected in different parts of the country. The details concerning the collection of strains are presented in the table below.

Number	Date	City*	Type of Store & Commodity	Remarks
00	20.6.90	Niafunke	Concrete/Rice	Chemical treatments
04	20.7.90	Zangasso	Traditional granary/Millet	Unknown treatment
08	23.7.90	Mopti	Concrete/Rice	Chemical treatments
09	23.7.90	Mopti	Private concrete store/Miscellaneous	Fish store around treated with unidentified chemicals
10	23.7.90	Mopti	OPAM[1]/Sorghum	Spraying & fumigation
14	19.1.91	Bamako	OPAM[1]-SNS[2]/Millet	Spraying & fumigation
17	13.2.91	Sevaré	OPAM-SNS	Spraying & fumigation
19	13.2.91	Gao	Private concrete/ Miscellaneous	No treatment
21	13.2.92	Gao	Private concrete/ Miscellaneous	No treatment
28	11.7.91	Kayes	Family granary/ Sorghum	No treatment

1: OPAM = Office des Products Agricoles du Mali (The Malian Cereal Marketing Board)

2: SNS = Stock National de Sécurité (National Security Stock)

*: Refer to the map in the Introduction for the locations of the cities.

After collection, insects were transferred to the experimental culture medium and kept at laboratory conditions which were recorded using a thermo-hygrograph.

2.2.2 Rhyzopertha dominica (Fab.)

Rhyzopertha dominica is a coleopteran of the family Bostrichidae. Species of this family are generally wood-borers, but some of them including R. dominica attack stored grain. Literature on the origin, the distribution and the biology of R. dominica is very abundant, eg Chittenden (1911), Lesne (1924b), Potter (1935). More detailed studies of the biology are reported by Birch (1944, 1945, 1945c) and Howe (1950).

R. dominica is a cosmopolitan insect believed to have originated in India (Chittenden, 1922; Potter, 1935). For Lesne (1910-1911), R. dominica is cosmopolitan in tropical regions. The occurrence of R. dominica in the Malian cities of Nioro and Timbuctu is reported in Lesne (1924b).

R. dominica is one of the smallest of the beetles which are injurious to grain in kernel (Chittenden, 1911). The adult is 2-3mm long and a characteristic form of the Bostrychidae: pronotum rounded at the front with transverse rows of teeth. Sinclair (1981) provides a review of external sex differences, and the sexual dimorphism between the male and the female pupae was described for the first time by Potter (1935).

The female lays eggs in crevices or on rough surfaces of seeds. On emergence larvae bore into the grain. At the optimum temperature of 34°C and relative humidity of 70%, development from egg to adult takes about 20 days (Howe, 1950). The maximum temperature at which development is possible is 38.2°C and the minimum temperature is 18-22°C depending on the moisture content of the grain.

The egg is white, of elongate pear-shaped form, one end forming a neck (Chittenden, 1911; Pers. obs.) and is about 0.59mm in length (Potter, 1935). The fully grown larva is about 2.8mm in length. The body is slightly curved. At

emergence the larvae bore into the grain where development takes place. According to Lesne (1924) the larvae undergo 4-5 moults inside the grain. The pupal stage is characterized by the larvae straightening out and becoming motionless (Potter, 1935). The pupal stage lasts about 6.5 days after which adults emerge. After emergence adults fast for 3-5 days and that they feed for 15 days before laying eggs (Potter, 1935) . Adults are frequently observed to fly (Pers. obs.) particularly in late afternoon.

R. dominica attacks a wide range of commodities. Besides whole cereals, R. dominica is a major pest of cassava. Severe infestation of cassava can lead to very heavy weight losses. Both adults and larvae are capable of boring into commodities. They can also cause damage to wooden storage structures by boring into them. There have been reports of small populations of R. dominica on cereals in the field before harvest although infestation is mostly post-harvest (Dobie et al., 1991).

R. dominica is a very serious pest in Mali and is found in all storage conditions (State and private stores, traditional family granaries in rural areas) and has a characteristic name in the bamanan language referring to its boring behaviour: N'SORONIN, literally meaning borer. Before chemical control was introduced, R. dominica was reported by British technical advisors to be present in all OPAM stores. Although a good degree of control is achieved in most stores, R. dominica still remains troublesome in some stores (Pers. obs.). The two-dose fumigation technique has been introduced to OPAM by NRI advisors especially because it is difficult to control this pest with usual procedures of fumigation under gasproof sheet.

Several strains of R. dominica were collected for experiments but many of them were destroyed by a mite infestation. Reculture for cleaning was successful with only one strain. Details concerning the strains collected are given below.

Number	Date	City	Type of Store & Commodity	Remarks
02	19.7.90	Sikasso	OPAM concrete store/ Millet	Spraying and fumigation
06	21.7.90	Koutiala	Private store/ Sorghum	No treatment
13	15.1.91	Bamako	Private store/ Sorghum	Unknown treatment
15	19.1.91	Bamako	SNS store/Millet	Spraying and fumigation
18	13.2.91	Sevaré	SNS store/Millet	Spraying and fumigation
20	13.2.91	Gao	Private store/ Miscellaneous	Unknown treatments
25	28.2.91	Bamako	Private store/ Sorghum	Unknown treatments
27	11.7.91	Kayes	Family granary/ Sorghum	No treatment

All these strains were attacked and destroyed by a mite infestation, only strain N. 06 was recultured successfully.

2.3 THE SELECTION LINES

The method of selection is described in detail in Chapter 6. But as the selection lines are referred to in earlier chapters, here the intention is to describe them briefly. Selection was carried out using two different culture-intervals: 6 week-culture interval and 8 week-culture interval .

In the 6 week-line, new cultures were set up every 6 weeks with adult offspring of the previous generation. The 6 week-line was also referred to as the fast line.

In the 8 week-line reculturing was done every 8 weeks. This line was then called the 8 week-line or the slow line. The intention was to select for fast and slow development respectively and to examine correlated responses to selection.

Chapter 3 WEIGHT LOSS AND OXYGEN CONSUMPTION

3.1 WEIGHT LOSS

3.1.1 Introduction

The measurement of weight loss due to insect damage has been for a long time a subject of interest to post-harvest entomologists for both economic and scientific reasons.

There are several types of losses caused by insects attacking stored grains: weight loss, quality loss, loss of nutritional value, monetary loss, loss of grain viability (Adams, 1977).

Weight loss or quantitative loss is a direct result of insects feeding on stored food, hence subtracting a portion of the food from consumers' use. One of the specificities of stored grain management is that any damage caused is irreversible, whereas when, for example, crops attacked by insects are treated early enough, they may be able to compensate for the damage and yield the normal amount of product. In a situation of food shortage, weight loss due to storage insects and for any other factor can lead to political and social problems. Economically, weight loss means less grain available for selling, hence less income for the producer. This loss is particularly important with cash crops. Also, when the remaining grain is of poor quality due to a high level of attacked and broken grain, it is sold for less on the market.

Measuring weight loss due to insect infestation has proven to be very difficult in practice, the reason being that there are many other causes of loss such as desiccation (loss of humidity when the grain dries), losses caused by handling during the various activities (eg loading trucks, stacking in stores etc), rodents, birds ..... and theft as argued by Rowley (1984). To determine the portion of total loss due to insects, one has to be able to control all the other factors; that is very difficult in practical storage situations as reported by Rowley (1984). But in the

laboratory, various methods for loss assessment have been developed to meet different needs. A review of methods is presented by Harris and Lindbad (1976) and by Boxal (1986).

Many estimates of weight loss are found in the literature, usually with limited reliability. Many assessments were meant to make international organizations aware of the loss problem for funding. In the 1970's various sources (including FAO) estimated grain loss in the Sahelian regions to be more than 30% calling for urgent technical assistance, and the Seventh United Nations General Assembly passed a resolution calling for the reduction of post-harvest losses to be given high priority, with a target of a 50% reduction in these losses by 1985 (FAO, 1977). Various measures were taken in the Sahelian regions. In Mali the severe drought which began in the early 1970s made it even more urgent to reduce post-harvest losses and as a result a Technical Co-operation Agreement between the United Kingdom and the Republic of Ireland on one hand and the Republic of Mali on the other led to the creation of the Stock Protection Project in 1976; this project was later replaced by the Division de la Protection des Stocks in OPAM. A recent study carried out in Mali in two specific zones of CMDT (Compagnie Malienne pour le Development Textile, which is the cotton growing company but also grows a lot of maize) and of OHV (Operation Haute Vallée, a company which grows various crops) by Berthé (1990) indicated a weight loss of 1% in 5 to 6 months at farm level.

Basically there are two ways for measuring weight loss. One method described by McFarlane (1990) as the determination of the apparent weight loss is based on the measurement of the weight of experimental cultures at the beginning and at the end of the experimental period. The other way described by Harris and Lindbad (1976) is based on the detection of damaged and undamaged grains, counting and weighing them, then deducing the loss due to insect damage. There are variants of both methods. A more recent method known as the TGM (Thousand Grain Mass Method) is described in Proctor and Rowley (1983) and assessed against the Standard Volume Method by Reed (1987).

Due to the anatomical configuration of their mouth parts and possibly to their physiological requirements (metabolism), different storage insects exhibit different abilities to damage grain. For example, species such as Rhyzopertha dominica (Bostrychidae), Trogoderma granarium (Everts) (Dermestidae) and Sitophilus spp. (Curculionidae) are described as primary pests because they have strong mandibles which enable them to attack healthy undamaged grain (NRI, 1990). Species such as Tribolium spp. (Tenebrionidae) and Cryptolestes ferrugineus (Silvanidae) are described as secondary pests because they are not able to attack undamaged grain and require the grain to have been damaged by primary pests or by mechanical means.

The aim of the present study was to carry out a preliminary survey to identify major differences in the ability to damage grain of strains of the same species and/or selection lines of the same strains with the goal of possible biotype characterisation. Throughout, weight loss has been assumed to be a measure of the ability of insects to damage grain. Part of the experiment was performed in Mali and part in the UK.

After the weight loss experiment, the oxygen consumption of the different strains and/or selection lines was measured using the Gilson Differential Respirometer in order to compare their metabolic rate. More detail is given in the section dealing with oxygen consumption.

3.1.2 Materials and Methods

Two experiments were performed in Bamako, the first at ambient laboratory conditions and the second at controlled temperature (30°C). The following six strains were used in the first experiment: strains N.00, N.04, N.08, N.09, N.17 and N.21. A mite infestation destroyed three strains and the experiment was completed with the following three strains: strains N.00, N.09 and N.17. Two strains were used in the second experiment: strains N.04 and N.21 which were

successfully cleaned from the mite attack by reculturing from eggs. In Leicester the selection lines (see Chapter 6) derived from strains N.04 and N.21 were also used, these lines were as follows:

- 6 week-line and 8 week-line from strain N.04;
- 6 week-line and 8 week-line from strain N.21.

It was also possible to include a parent strain in the experiment in Leicester, namely strain N.21, making it possible to compare the performance of a parent line to the performance of the lines resulting from selection.

The culture medium was as described in general materials and methods, consisting of 12 parts of sorghum flour and one part of yeast.

In Bamako, when cultures were kept at ambient laboratory conditions, a thermo-hygrograph was set to record temperatures and relative humidities. The maximum and minimum temperatures and relative humidities recorded during the experiment were as follows:

	Temperature	Relative Humidity
Minimum	22°C	29%
Maximum	36°C	66%

In Leicester, cultures were kept in a constant temperature room where the temperature was 30°C (± 1°C) and the relative humidity was 45-55%.

For insect weight measurement an OHAUS Triple Beam balance with a precision of $\frac{1}{1000}$g (1mg) was used in Bamako and a CAHN microbalance with a precision of $\frac{10}{1000}$mg (10µg) was used in Leicester. For the measurement of the weight of experimental cultures (jars + flour + insects) an OHAUS Triple Beam balance with a higher capacity (1600g) and a precision of 1mg was used in both places. At the end of the experiments the final numbers of adult insects were counted using a hand tally.

The experimental method used was based on the measurement of weights of cultures at the beginning and at the end of the experimental period. The final numbers of live and dead adult insects were also determined at the end of the experimental period.

Experimental cultures were set up as follows:

- In Bamako: 30 unsexed adults of unknown age collected at random were introduced into culture jars containing 50g of culture medium. There were three replicates for each strain.
- In Leicester: adults were collected at random and sexed using the setiferous puncture on the anterior femur of the male as described by Hinton (1942) and 15 pairs were introduced in jars containing 60g of medium.

The experimental period was 3 months in Bamako at ambient laboratory conditions and 2 months at controlled conditions and was 2 months also in Leicester.

3.1.3 Results and Discussion

3.1.3.1 Results

Results were analysed using the Minitab package. Differences between strains in Table 3.1 were tested using pairwise t tests because data were not balanced, elsewhere analysis of variance was performed. In strain N.17 (Table 3.1) for an unknown reason insects died in one replicate. In all the experiments weight loss was corrected for weight change in the controls.

The weight loss caused by strains of T. castaneum and R. dominica in the different experimental conditions are shown in Tables 3.1 to 3.4. At laboratory conditions in Mali (Table 3.1), strains of T. castaneum did not cause significantly different weight loss ($P > 0.05$). Table 3.2b shows that the strains tested at constant temperature caused significantly different weight loss ($P = 0.026$). Tables 3.2a and 3.3a show

that strains causing the highest weight loss are those which have also produced the highest final number of adults, indicating perhaps a higher productivity. Table 3.3b shows the analysis of variance of apparent weight loss caused by the different culture lines of the two strains tested at controlled conditions , the unselected parent N.21P was not included otherwise the data would be unbalanced. Table 3.3b shows that selection lines also caused significantly different weight loss, but the strains did not differ significantly. The correlation between weight loss and final number of adults is illustrated in Fig 1. In Tables 3.2a and 3.3a mortality rates appear to be positively correlated to final number of adults. In Table 3.4, weight losses caused by the three strains of R. dominica are not significantly different .

Table 3.1: Apparent weight loss caused by three strains of T. castaneum at room temperature (uncontrolled) in Bamako over a period of 3 months.

Strain		Weight Loss	
No	Replicates	g	%
	1	31	9.45
	2	3.2	0.973
N.00	3	7.0	2.176
	Mean	13.73	4.200
	St dev	15.07	4.587
	1	24.3	7.279
	2	31.3	9.708
N.09	3	17.9	5.327
	Mean	24.5	7.438
	St dev	6.70	2.19
	1	30.9	9.620
	2	13.8	4.236
N.17	3	-	-
	Mean	22.35	6.928
	St dev	12.09	3.81

Table 3.2a: Apparent weight loss caused by two strains of T. castaneum at constant temperature of 30°C in Bamako over a period of 2 months.

Strain		Weight Loss		Final Number of Adults		
No	Replicates	g	%	Live	Dead	Total
	1	7.63	15.62	321	45	366
	2	5.53	11.25	219	43	262
N.04	3	7.98	16.22	304	52	356
	Mean	7.04	14.37	281.33	46.66	328
	St dev	1.32	2.71	54.65	4.72	57.38
	1	10.15	21.41	430	74	504
	2	9.41	19.06	342	71	413
N.21	3	9.88	20.27	437	81	518
	Mean	9.81	20.25	403	75.33	478.33
	St dev	0.37	1.18	52.9	5.13	57.01

Table 3.2b: Analysis of variance of apparent weight loss caused by strains N.04 and N.21 of T. castaneum.

Source	df	SS	MS	F	P
Strain	1	51.920	51.920	11.88	0.026
Error	4	17.481	4.370		
Total	5	69.402			

Table 3.3a: Apparent weight loss caused by selection lines (-6 or -8 week or -P unselected) of two different strains of T. castaneum at controlled conditions (30°C and 45-55%RH) in a period of 3 months in Leicester.

Strain		Weight Loss		Final Number of Adults		
No	Replicates	g	%	Live	Dead	% Mortality
	1	13.69	22.568	635	231	26.67
	2	14.02	23.086	623	165	20.94
N.04-6	3	14.060	23.144	548	145	20.92
	Mean	13.923	22.933	602	180.33	22.84
	St dev	0.203	0.317	47.15	45	3.31
	1	15.010	24.749	476	362	43.19
	2	15.49	25.490	503	323	39.10
N.04-8	3	15.390	25.333	517	351	40.44
	Mean	15.297	25.191	498.67	345.33	40.91
	St dev	0.253	0.390	20.84	20.11	2.08
	1	15.090	24.852	664	254	27.67
	2	14.66	24.144	505	445	46.84
N.21-6	3	14.60	24.057	778	111	12.49
	Mean	14.783	24.351	649	270	29
	St dev	0.27	0.44	137.12	167.57	17.21
	1	15.18	25.008	495	491	49.79
	2	14.810	24.399	452	415	47.87
N.21-8	3	14.85	24.469	406	510	55.68
	Mean	14.947	24.625	451	472	51.11
	St dev	0.20	0.33	44.51	50.27	4.07
	1	13.460	22.164	167	410	70.06
	2	13.630	22.444	95	476	83.36
N.21-P	3	12.14	19.977	268	215	44.51
	Mean	13.077	21.528	176.67	367	65.98
	St dev	0.82	1.35	86.90	135.71	19.74

Table 3.3b: Analysis of variance for apparent weight loss caused by culture-lines of different strains of T. castaneum at controlled conditions.

Source	df	SS	MS	F	P
Line	1	4.8095	4.8095	34.71	0.000
Strain	1	0.5457	0.5457	3.94	0.082
Interaction	1	2.9512	2.9512	21.30	0.000
Error	8	1.1085	0.1386		
Total	11	9.4149			

Table 3.4a: Apparent weight loss caused by three strains of R. dominica at uncontrolled conditions over a period of 3 months in Bamako.

Strain No	Replicates	Weight Loss g	Weight Loss %
N.06	1	17.01	24.328
	2	16.90	24.893
	3	17.93	26.257
	Mean	17.28	25.159
	St dev	0.56	0.99
N.25	1	16.27	23.808
	2	18.47	26.310
	3	15.82	23.271
	Mean	16.85	24.463
	St dev	1.42	1.62
N.27	1	21.79	31.992
	2	18.29	26.945
	3	16.62	24.747
	Mean	18.90	27.895
	St dev	2.64	3.71

Table 3.4b: Analysis of variance for apparent weight loss caused by strains of R. dominica.

Source	df	SS	MS	F	P
Strain	2	19.743	9.872	1.70	0.261
Error	6	34.826	5.804		
Total	8	54.569			

Fig.1: CORRELATION BETWEEN WEIGHT LOSS AND FINAL NUMBER OF ADULTS IN THE 6WEEK LINE OF STRAIN N.21 OF T. CASTANEUM

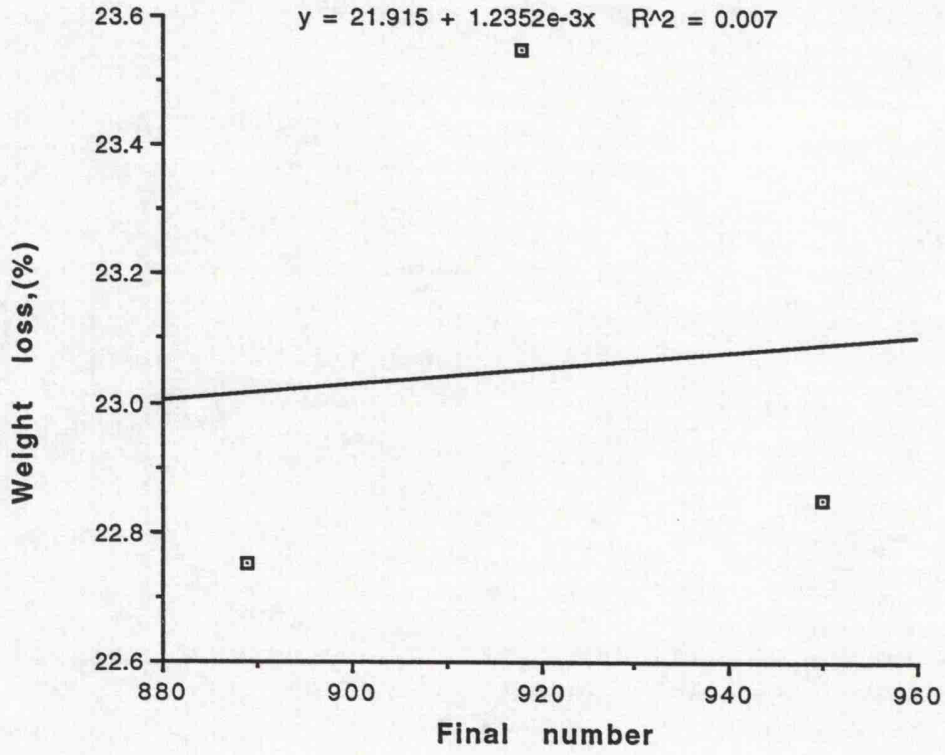
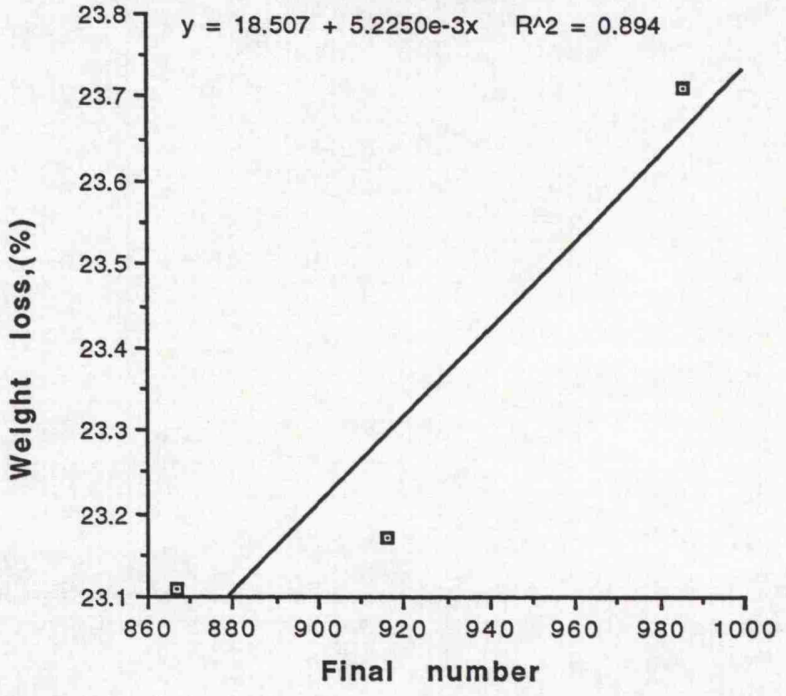


Fig.2: CORRELATION BETWEEN WEIGHT LOSS AND FINAL NUMBER OF ADULTS IN THE 8WEEK LINE OF STRAIN N.21 OF T. CASTANEUM



3.1.3.2 Discussion

In the experiment reported here, there were no significant differences between strains of T. castaneum at uncontrolled conditions in their ability to cause weight loss, (Table 3.1, $P > 0.05$). The lack of significant difference could be the result of the great variability of the weight losses due to unstable experimental conditions. Figs 1 and 2 show how variable the data were. At constant temperature, the strains of T. castaneum tested were significantly different (Table 3.2b). Differences between strains of the same species of storage insect pests are reported by several authors, eg White et al. (1988), McFarlane et al. (1990). Such difference between strains could be the result of significant variation in some of their life history traits. Strains having higher fecundity for example or shorter development period hence having higher fitness, would produce more progeny in the same period of time than strains less fit and therefore might consume more food. For example in Table 3.2a and Table 3.3a the weight loss appears to be higher where the final numbers of adults are greater, Fig.1 illustrates the trend of the relationship between weight loss and final number of adults. The correlation between weight loss and productivity could not be correctly described because the larval stage was not measured.

The mortality rate might also have affected the weight loss by controlling the final number of adults alive and, as can be seen in Table 3.2a and Table 3.3a, mortality rates were greater where the final number of adults were higher (perhaps indicating a density-dependent mortality).

The difference between strains in their ability to damage grain could also be the result of a possible significant difference in their metabolic rate, the strain having the highest metabolic rate might consume more food and hence cause higher damage.

The significant difference between selection lines in their ability to damage grain (Table 3.3b) could be the result of the effect of selection on one or several life

history traits. The non-significance of the difference between the strains of R. dominica in their ability to damage grain (Table 3.4b) might also be due to the uncontrolled conditions or might simply be an indication that the strains tested were not different. Relating the results of the weight loss experiment to the results of the resistance tests reported in the next chapter shows that in both R. dominica and T. castaneum the most resistant strains cause the highest loss, though of course the number of strains tested was small and the difference between the strains of R. dominica was not significant. Differences between resistant and susceptible strains in respect of their relative fitness have been reported by some research workers, a review of which is given in Rousch and Croft (1986). In the next section, oxygen consumption, which depends on metabolic rate, will be measured in two strains to find out whether it has a correlation with weight loss.

3.2 RESPIROMETRY STUDIES

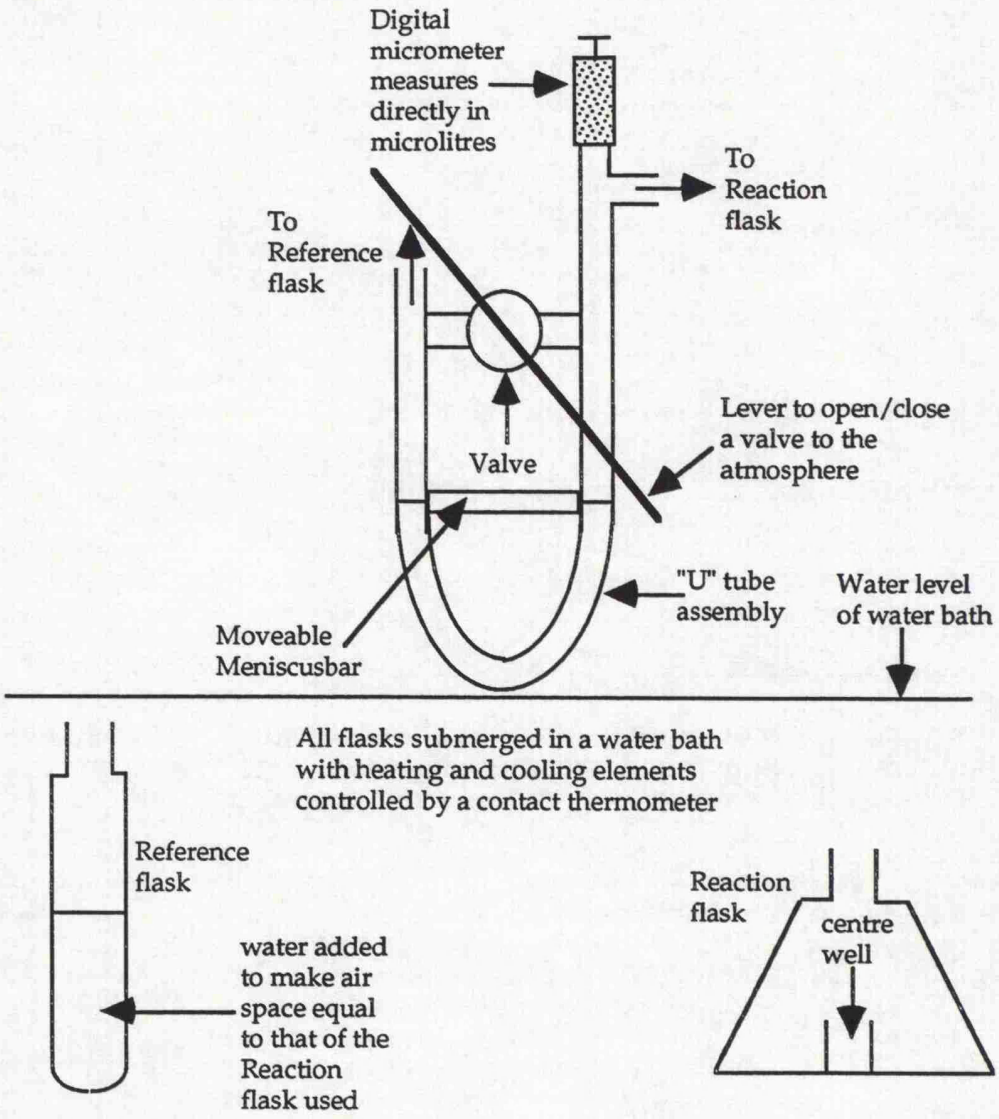
3.2.1 Introduction

In the weight-loss experiments, the strains studied (strain N.04 from Zangasso and strain N.21 from Gao) showed a significant difference in their ability to damage grain (Tables 3.2a and b). Weight loss appeared to be greater in strains producing higher final number of adults although there was no significant correlation perhaps due to the fact that only the adult stage was measured. Therefore there seemed to be some indication that progeny production could be one of the causes of the observed difference between strains in their ability to damage grain. But since the food consumed is metabolised to provide energy for the various physiological requirements, the difference in weight loss could also be the result or the consequence of different metabolic rates. The chemical processes taking place during metabolism require oxygen which is provided during respiration; oxygen consumption can then be used as a measure of metabolic rate (Schmidt-Nielsen, 1984). It was interesting to find out whether the strains used for the weight loss experiment would have different metabolic rates; respirometry studies were

therefore carried out and it was predicted that the strain which caused the highest weight loss (N.21 from Gao) would show the highest oxygen consumption.

3.2.2 Materials and methods

The respirometry studies were performed using the Gilson Differential Respirometer (GDR) (Gilson, 1963). The GDR is based on a manometric procedure in which the insect is retained in a confined space and oxygen consumption is measured directly by volume change because the CO_2 produced is immediately absorbed in a concentrated alkaline solution. As shown in the diagram below, each respirometer consists of a reference flask, a digital micrometer and a reaction flask connected by constant bore microtubing. The full operating instructions of the GDR are given in Appendix 2.



Gilson Respirometer diagramatic layout

The procedure used in the present study was different from the procedure described by Daniel (1992) because in the model used by Daniel (1992) there was one reference flask connected to all the reaction flasks whereas in the model used here each reaction flask was connected to an independent reference flask.

The following procedure was followed:

- open all valves by means of master lever (handle up);
- clean respirometry flasks and pre-cut strips of filter paper;
- roll filter paper strips and insert in the central well of the flask;

- using a pasteur pipette place 5 drops of 30% KOH* onto the filter paper in the well (KOH is used to absorb CO_2);
- cover the well with a perforated polythene cap to prevent insects from climbing into the well;
- place insects in the flasks, leaving three flasks without insects to serve as controls;
- lightly grease all glass connectors;
- attach the reaction vessels containing insects to the glass connectors and secure by means of springs;
- submerge all the reaction vessels in the water bath to maintain constant temperature, the level of water in the bath should be above the greased joint;
- set all the micrometers to be used at a convenient even number (here they were set at 100);
- set the moveable black index line to the meniscus of the red fluid at the front of each manometer;
- allow system to equilibrate for two* hours, then to commence measurements, close the operating valves by means of the master valve lever (handle down);
- take readings at regular intervals (half hour or one hour);
- take readings by turning the micrometer so that the meniscus of the dye is level with the pre-set meniscus bar again, values are then read directly in microlitres;
- for each reading session take the mean of the controls and correct experimental flasks by subtracting the control mean from that recorded;
- to convert the results to STP, multiply the micrometer readings by

$$\frac{(273)(P_b - 3 - P_w)}{(273 + t)(760)}$$

where,

t = temperature of water bath (°C)

P_b - 3 = operating pressure (barometric pressure minus three to compensate for mercury specific gravity at room temperature

P_w = vapour pressure of water.

- when finished open all valves (lever up) and remove reaction flasks;
- transfer each experimental insect to a numbered plastic vial;
- kill and dry insects by leaving vials containing them in the electric oven set at two (approximately 60°C) for 24 hours;
- measure dry weight of each insect using the CAHN Microbalance;
- finally express results in microlitre per minute per mg dry body weight.

Instructions marked * were set following some preliminary tests hence differ from the recommendations given in the operating instructions.

At first, tests were performed in a constant temperature room where the temperature was maintained at 25°C, and the stirrer and the heater of the respirometer were switched on before each test according to the operating instructions to bring the water bath temperature up to 30°C. When the respirometer was later moved to a new constant temperature at 30°C, the equilibrating temperature of the water bath was 29°C.

Oxygen consumption in insects is affected by age and body size (Roeder, 1953). Therefore it was better to perform tests on individuals of the same age. For this reason adults of the strains to be used were allowed to oviposit for one day. Adults were then removed and cultures were kept undisturbed until required for the tests. All the individuals therefore were the same age. The first tests were performed using the last larval instar (21 days after hatching) and then on adults emerged in the same cultures. An attempt was also made to control the density in the cultures as density is known to affect body size; this was done by initially leaving the same number of females in each strain (10) on the same amount of flour (30g) for one-day oviposition. The strains used were:

- the 6 week-line and 8 week-line of strain N.04 from Zangasso;
- the 6 week-line and 8 week-line of strain N.21 from Gao;

- the unselected strain N.21 – the unselected strain N.04 was not included because it was found to have a light infestation of mites (Acarophenax sp.).

The barometric pressures were obtained from the Cosby Weather Station. No correction for the altitude of the Environmental and Population Biology laboratory was required as it was estimated by the Geography Department of the University that the altitude of the second floor of Adrian Building is approximately 251ft (76m) above sea level, which is the same as the altitude of the Cosby Weather Station.

For both adults and larvae each session was carried out with 10 individuals because it was found, following several pressure tests, that only 13 out of the 20 flasks of the respirometer were good enough to be used. It was also found after a few tests that 30 minutes was too short an interval and that 10 hours was not an appropriate duration for one session because the water resulting from the condensation inside the reaction flasks affected insects often up to death and finally that the time for equilibration should be at least 1.5 hours otherwise negative readings were recorded. Therefore for each session, the system was allowed to equilibrate for 2 hours and readings taken every hour for 5 hours or sometimes 6 hours, each session then lasted 7 to 8 hours.

It was not possible to perform as many sessions with the larvae as with the adults, the reason being that, having the same age, all the larvae emerged into pupae within a few days from the start of the study.

The insects being individually confined inside flasks with no food, without partner (no mating), it was assumed that they had minimal activity during the experiment, therefore it was the basal metabolic rate which was estimated.

3.2.3 Results and Discussion

3.2.3.1 Results

Results are summarized in Tables 3.5 to 3.8. As explained above, with adults, at least five sessions were performed with each strain using 10 individuals and taking a minimum of five readings during each session. Therefore each figure in Table 3.5a is a mean of a minimum of 250 readings. With larvae, each reading in Table 3. 6a is a mean of a minimum of 100 readings.

Results were analysed using the Minitab statistical package.

In the literature, oxygen consumption is expressed in several ways; eg µg/minute/mg, µg/day/mg, µl/minute/mg mm^3/minute/mg etc. Here results are expressed in µl/minute/mg, but to make comparison with results from other sources easy, a conversion was also done into µg/minute/mg and µg/minute/insect (Table3. 5a).

In Table 3.5b the analysis of variance was performed on the means of days. For example, when five readings were taken the same day, the mean oxygen consumption from the five data was obtained after all the calculations and corrections. The same explanation applies to Table 3.6b.

In adults, the analysis of variance (Table 3.5b) showed that there was a significant difference in oxygen consumption between strains but not between selection lines: strain N.04 from Zangasso had a metabolic rate significantly higher than strain N.21 from Gao, which contradicts the a priori prediction.

In larvae, the analysis of variance (Table 3.6b) indicated that the difference between strains in oxygen consumption was not statistically significant but that selection lines differed significantly. Figures 3 and 4 show the increase in oxygen consumption with time. Figures 5 and 6 show a negative correlation between dry

body weight and oxygen consumption. Overall, the 8-week lines had significantly higher metabolic rates than both the 6-week and the unselected lines.

Tables 3.7b and 3.8b show the variation in dry body weight respectively in adult and larvae. In both adult and larvae were significantly different, but the difference between selection lines was significant only in larvae, not in adults.

Table 3.5a: Mean oxygen consumption of adults of fast (6 week) and slow (8 week) lines of strains N.04 (Zangasso), N.21 (Gao) and unselected lines of T. castaneum (in µl or µg per unit time per mg dry weight).

Strains	Oxygen Consumption		
	(µl/min./mg)*	(µg/min./mg)	(µg/min./insect)
N.04 – 6	0.084	0.1200	0.1039
N.04 – 8	0.091	0.1294	0.0967
N.21 – 6	0.066	0.0943	0.0904
N.21 – 8	0.049	0.0697	0.0677
N.21	0.030 (± 0.004)**	0.0431	0.0426

*: Standard error of a difference between two means = 0.009

**: Strain N.21 was not included in the anova, therefore its individual estimated standard error is given.

NB. Each figure is the mean of a minimum of 250 recordings.

Table 3.5b: Analysis of variance of oxygen uptake of adults in fast (6 week) and slow (8 week) lines of strains N.04 (Zangasso) and N.21 (Gao) of T. castaneum. The unselected strain N.21 could not be included else the data would have been unbalanced.

Source	df	SS	MS	F	P
Strain	1	12.745	12.745	7.74	0.017
Selection line	1	0.384	0.384	0.23	0.641
Interaction	1	2.088	2.088	1.27	0.282
Error	12	21.844	1.820		
Total	15	34.973			

Table 3.6a: Mean oxygen uptake of larvae in fast (6 week) and slow (8 week) lines of strains N.04 (Zangasso) and N.21 (Gao) and unselected lines of strain N.21 of T. castaneum.

Strains	Oxygen Consumption		
	(µl/min./mg)*	(µg/min./mg)	(µg/min./larva)
N.04 – 6	0.1119	0.1600	0.1129
N.04 – 8	0.1656	0.2365	0.1502
N.21 – 6	0.0882	0.1259	0.1464
N.21 – 8	0.1328	0.2009	0.2059
N.21	0.0960 (± 0.016)**	0.1372	0.1250

*: Standard error of difference = 0.028

**: Strain N.21 was not included in the anova, therefore its individual estimated standard error is given.

Table 3.6b: Analysis of variance for oxygen uptake of larvae in fast (6 week) and slow (8 week) lines of strains N.04 (Zangasso) and N.21 (Gao).

Source	df	SS	MS	F	P
Strain	1	28.75	28.75	2.02	0.164
Selection line	1	86.85	86.85	6.10	0.018
Interaction	1	0.73	0.73	0.05	0.822
Error	36	512.69	14.24		
Total	39	629.02			

Table 3.7a: Mean dry weight of selection lines and strains of adult T. castaneum.

		Lines		Strain Mean
		6 Week	8 Week	
Strains	N.04	0.866	0.747	0.807
	N.21	0.959	0.972	0.966
Line mean		0.913	0.860	

(n = 60 for each factor)

Table 3.7b: Analysis of variance for adult dry weight in the two selection lines of strains N.04 (Zangasso) and N.21 (Gao).

Source	df	SS	MS	F	P
Strain	1	0.7567	0.7567	30.37	0.000
Selection line	1	0.0836	0.0836	3.35	0.070
Interaction	1	0.1316	0.1316	5.28	0.023
Error	116	2.8903	0.0249		
Total	119	3.8622			

Table 3.8a: Mean dry weight of selection lines and strains of larvae of T. castaneum.

		Lines		Strain Mean
		6 Week	8 Week	
Strains	N.04	0.706	0.635	0.670
	N.21	1.163	0.992	1.077
Line mean		0.934	0.814	

(n = 20)

Table 3.7b: Analysis of variance for larval dry weight in the two selection lines of strains N.04 (Zangasso) and N.21 (Gao).

Source	df	SS	MS	F	P
Strain	1	1.6545	1.6545	53.74	0.000
Selection line	1	0.1451	0.1451	4.71	0.037
Interaction	1	0.0252	0.0252	0.82	0.371
Error	36	1.1083	0.0308		
Total	39	2.9331			

3.2.3.2 Discussion

Most of the data on oxygen consumption found in the literature concern T. confusum and are slightly lower than the results reported here. In adult beetles, for example, Sokoloff (1977) reports an oxygen consumption of 0.062µg/minute/mg at 30°C and Kennington (1957) found oxygen consumption in the range of 0.0381–0.0957µg/minute/mg. In the present study oxygen consumption for adults was found to be in the range of 0.0431–0.1708µg/minute/mg (Table 3.5a). According to Bowker (1974) T. castaneum has a higher metabolic rate compared to T. confusum and that may be the reason why the figures reported here are higher than those found in the literature.

Although comparative studies of their biology (eg life history parameters, resistance status) give indirect indications that strains of T. castaneum might have different metabolic rates, there are no published reports of differences between metabolic rates of different strains of T. castaneum compared by direct measurements of their oxygen consumption. The two strains studied were found to have significantly different metabolic rates but the results did not agree with the a priori prediction made. In the weight loss experiment, strain N.21 from Gao caused the highest weight loss and produced significantly more adult individuals than strain N.04 from Zangasso, therefore it was predicted that strain N.21 consuming more food would have higher metabolic rate, but the results showed that it had a significantly lower metabolic rate than strain N.04 in adults. The reason might be the difference between the two strains in body size. Tables 3.7a and b show that strain N.04 is significantly lighter than strain N.21 and Zeuthen (1947) reports works showing that among insects, in general the metabolic rate decreases with increasing size, the same statement was made by Peters (1983), and there was a negative correlation between oxygen consumption and dry body weight illustrated in Figs. 5 and 6.

Although in Figs. 3 and 4 the 6 week-line is above the 8 week-line indicating that oxygen consumption was consistantly higher in the former no significant difference in oxygen consumption was found between culture-lines in adults, hence selection had no effect on oxygen consumption in adults. In larvae (Tables3.6 a and b) the difference between culture lines was statistically significant indicating that selection has affected metabolic rate in larvae. These results suggest a decoupling of adult from larval metabolic rate indicating that metabolic rate is controlled by different genes in adults and larvae, Daniel and Smith (in press) obtained the same result in Callosobruchus maculatus.

The two strains studied were found to have the same resistance status at the discriminating doses, they were both susceptible to fenitrothion and pirimiphos-

methyl (100% mortality) and resistant to malathion (41.25% and 48.75% mortality respectively for strains N.04 and N.21). Therefore the difference between the two strains in oxygen consumption is not related to the resistance status in the present work.

3.3 CONCLUSION

When a significant difference in weight loss was found between strains (eg strains N.00 and N.09) and/or between culture lines (eg strains N.04–6 and N.04–8), it seemed to be correlated to the final number of adults. Selection had an effect on the ability to damage grain in strain N.04 but not in strain N.21. This might be an indication of a genetic difference between the two strains.

The strain which caused the highest weight loss did not have the highest metabolic rate. The difference between strains found in adult metabolic rate might be the result of the difference between adult body weight in the two strains and, as shown in Figs. 5 and 6, the smaller strain had the highest metabolic rate.

In the two strains used for the respirometry studies, a decoupling of adult for larval metabolic rate was found suggesting that metabolic rate might be controlled by different genes in adults and larvae.

Fig.3: CORRELATION BETWEEN TIME AND OXYGEN CONSUMPTION IN a-6WEEK-LINE AND b-8WEEK-LINE OF STRAIN N.04 OF T. CASTANEUM

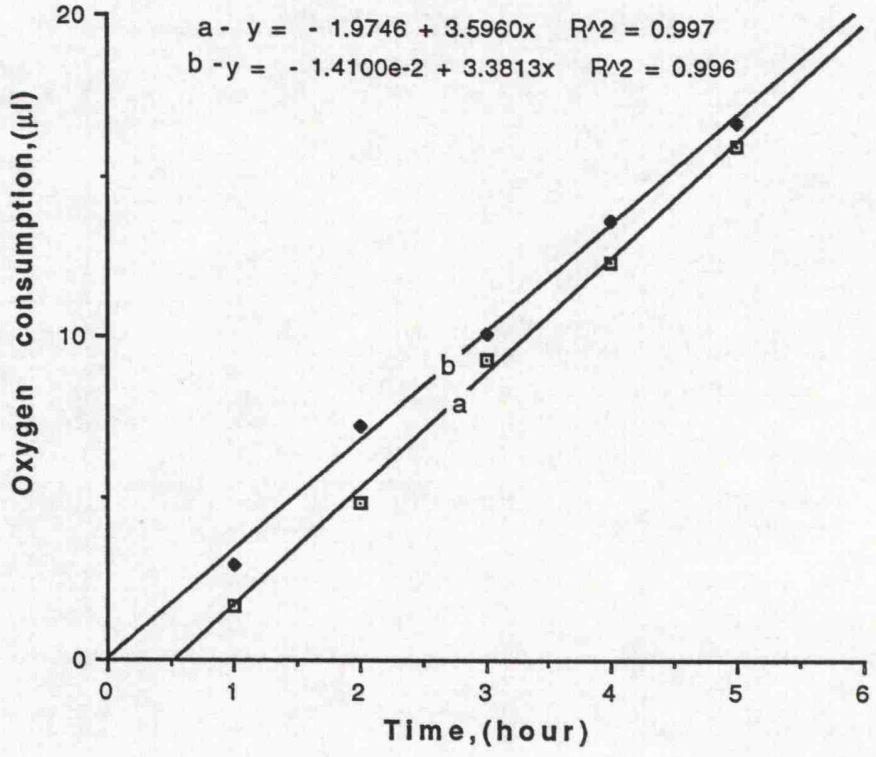


Fig.4 CORRELATION BETWEEN TIME AND OXYGEN CONSUMPTION IN a-6WEEK-LINE AND a-8WEEK-LINE OF STRAIN N.21 OF T. CASTANEUM

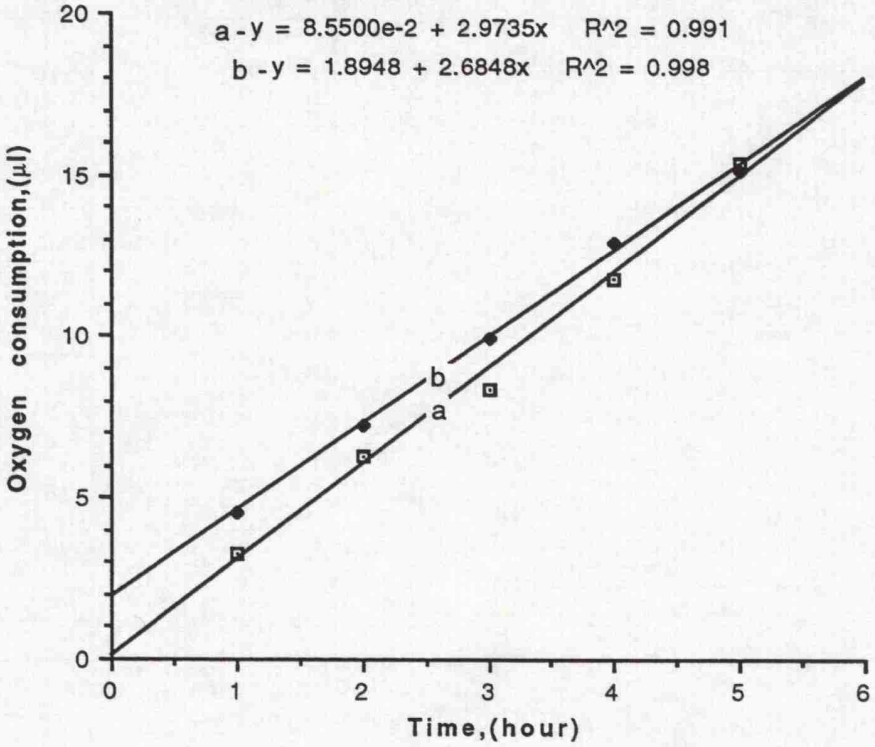


Fig.5: CORRELATION BETWEEN DRY BODY WEIGHT AND OXYGEN CONSUMPTION IN a-6WEEK AND b-8WEEK-LINES OF STRAIN N.21 OF T. CASTANEUM

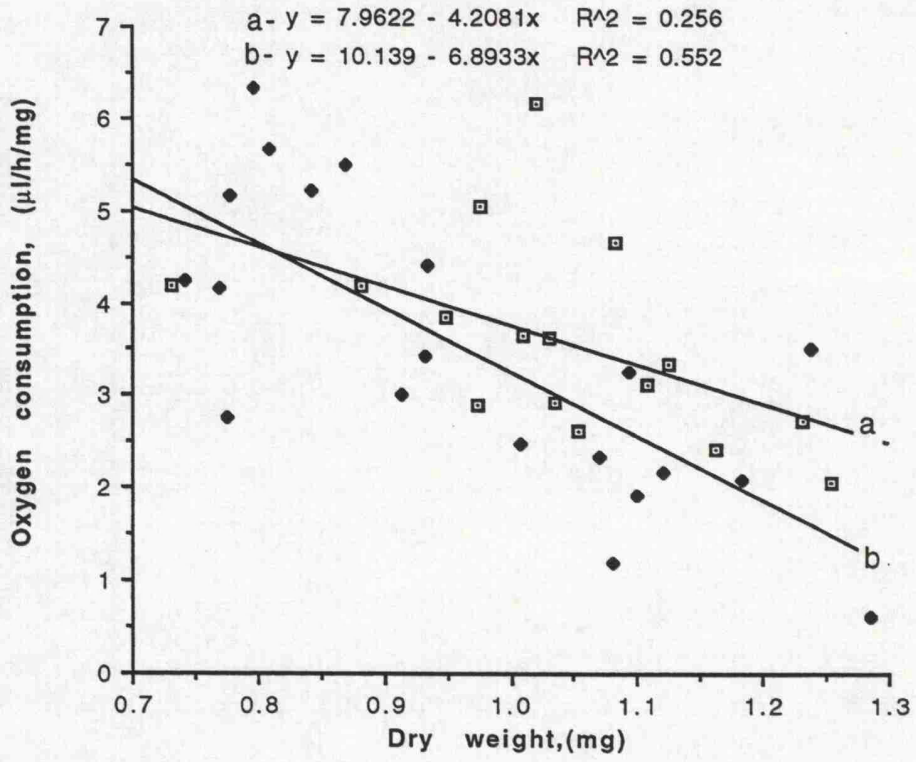
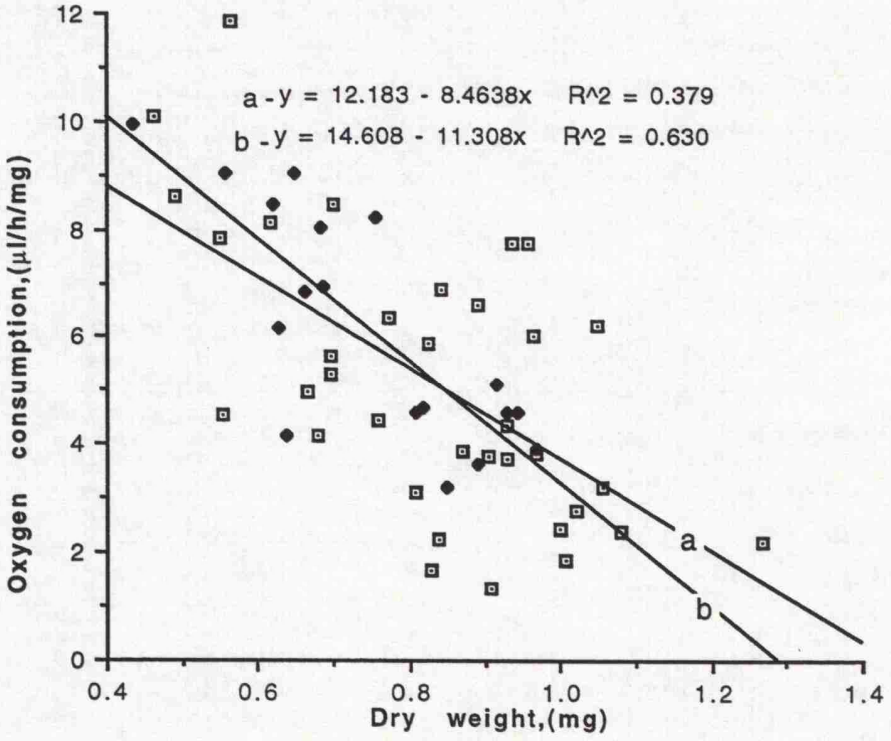


Fig. 6 CORRELATION BETWEEN DRY BODY WEIGHT AND OXYGEN CONSUMPTION IN a-6WEEK AND b-8WEEK-LINES OF STRAIN N.04 OF T. CASTANEUM



Chapter 4 INSECTICIDE RESISTANCE AND FITNESS

Many references found in the literature suggest that resistant and susceptible phenotypes differ in fitness. For example, metabolic rate decreases with increasing body weight (Zeuthen, 1953); as a consequence, significant differences in body weight (a fitness trait) might result in differences in resistance to insecticides as reported by Roeder (1953). McGraig (1956) (cited in Holloway, 1984) reported that heavier individuals of Schistocerca gregaria (Forsk) and Locusta migratoria migratoriodes (R & F) were less susceptible to an organochlorine insecticide (DNC) than lighter individuals. Muggleton (1983) working with Oryzaephilus surrinamensis reported differences in fitness between susceptible and resistant phenotypes.

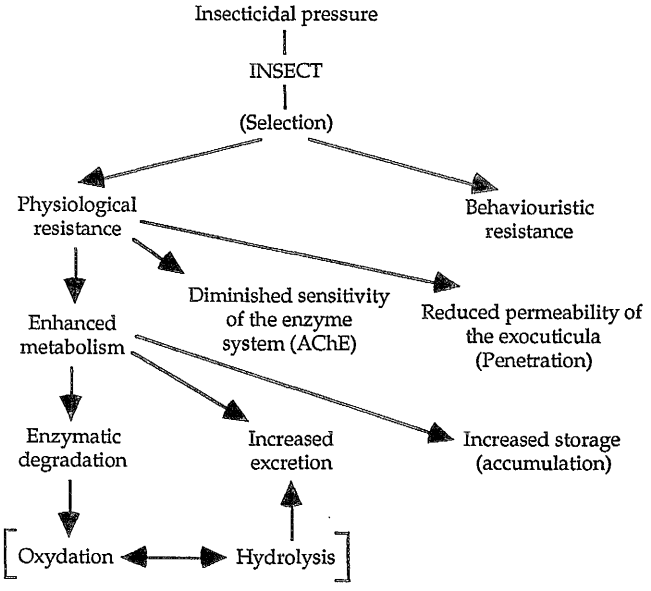
The frequency of resistance genes often decreases in the absence of insecticides, possibly indicating that these genes have pleiotropic effects that reduce the fitness of resistant genotypes (Dyte, 1990). In T. castaneum, Dyte (1990) described two types of resistance to malathion: specific and non-specific resistance. Analysing the frequency of each type in the samples collected during the FAO survey (Champ and Dyte, 1976), Dyte (1990) indicated that individuals with non-specific resistance were much less fit than susceptible ones whereas individuals with specific resistance were only a little less fit compared with susceptible.

In this chapter an attempt is made to relate components of fitness (life-history traits) of different strains to their resistance status in order to find a possible correlation. The chapter will consist of three parts. The results of the resistance test using the NRI Test Kit (Taylor, 1990) will be reported in the first part and the results of a study of life-history traits that contribute to fitness (eg body weight, development period etc) will be reported in the second part of the chapter. In the third part, the relationship, if any, between fitness and resistance status will be discussed.

4.1 INSECTICIDE RESISTANCE IN MALI STRAINS OF T. CASTANEUM AND R. DOMINICA

4.1.1 Introduction

The problem of resistance of insects to insecticides is now well documented. Many specialists have been working for quite a long time on various aspects of this important subject. One of the most cited references is certainly the FAO Global Survey of Insecticide Resistance (Champ and Dyte, 1976) which reports insecticide resistance in various species from different parts of the world. To show the extent of the problem, Champ (1986) lists 31 storage pests resistant to residual insecticides and 9 resistant to fumigants only. Several other workers have reported insecticide resistance, eg Parking (1965), Kamel & Fam (1973), Tyler & Binns (1982). Resistant insects are not only able to tolerate doses of pesticides that would kill susceptible ones, they also have the capacity to pass on this ability to their offspring (Dyte, 1990). Fest et al. (1973) illustrate the development of resistance in the diagram below:



Mechanisms of insect resistance (after Fest and Schmidt, 1973)

Resistance to insecticide can appear in a population of insects as a consequence of the use of insecticide (see diagram above) or simply through introduction of resistant strains brought from elsewhere. One of the tasks of OPAM is the management of food aids donated by various countries and organizations. Grains from various parts of the world (America, Asia and Europe) have been supplied in important quantities since the serious drought of the early 1970s. Introduction of resistant strains has been suspected (G A Gilman, personal communication).

Insecticide resistance results in an increase of direct losses due to insect damage and the cost of chemical treatments. Storage pests inflict these losses after all the effort and expenses are put into production and harvesting (Wool et al., 1982).

Insecticides have been in use in Mali for many decades, particularly in public hygiene (eg chemical control of mosquitoes). Official records are difficult to obtain but it is commonly admitted that DDT was widely used for this purpose. In agriculture, pesticides were mostly used by the cotton growing company, CMDT (Compagnie Malienne pour le Developpement Textile) and the rice growing company, ON (Office du Niger). The pesticides used were organophosphorous according to the authorities of these companies.

The use of pesticides in the protection of grain stored by the Malian Cereal Marketing Board (OPAM) was introduced and developed by the British Technical Advisors of the Tropical Product Institute (TPI) now known as the Natural Resources Institute (NRI), located in Chatham. Contact insecticides, fumigants and rodenticides were introduced. Malathion was the first contact insecticide used and phostoxin (aluminium phosphide) the first fumigant. Later, other organophosphorous insecticides were introduced when resistance to malathion was suspected, fenitrothion in particular was widely used followed by pirimiphos-methyl (Actellic). Dieldrin, an organochloride insecticide, was used to a much lesser extent for the control of termites causing damage to storage structures.

Malathion is one of the most generally useful organophosphorous compounds. It is principally used to control sap-sucking insects, although weevils and small beetles are also susceptible. It owes its popularity to the relative safety (rat LD_{50}: 2800mg/kg) with which it can be handled (Hassall, 1982). Within a decade of the introduction of malathion, several species of insects had developed resistance to it. During the FAO Global Survey of Pesticide Susceptibility of Stored Grain Pests, resistance to malathion in T. castaneum was found in 439 strains out of 505 strains tested, in R. dominica resistance to malathion was found in 50 strains out of 158 strains listed (Champ et al., 1977).

Fenitrothion is a more toxic organophosphorous (LD_{50} to rats: 520mg/kg) compared to malathion. Its agricultural uses include the control of flour beetles, grain beetles and grain weevils (Hassall, 1982). Fenitrothion was found by many workers to be one of the most effective insecticides against T. castaneum (eg Tyler and Binns, 1977; Coulibaly, 1986; Weaving, 1975).

Pirimiphos-methyl, best known as actellic, is an organophorous insecticide developed by the British firm Zeneca, formerly ICI (Imperial Chemical Industries). In Britain pirimiphos-methyl is used primarily to control mites, flour beetles, and grain weevils in stored products and it is less toxic than fenitrothion (LD_{50} to rats: 2000mg/kg) (Hassall, 1982).

The use of insecticides in OPAM stores began in 1976-77. The stores were sprayed with insecticides in the form of wettable powder or emulsifiable concentrates diluted in water. For about six years, treatments were carried out according to a plan based on three treatments per store per year. Operators were initially trained on the job by NRI advisors. A training manual was later elaborated when operators were selected among people with a higher level of education. The doses recommended for application were 1-2% and the rates of application of the insecticide diluted were 10 l/100m^2 for porous surfaces and 5 l/100m^2 for non-porous surfaces (OPAM Training Manual).

The planning of treatments was changed later. Stores were only treated when infestations were found following inspections. This new approach resulted in a reduction of the number of treatments per year, in particular in security stores where the rate of reinfestation was lower due to limited handling in these stores.

Nowadays, very many types of pesticides are found in Mali due to total absence of control and lack of efficient regulations. Several agrochemical companies have sales representatives in Mali and manufacturing branches in some neighbouring countries eg CIBA-GEIGY, BAYER, ICI. There is also a national chemical manufacturing company; Société Malienne des Produits Chimiques (SMPC). Besides these there are many illegal traders. Pesticide misuse and abuse are very common. Unknown chemicals are offered by various salesmen in containers that do not meet the safety requirements (Pers. obs.). They are offered to people like farmers having no knowledge of the use and the hazards attached to them.

Only recently have attempts been made to train people likely to use pesticides. The pest control division of OPAM has been requested by Donor's Committee (PRMC: Programme de Restructuration du Marché Cèréalier) to train about 100 village associations. On the other hand a national committee has been set up to make pesticide regulation proposals. This committee is also supervised by the pest control division of OPAM.

Insecticide resistance in storage insects in Mali was first reported by British Technical Advisors, eg Gilman (Pers. comm.), Taylor and Halliday (1986). Resistance to contact insecticides, namely to fenitrothion and pirimiphos-methyl, in Malian strains of T. castaneum and R. dominica was also found by Coulibaly (1986). More recently Ansell (1992) reported resistance to phostoxin in Malian strains of T. castaneum.

Investigations concerning insecticide resistance may be carried out for several purposes. In certain circumstances, it may be necessary to monitor the extent and the level of resistance; this may be relevant particularly to pest control officers. At a more academic level, the aim might be to study the mechanism of the phenomenon. Many works have focused on comparing susceptible and resistant individuals. For example, Muggleton (1983) studied the relative fitness of malathion-resistant phenotypes of Oryzaephilus surinamensis whereas Wool et al. (1983) studied the inheritance pattern and possible enzymatic mechanisms of malathion-resistance in Tribolium strains and hybrids. In a more recent paper, Dyte (1990) gives a comprehensive review of various aspects of resistance.

In the present work, using the field technique developed by NRI, comparative studies on resistance in different field strains of T. castaneum and R. dominica are carried out to gather information on strains to be used in subsequent experiments.

4.1.2 Materials and Methods

Champ and Campbell-Brown (1970b) provide an evaluation and summary of various methods for detecting insecticide resistance. The basic principle of testing for resistance consists of measuring the response of insects to doses of insecticides applied (FAO, 1969). Test methods differ in the way insecticides are applied and the criteria used to measure insect response. The topical method, for example, consists of applying a known amount of chemical to individual insects, making it possible to relate the response observed directly to the amount of chemical applied. Arthur and Zettler (1992) used the topical method for their investigations in malathion resistance in T. confusum. The topical method contrasts, for example, with the filter paper method where experimental beetles are exposed to filter papers treated with known doses of insecticides and where the actual amount of chemical picked up by the insects is not known. The most widely used method is the FAO filter paper technique (FAO, 1969a), from which a field method has been developed by NRI (Taylor, 1990). This method consists of filter paper pretreated

with discriminating doses of chemicals. A kit has been designed based on that field method by NRI and was used in the present study.

The NRI test kit permits the identification of resistant insects by exposing them to a single discriminating dose of insecticide on treated filter papers. The Watman No 1 filter papers are a standard 7cm diameter and 0.5ml of a solution of active ingredient (a.i.) is applied to each according to FAO (1969). Different insecticides have different discriminating doses, expressed below as %a.i. The kit used in the work reported here permitted identification of resistance only for malathion, pirimiphos-methyl and fenitrothion and only for T. castaneum and Sitophilus spp. Discriminating doses were not available for R. dominica. The discriminating doses for T. castaneum were as follows:

Malathion	=	0.5%
Pirimiphos-methyl	=	0.7%
Fenitrothion	=	0.5%

Insects are exposed to the pretreated filter papers for periods specific to each species. For T. castaneum the exposure period is 5 hours at 30°C.

The kit comprises test papers treated with insecticide, control papers treated only with the solvent mixture, plexiglass rings (5cm diameter, to retain insects), perforated covers, fluon and brushes. Test papers are supplied in packets, labelled with the insecticide and insect species for which they are to be used. When kept in a refrigerator (5°C) papers can be expected to remain effective for six months.

For a full test, three rings on insecticide-treated papers and one ring on untreated control were used and 40 insects were used for each ring. Insects were unsexed, of unknown age and counted at random in groups of 40. For a quick test the method allows the use of only a few insects. Insects were held without food for at least one hour before application into the rings which were then transferred to an oven set at the experimental temperature of 30°C. At the end of the exposure period the

numbers of insects alive or dead were counted. The numbers of insects dead or "knocked-down" were combined and assessed as susceptible ("knocked-down" insects cannot walk straight, or right themselves easily when lying on their backs). When the test method was followed correctly, all control insects remained alive at the end of the exposure period. If there was more than 10% control mortality, the test was repeated as recommended in the instructions. For control mortality less than 10% results had to be corrected using Abbot's formula given below:

$$\text{Corrected mortality} = \frac{P - C}{100 - C} \times 100$$

where: P = % mortality in treatment

C = % mortality in control

The following strains of T. castaneum were used:

- strain N.00 from an OPAM store of Niafunké;
- strain N.04 from a village granary of Zangasso;
- strain N.17 from an OPAM store of Sévaré;
- strain N.09 from a private store of Gao;
- strain N.21 from a private store of Gao;
- strain N.28 from a family granary of Kayes.

Although no discriminating dose was provided for R. dominica, an attempt was still made to use the method to make comparative tests on different strains of this species. Considering that the concentrations of fenitrothion and pirimiphos-methyl required to achieve various levels of "knock-down" (KD_{50}, KD_{99} and $KD_{99.9}$) in R. dominica were higher than in T. castaneum as well as in S. oryzae (Coulibaly, 1986), the highest doses available were used and were as follows:

Fenitrothion	=	0.8%
Pirimiphos-methyl	=	2%
Malathion	=	0.5%

The exposure period observed with R. dominica was 24 hours in accordance with the FAO filter paper method. Three strains of R. dominica were used: strains N.06, N.25 and N.27 respectively from Koutiala (private); Bamako (private) and Kayes (private).

4.1.3 Results and Discussion

4.1.3.1 Results

Tables 4.1 and 4.2 give the number of insects knocked-down at the discriminating doses of the different insecticides tested in strains of T. castaneum and R. dominica. Data were analysed using the Minitab package. Each treatment was tested for homogeneity (Chi square) and analysis of variance was then performed. In both species, the analysis of variance indicated significant differences between strains as well as between insecticides. The strain-insecticide interaction was found to have a significant effect on the number of insects knocked-down.

Results expressed as mortality (%) at discriminating doses (% of active ingredient or %a.i.) of the different insecticides are shown in Table 4.3, where each figure is a mean of three replicates corrected when necessary for the mortality in the controls. Table 4.4 shows strains in decreasing order of resistance for each chemical. For example, it shows that for fenitrothion, malathion and pirimiphos-methyl, the most resistant strains are respectively strain N.00, strain N.19 and strain N.17 in T. castaneum. In R. dominica the three strains tested are all susceptible to fenitrothion (100% mortality), whereas for malathion and pirimiphos-methyl the most resistant strain is strain N.27 for both. It seems that strains of T. castaneum collected from OPAM stores (N.00, N.17) where regular insecticide treatments are known to be carried out, exhibit resistance to the three chemicals tested. It is also interesting to note that all strains of T. castaneum show a significant level of resistance to malathion. In R. dominica, strain N.27 shows the highest level of tolerance to both malathion and pirimiphos-methyl although the mortalities recorded are high (96% with malathion and 98% with pirimiphos-methyl).

Table 4.5 shows a comparison of the relative effectiveness of the three insecticides used against the different strains of T. castaneum and R. dominica. In T. castaneum, fenitrothion and pirimiphos-methyl show equal effectiveness for all strains except strain N.00 (where pirimiphos-methyl is best) and strain N.17 (where fenitrothion is best). Malathion is the least effective for all strains. In R. dominica, fenitrothion is most effective against all strains; the difference between malathion and pirimiphos-methyl is not significant although pirimiphos-methyl is slightly more effective. In fact, to increase its effectiveness, particularly against R. dominica, pirimiphos-methyl has recently been mixed with a pyrethroid to give an insecticide known under the trade name of Actellic "plus".

Table 4.1a: Number of adult insects knocked-down at discriminating doses of fenitrothion, malathion and pirimiphos-methyl in strains of T. castaneum.

Chemicals	Replicate No	N.00	N.04	N.09	N.17	N.21	N.28	Chi square
Fenitrothion (0.5%)	1	11	40	40	34	40	40	Not significant (Treatment homogen-ous)
	2	15	40	40	38	40	40	
	3	9	40	40	38	40	40	
	Control	0	0	0	0	0	0	
Malathion (0.5%)	1	2	24	0	1	21	37	Not significant (Treatment homogen-ous)
	2	0	18	0	1	18	37	
	3	2	23	1	0	19	37	
	Control	0	0	0	0	0	2	
Pirimiphos-methyl (0.7%)	1	40	40	40	30	40	40	Not significant (Treatment homogen-ous)
	2	39	40	40	34	40	40	
	3	40	40	40	30	40	40	
	Control	0	0	0	0	0	0	

Table 4.1b: Analysis of variance of number of insects knocked-down in different strains of T. castaneum at discriminating doses of fenitrothion, malathion and pirimiphos-methyl.

Source	df	SS	MS	F	P
Strain	5	2825.65	565.13	290.67	0.000
Compound	2	6599.70	3299.85	1697.07	0.000
Interaction	10	2716.74	271.67	139.72	0.000
Error	36	70.00	1.94		
Total	53	12212.09			

Table 4.2a: Number of adult insects knocked-down at chosen doses of fenitrothion, malathion and pirimiphos-methyl in strains of R. dominica.

Chemicals	Replicate No	N.06	N.25	N.27	Chi square
Fenitrothion (0.8%)	1	40	40	40	Not significant (Treatment homogen-ous)
	2	40	40	40	
	3	40	40	40	
	Control	0	0	1	
Malathion (0.5%)	1	39	39	35	Not significant (Treatment homogen-ous)
	2	40	40	35	
	3	40	40	37	
	Control	0	0	1	
Pirimiphos-methyl (0.7%)	1	39	38	36	Not significant (Treatment homogen-ous)
	2	40	40	39	
	3	39	40	39	
	Control	0	0	1	

Table 4.2b: Analysis of variance of number of insects knocked-down in different strains of R. dominica at experimental doses of fenitrothion, malathion and pirimiphos-methyl.

Source	df	SS	MS	F	P
Strain	2	12.9630	6.4815	8.75	0.002
Compound	2	18.9630	9.4815	12.80	0.000
Interaction	4	16.5926	4.1481	5.60	0.004
Error	18	13.3333	0.7407		
Total	26	61.8519			

Table 4.3: Mortality rate (%) at discriminating doses of three organophosphorous in strains of T. castaneum and R. dominica.

a) Tribolium castaneum

Compounds	%a.i.	N.00	N.04	N.17	N.09	N.21	N.28
Fenitrothion	0.5	29.16	100	91.66	100	100	100
Malathion	0.5	3.33	41.25	1.66	0.83	48.75	92.94
Pirimiphos-methyl	0.7	99.16	100	78.33	100	100	100

b) Rhyzopertha dominica

Compounds	%a.i.	N.06	N.25	N.27
Fenitrothion	0.8	100	100	100
Malathion	0.5	99.66	99.66	96
Pirimiphos-methyl	2	99.33	99.33	98

Table 4.4: Comparison between strains of T. castaneum and R. dominica in their tolerance to three organophosphorous: for each chemical, strains are in order starting from the most tolerant (or most resistant) to the most susceptible.

a) T. castaneum

Fenitrothion	N.00	N.17	N.04 = N.09 = N.21 = N.28 = 100%
Malathion	N.09	N.17	N.00 N.04 N.21 N.28
Pirimiphos-methyl	N.16	N.00	N.04 = N.09 = N.21 = N.28 = 100%

b) R. dominica

Fenitrothion		100% in all strains
Malathion	N.27	N.06 = N.25 = 99.66
Pirimiphos-methyl	N.27	N.06 = N.25 = 99.33

Table 4.5: Comparison between three organophosphorous chemicals in their effectiveness (mortality caused) against strains of T. castaneum and R. dominica.

a) T. castaneum

	N.00	N.04	N.17	N.09	N.21	N.28
1st	PM	F = PM	F	F = PM	F = PM	F = PM
2nd	F	-	PM	-	-	-
3rd	M	M	M	M	M	M

b) R. dominica

	N.06	N.25	N.27
1st	F	F	F
2nd	PM	PM	PM
3rd	M	M	M

4.1.3.2 Discussion

Table 4.3a shows quite clearly that resistance to fenitrothion and pirimiphos-methyl appears in strains of T. castaneum collected in OPAM stores where regular chemical treatments are carried out. The development of insecticide resistance following the use of the same chemical for a longer period of time is explained in FAO (1969). Every treatment kills the most susceptible individuals, leaving behind the most tolerant ones, and resistance is brought about by selective breeding of these survivors. This leads to the decrease of the mean susceptibility of the population and, in genetical terms, an increase in the frequency of the genes conferring resistance. The appearance of resistance to malathion in all the strains could be explained by the fact that this chemical might have been widely used in agriculture, particularly in the "CMDT" zones (eg Zangasso where strain N.04 was collected), and in public hygiene.

The same observations do not apply to R. dominica where all the strains show total susceptibility to fenitrothion and very limited levels of resistance to both pirimiphos-methyl and malathion, although a valid comparison between R. dominica and T. castaneum cannot be made as the method used did not provide a discriminating dose for R. dominica at the time of the present work. In practice the fact that R. dominica has been more difficult than T. castaneum to control in OPAM stores (Pers. obs.) may be because in R. dominica, the immature stages in particular, living inside the grain are not reached sufficiently by the chemicals applied.

The results obtained here confirm the wide spread of insecticide resistance in storage insects reported by workers cited earlier in this chapter. Different strains, in T. castaneum particularly, show very significant differences in their susceptibility to the chemicals used. According to Dyte (1990) resistant and susceptible strains should differ in properties other than their ability to tolerate particular pesticides. It is well established that reproductive disadvantages are not always associated with resistance (Rousch and Croft, 1986) although Bhatia and Pradhan (1968) found in laboratory-selected strains that resistant strains were less fit than susceptible strains.

Although pesticide resistance has long been recognised as evolutionary change (Dobzhansky, 1937), very little is known about the initial frequency of resistant alleles (Rousch and Croft, 1986). Biotypes represent evolutionary transients in the process of speciation and development through natural selection (Saxena and Barrion, 1987). Resistance to insecticides might help to characterize biotypes in T. castaneum since strains showed different resistance status, though no correlation was found between the resistance status of strains and their geographic origins. As insecticide toxicity increases and resistance factor decreases with increasing temperatures (Coulibaly, 1986), the resistance level would be expected to be higher in cooler regions (eg Zangasso) compared to hotter regions (eg Gao, Kayes), but this was not the case. The reason might be that the different populations are

continuously mixed because of the permanent introduction of strains from the agricultural surplus areas (cooler regions of the south) to the agricultural deficit areas (hotter regions of the north) through grain trade.

4.2 LIFE HISTORY STUDY

4.2.1 Introduction

Life history includes all the events that mark an organism's path from conception to death (Peters, 1983). In coleopteran insects, life history is the sequence of the following stages: egg, larva, pupa and adult. It is characterized by parameters that are affected by various factors, eg temperature, relative humidity, food. They are also affected by genetical factors (Sokoloff, 1977) and by population density.

Life-history parameters have been subject to extensive investigations by many workers for various aims. The life-history parameters commonly studied are the fecundity, the productivity, the adult developmental period, egg hatchability, time to sexual maturity, the pre-oviposition period. They are sometimes combined as a measure of fitness (Smith, 1991).

Adult developmental time, also referred to as time to adult emergence, is the period of time between egg laying and adult emergence. The effect of temperature and relative humidity on the development of T. castaneum and R. dominica have been studied by several research workers. Soliman and Hardin (1972) for example found that in T. castaneum developmental time can vary from 24.6 days to 33.3 days according to the experimental conditions. The most detailed studies on these two species are provided by Howe (1956, 1962), Dawson (1964a), Birch (1944, 1945, 1945c), White (1987).

Fecundity and productivity relate respectively to the number of eggs and the number of progeny produced by a female in a given period of time (a day or the entire life of the individual). Howe (1962) found that oviposition rate in T.

castaneum is highest at 32.5°C and Sonleitner and Guthrie (1991) showed that it is affected by crowding.

Genetic variations in life history parameters have been reported in many sources, an account of these variations is given in Sokoloff (1977). For example, it has been shown by Sokoloff, Shrode and Bywaters (1965) that productivity is influenced by genetic and environmental factors and interactions. The same authors stated that homozygous mutants are less productive than heterozygous and wild types.

In the work presented here the aim was to characterize life-history parameters of different Malian strains of T. castaneum and R. dominica.

Some preliminary investigations were carried out initially on several strains of the two species mentioned above after which two strains of T. castaneum were retained for the selection experiments.

Reciprocal or simple transplant experiments can be used to compare populations; the method consists in transplanting individuals from a number of habitats to a common habitat in which their life history components are measured, population difference would then reflect genetic differences (Sibly & Antonovics, 1992). It was intended to perform such study during the present research; that would require regular trips to experimental cities. The insecurity then in the northern regions of the country made the transplant experiments impossible and they were cancelled.

4.2.2 Materials and Methods

The life-history experiments were performed in three different situations according to availability:

- in uncontrolled, ambient conditions of the OPAM laboratory in Mali;
- in controlled conditions (still in the OPAM laboratory) using desiccators containing saturated salt solution (as described by Solomon, 1951) and placed in an incubator set at the temperature of 30°C;

- in constant temperature room (CT-room) of the Department of Zoology of the University of Leicester where conditions were 30°C and 45-55%RH.

The culture medium described in the general Materials and Methods was used for the experiments.

The following strains of T. castaneum were used in the preliminary investigations at laboratory uncontrolled conditions:

- strain N.00 from Niafunké;
- strain N.04 from Zangasso;
- strain N.10 from Mopti;
- strain N.21 from Gao.

The strains of R. dominica used in the same conditions were:

- strain N.06 from Koutiala;
- strain N.25 from Bamako;
- strain N.27 from Kayes.

Strains N.17 and N.21 of T. castaneum were subject to more detailed investigations at uncontrolled conditions.

For quick reference the places of origin and the resistance status (% kill at discriminating doses) of the strains used are given below:

Species	Strain	Place of Origin (City)	Resistance Status		
			Fenitrothion	Malathion	Pirimiphos-methyl
	N.00	Niafunké	29.16	3.33	99.16
T. castaneum	N.04	Zangasso	100	41.25	100
	N.21	Gao	100	48.75	100
	N.06	Koutiala	100	99.66	99.33
R. dominica	N.25	Bamako	100	99.66	99.33
	N.27	Kayes	100	96	98

Controlled condition was achieved using desiccators containing saturated solution of sodium chloride giving a relative humidity of 75% (Solomon, 1951) and placed in a cooled incubator set at an experimental temperature of 30°C. Desiccators were regularly opened every two days to allow aeration.

Plastic vials, 5.5cm in depth and 3cm in diameter were used to hold the insects. Vials were closed with plastic lids which were perforated to allow aeration. A hand tally was used for counting insects and when experiments were performed at uncontrolled conditions, a thermo-hygrograph was set for recording temperature and relative humidity variations. In Mali an analytical balance (OHAUS Triple Beam Balance) was used to measure body weight. In the UK it was a CAHN C-31 Microbalance (accurate to $\pm$ 0.1µg).

The life-history study consisted of following the development of individuals from egg to adult stage making appropriate measurements according to the aims of the study. The methods described concern basically both T. castaneum and R. dominica. Where relevant, specific experimental details will be given for each species.

4.2.2.1 Egg hatchability and time to hatch

T. castaneum: Adults were put in pairs and each pair was left on 2g of culture medium and allowed to oviposit for one day. They were then removed. To collect the eggs, the flour was first sifted through a sieve with 850-600μ mesh, the fine flour and the eggs passing through were checked under microscope and the eggs were removed using a fine brush and transferred individually to empty vials. Eggs were then checked daily for hatch (larval emergence). Every day the number of eggs hatched and the date were recorded. Due to the difficulty in egg collection in T. castaneum a maximum of 20 pairs were treated at a time and two experiments were performed on each strain.

R. dominica: Strips of crepe paper were introduced in culture jars for one day and then removed and checked for eggs. Eggs were collected and treated as described in T. castaneum.

4.2.2.2 Egg to adult developmental period and body weight at emergence

As they emerged from eggs, larvae were transferred in individual vials containing 2g of culture medium. A treatment consisted of 20 individuals. Vials were then left undisturbed at the experimental conditions. At pupal stage, the flour was removed and pupae were kept in the vials and any adult emergence could be checked easily. At adult emergence the date was recorded and body weight measured. Egg to adult developmental period is the number of days elapsed between the date on which eggs were laid and the date on which adults emerged. Dividing one by the egg to adult developmental period gives the development rate which is a very important fitness trait. Adults were also weighed on the day of their emergence, the weight obtained was referred to as body weight at emergence.

4.2.2.3 Pre-oviposition period

This trait was studied in two strains of T. castaneum. Pupae were collected, sexed and sexes were kept separate until adult emergence began. As they emerged

virgin adults were paired and each pair was left in a separate vial containing 2g of culture medium. A treatment consisted of 25 pairs. Pairs were transferred every day to a new vial for two weeks. In this way vials were in series of 1 to 15 days of age. Vials were checked when they were at least two weeks old for the presence of larvae. When larvae were found in a vial, the age (in days) of its series was recorded. Pre-oviposition period or time to sexual maturity was the earliest age at which larval presence was recorded.

4.2.3 Results

Results are summarised in Tables 4.6 to 4.10. They were analysed using the Minitab package and the following tests were performed:

- Chi square contingency table test was carried out to test for homogeneity of strains in egg hatchability; a pair wise t-test was then done.
- weight of adults and/or pupae and developmental period were analysed using t-test or analysis of variance.

Table 4.6a gives the results of experiments performed during the month of April 1991 and the minimum and maximum temperatures and relative humidities recorded were as follows:

	Temperature	Relative Humidity
Maximum	30°C	62%
Minimum	31°C	29%

Experiments reported in Table 4.7 were performed during the month of December 1991 and conditions were as follows:

	Temperature	Relative Humidity
Maximum	28°C	60%
Minimum	23°C	42%

(see graph at Appendix 4).

4.2.3.1 T. castaneum

The strains show significant difference in egg hatchability (Tables 4.6 to 4.9), the values of χ^2 are given below the respective tables. Table 4.7 gives more detailed results concerning two strains of T. castaneum (N.17 and N.21) at OPAM's laboratory conditions. No significant difference was found between the two strains in developmental period and in body weight at emergence ($P > 0.05$ in both cases). In Tables 4.8 a and b, (at constant temperature) the difference between the two strains in adults' body weight was not significant but the difference in development period was highly significant: strain N.21 (Gao) showed a significantly shorter developmental period compared to strain N.04 (Zangasso). The t. test indicated that strains N.10 and N.00 (Table 4.9) had significantly different pre-oviposition periods ($P = 0.013$). No significant difference was found in time to hatch in strains used in the same experiment, but there was a difference between the results in experiments performed at different periods (eg Tables 4.6 and 4.7).

4.2.3.2 R. dominica

Tables 4.10a and b show differences between the strains studied in egg hatchability and in time to hatch.

Table 4.6a: Chi square analysis on number of eggs hatched in four strains of T. castaneum at OPAM's laboratory conditions.

	Hatched	Not Hatched	Total	% Hatch
N.00	69	20	89	77.5
N.04	63	26	89	70.8
N.10	33	7	40	82.5
N.21	48	26	74	64.9
Total	213	79	292	72.9

$\chi^2 = 5.456$ with 3df

Table 4.6b: Time to hatch (days) in four strains of T. castaneum at OPAM's laboratory conditions:

Strains	Time to hatch (no variance) (days)
N.00	3
N.04	3
N.10	4
N.21	3

Table 4.7: Life-history parameters of strain N.17 and strain N.21 of T. castaneum estimated at OPAM's laboratory uncontrolled conditions.

	Strain N.17	Strain N.21
Egg hatchability (%)	65 ($\chi^2 = 1.129$ df = 1)	80
Time to hatch (days)	5	5
Time to pupate (days)		
◦ Mean	50.3	49.53
◦ St deviation	2.83	3.31
◦ n	15	10
Weight of pupa at emergence (mg)		
◦ Mean	3.13	2.15
◦ St deviation	1.20	0.57
◦ n	10	15
Weight of adults at emergence (mg)		
◦ Mean ± SE	1.663 ± 0.118	1.90 ± 0.114
◦ St deviation	0.88	0.58
◦ n	8	12
Egg to adult development period (days)		
◦ Mean	56 ± 1.13	54.6 ± 0.855
◦ St deviation	3.39	3.31
◦ n	9	15
Sex ratio		
% Male	50	66.66

Table 4.8a: Life-history parameters of strains N.04 and N.21 of T. castaneum estimated at 30°C.

	Strain N.04	Strain N.21
Egg hatchability (%)	70 ($\chi^2 = 7.059$ df = 1)	100
Time to hatch (days)		
◦ Mean ± SE	3.4 ± 0.163	3 ± 0
◦ St deviation	0.516	0
◦ n	10	20
Egg to adult development period (days)		
◦ Mean ± SE	25.571 ± 0.896	23.250 ± 0.160
◦ St deviation	2.370	0.716
◦ n	7	20
Adult body weight (mg)		
◦ Mean ± SE	1.700 ± 0.100	1.74 ± 0.05
◦ St deviation	0.265	0.233
◦ n	7	20

Table 4.8b: Analysis of variance for adult developmental period in strains N.04 and N.21 of T. castaneum at 30°C.

Source	df	SS	MS	F	P
Strain	1	27.943	27.943	16.07	0.000
Error	25	43.464	1.739		
Total	26	71.407			

Table 4.9: Pre-oviposition periods (days) of strain N.00 and Strain N.10 under ambient (uncontrolled) conditions in the OPAM laboratory.

	Strain N.00	Strain N.10
Range (days)	5 – 11	5 – 8
Mean (days) ± SE	7.73 ± 0.452	6.22 ± 0.324
St deviation	1.751	0.972
n	25	25

Table 4.10a: Chi square analysis on number of eggs hatched in three strains of R. dominica at OPAM's laboratory conditions.

	Hatched	Not Hatched	Total	% Hatch
N.06	84	4	88	95.4
N.25	28	5	3	48.8
N.27	23	0	23	100
Total	135	9	144	

$\chi^2 = 6.432$ with 2df

Table 4.10b: Time to hatch (days) in three strains of R. dominica at OPAM's laboratory conditions:

Strains	Time to hatch (days)
N.06	6
N.25	6
N.27	7

4.2.4 Discussion

4.2.4.1 T. castaneum

When experiments were performed under ambient conditions, results were not similar to those reported in the literature because most of the results reported in the literature were obtained at controlled conditions. For egg hatchability, for example, Howe (1962) found a range of 76.1 to 92.2%; Shazali and Smith (1986) found 89.3% at 30°C and White (1987) obtained 89% at 37.5°C. The results obtained here at uncontrolled conditions, in particular in Table 4.6, are lower than the results given above. The reason for such difference might be the fluctuating conditions. The temperatures recorded on the thermo-hygrograph fluctuated between 31°C and 36°C in the period during which experiments reported in Table 4.6 were performed and fluctuations of temperature between 23°C and 28°C were recorded in the period during which experiments reported in Table 4.7 were carried out. For the same reason, the developmental period at laboratory conditions (Table4.7) is much longer than the figures reported in the literature, most of which were obtained under controlled conditions. Time to hatch at the high range temperatures (Table 4.6) was 3 to 4 days and at the low range temperatures (Table4.7) it was 5 days confirming the statement made by Abdelsamad et al. (1988) that time to hatch is significantly affected by temperature. Pre-oviposition period in the two strains studied was in the range of 5 to 11 days, which is wider than the range of 7 to 10 days reported by Howe (1962), the reason being again that Howe's study was done at controlled temperature and relative humidity (25°C and 70%).

When experiments were repeated under controlled conditions (Table 4.8) results were similar to those reported by other workers. For example, the egg to adult developmental periods were 25.57 days for strain N.04 and 23.25 days for strain N.21, these results are in the range reported in the literature. Howe (1956), for example, stated that under optimal conditions, the development of T. castaneum can be completed in 20 days; Park and Frank (1948) found a development period of 25.5 days at 29°C.

Among the life-history traits studied, significant differences between strains were found in egg hatchability (Table 4.6) and adult development period (Table 4.8b) and in pre-oviposition period (Table 4.9). Soliman & Hardin (1972) found also significant differences between strains of T. castaneum in developmental period, but White (1984) failed to find significant differences among field strains although he found laboratory strains were significantly different from field strains. The difference between strains in developmental period can be the effect of various factors. The strains may be different genetically; developmental time is known to be partly under genetic control (Dawson, 1965). The difference in their habitat may have produced a difference in the developmental period of the two strains (Table 4.8). As suggested by Li et al. (in press), differences in life history traits between strains may reflect difference in their habitats. Bradshaw (1984) gives good examples of population difference in response to climate and reviews some transplant experiments where it was found that extreme populations of the same species could not survive in each others' habitats. Strain N.04 (Zangasso) and strain N.21 (Gao) are from contrasting climatic areas (see map in the introduction). Basically Zangasso (in the south) is a wetter and cooler place with an annual rainfall over 1400mm (Reuben, 1987) compared to Gao in the drier and hotter northern regions with annual rainfall below 250mm and daily maximum temperatures exceeding 38°C during the hottest months (Reuben, 1978).

4.2.4.2 R. dominica

All the results were obtained under ambient laboratory conditions. The strains used did not differ significantly in the traits studied. No detailed investigation was performed on strains of R. dominica. Because development of this species takes place inside the grain, in practice it is difficult to estimate parameters such as developmental period, body weight at emergence. To make such measurements would require to dissect grains and would complicate experimental design. Therefore, partly because of practical reasons with regard to the facilities available in the OPAM laboratory but mostly because of lack of variation among R.

dominica strains, T. castaneum alone was used for the quantitative genetics and selection experiments.

4.3 CONCLUSION

Relating the results of life-history traits reported in the second part to the results of resistance tests reported in the first part leads to no consistent relationship. In Table4.6a, strain N.00 showed an egg hatchability significantly higher (77.5%) than strain N.04 (70.8%) and N.21 (64.9%), in other words the fitness of strain N.00 was higher than the fitness of both strains N.04 and N.21. In Table4.1a giving the number of insects knocked-down at discriminating doses of the three chemicals tested, of the above strains, strain N.00 had the lowest number knocked-down hence the highest level of resistance. Therefore the fittest strain appeared to be the most resistant. In Table 4.8a, strain N.21 had higher egg hatchability and shorter development period than strain N.04 nevertheless in Table4.1a the two strains showed the same resistance level to all the pesticides tested. Therefore one can only agree with Dyte (1990) when he states that, if we are to understand the effects of resistance on fitness, we need to distinguish the true pleiotropic effects of the resistance gene(s) from incidental strain differences which may be inherited independently and are thus not an inevitable concomitant of the resistance being studied.

For such study, the use of a single discriminating dose may not be the most appropriate method. The NRI Test Kit is really designed for field screening (Taylor, 1990). Better comparison could be done using the filter paper technique as in Coulibaly (1986) or the topical method as in Dyte & Daly (1970) and Arthur and Zettler (1992). These methods permit the determination of particular doses (eg LD_{50}, LD_{99}) for the strains studied making comparison between strains more precise. None of these methods was available in Bamako where the resistance tests were performed.

Chapter 5 QUANTITATIVE GENETICS OF LIFE-HISTORY TRAITS IN TRIBOLIUM CASTANEUM

5.1 INTRODUCTION

Quantitative genetics is concerned with the inheritance of those differences between individuals that are of degree rather than kind, quantitative rather than qualitative (Falconer, 1989). Qualitative characters are those which modify a trait in a visible way (eg colour), quantitative traits are those which are controlled by numerous genes at many loci, their effect is measured by statistical method, eg body weight, developmental period (Sokoloff, 1977). Variations in quantitative characters are continuous. Any attribute that varies continuously and can be measured might be studied as a metric character (Falconer, 1989). Falconer (1989) states also that the genetics of a metric character centres round the study of its variation. Explaining how genetic variability might be maintained under natural selection has preoccupied population geneticists for many years (Smith et al., 1987). Genetic variability may be maintained by a life-history trade-off (Smith et al., 1987). Trade-offs are linkages between traits that constrain the simultaneous evolution of two or more traits (Stearns, 1992). The importance of demonstrating trade-offs is that they reveal some of the constraints on an animal's evolutionary options in a given environment (Sibly & Calow, 1986). A negative genetic correlation between traits is taken as an indication of a genetic trade-off between them (Møller et al., 1990), which will act as a genetic constraint on evolution. Møller et al. (1989) for example found that in Callosobruchus maculatus, development rate is genetically negatively correlated with both adult longevity and fecundity. Stearns (1992) distinguishes a physiological trade-off, which is the energy allocation between two or more functions competing for the same resources within a single individual, from the evolutionary trade-off defined by manipulation experiments on phenotypes and by correlated response on populations. Stearns (1992) summarizes the energy-allocation principle in the following words, "the optimal animal, born with some amount of energy, proceeds

through life gaining and expending energy according to some schedule that maximizes total reproduction output."

The value observed when a character is measured on an individual is the phenotypic value (Falconer, 1989). In order to analyse the genetic properties of the population the phenotypic value has to be divided into parts attributable to different causes. Individuals have different phenotypes because their genotypes are different and because they are exposed during development to different environments (Bowman, 1974).

The variance of phenotypic values (V_P) is the sum of the variance of genetic values (V_G) and the variance of environmental values (V_E):

$$V_P = V_G + V_E \qquad \text{(Falconer, 1989).}$$

The genetic variance is composed of the additive variance (V_A), the dominance variance (V_D) and their interaction (V_I) hence:

$$V_G = V_A + V_D + V_I \qquad \text{(Falconer, 1989).}$$

The additive variance is the important component and the main cause of resemblance between relatives. In practice phenotypic variance is partitioned into additive genetic variance versus non-additive genetic and environmental variance (Falconer, 1989). The proportion of total phenotypic variance among individuals accounted for by genetic variance is the heritability, h^2 (Bowman, 1974; Falconer, 1989; Stearns, 1992):

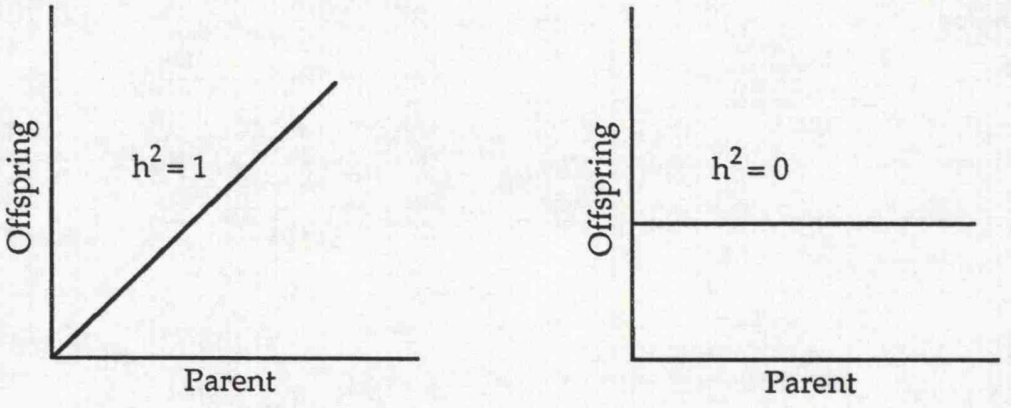
$$h^2 = {}^{V_A}\!/_{V_P}$$

There are two measures of heritability:

a) broad-sense heritability which is the proportion of total phenotypic variance among individuals in a population that is accounted for by genetic effects in general. It can include maternal effects as well as effects of dominance and epistasis;

b) narrow-sense heritability is the proportion of phenotypic variance among individuals in a population that is accounted for by additive genetic variance (Stearns, 1992).

The heritability can be estimated by several methods based on the degree of resemblance between relatives. Here the method used is based on the offspring-parent regression where the values for a character in offspring are regressed on the values of the same character in parents. The regression coefficient, b is used to estimate heritability (h^2). As illustrated in the figures below, $h^2 = 1$ indicates that all the variation in the offspring is caused by genetic factors and $h^2 = 0$ indicates that there is no genetic factor involved in the variations observed in the offspring in the trait under consideration:



The table below shows the composition of the phenotypic covariances and the heritability estimated by regression (b) or correlation (t).

Relatives	Covariances	Regression (b) or Correlation (t)
Offspring and one parent	$\frac{1}{2}V_A$	$b = \frac{1}{2}h^2$
Offspring and mid-parent	$\frac{1}{2}V_A$	$b = h^2$
Half sibs	$\frac{1}{4}V_A$	$t = \frac{1}{4}h^2$
Full sibs	$\frac{1}{2}V_A + \frac{1}{4}V_D + V_{EC}$	$t \geq \frac{1}{2}h^2$

(from Falconer, 1989).

In the above table the mid-parent value of a trait is the mean of the male parent value and the female parent of that trait.

Characters can have relationships known as correlation. A correlation may be caused by different factors. Falconer (1989) describes the following correlations:

- the phenotypic correlation which is the association between two characters that can be directly observed in the correlation of phenotypic values;
- the genetic correlation which is the correlation of breeding values (see Falconer, 1989, for the definition of breeding values);
- and the environmental correlation which is the correlation of environmental deviation together with non-additive genetic deviation.

Since it was found in 1958 that Tribolium spp was a potentially useful material for genetic studies (Sokoloff, 1977), many investigations have been carried out on that species, a good review of which is provided by Sokoloff (1972, 1974 and 1977). Heritability of pupal weight in T. castaneum has been estimated by many research workers, eg Wilson et al. (1965); Boyland and Wong (1965c). Maternal effects on quantitative characters in the development of T. castaneum were estimated by Dawson (1964b). Correlations between life-history traits, in particular between body weight and other parameters such as developmental period and productivity, were studied by various workers. Li et al. (in press) for example found a positive linear correlation between female body size and productivity in field strains of T. castaneum, but they did not estimate the genetic correlation.

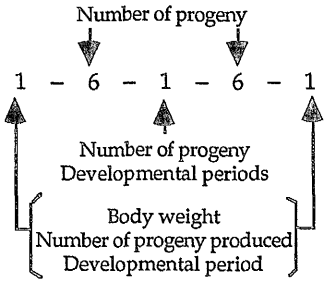
The aim of the present study was to investigate the heritability in and the correlation between components of fitness in a field strain of T. castaneum from Mali in order to examine the evidence for life-history trade-offs.

5.2 Materials and Experimental Design

Strain N.21 was used in this experiment which was designed as follows.

Several adult females were allowed to oviposit for one day on a small amount of culture medium. After the removal of adults, the amount of culture medium was made up to 100g. As they emerged, pupae were collected and sexed until the required numbers of 200 females and 100 males were obtained. The two sexes were kept separate and as adults emerged, their body weights were measured using the CAHN microbalance C-31 and their developmental period was recorded. Females were divided into two sets of 100 individuals. Females in both sets were kept in individual vials (vial specifications are as described in previous chapters) which were numbered from 1 to 100 in the first set and from 101 to 200 in the second set. Females in set one were each paired with one of the 100 males at random, and were then allowed to mate for three days starting from the day on which both females and males emerged. Males were then transferred to set two so that the male which was paired with the female in vial number 1 went to vial number 101 in the second set, the male from vial number 2 to vial number 102 and so on. Thus each male was mated to two females. Females in set two were also allowed to remain with their male mates for three days. In both sets pairs were in the presence of 2g of culture medium. In each set, at the end of the mating period females were transferred to new vials containing fresh food following successive periods of 1-6-1-6-1 days. In the 1 day periods, females were allowed to oviposit for 1 day in the presence of 5g of culture medium and in the 6 day periods they were allowed to oviposit for 6 days in the presence of 15g of culture medium. Cultures were kept in a constant temperature room where the temperature was 30°C and the relative humidity varied between 45 and 55%.

The diagram below shows when the different measurements were carried out.



Body weight was measured on four offspring (two males and two females) of each female parent. To find developmental period, after pupation vials were checked daily, emergences and dates of their occurrence were recorded. Developmental period was calculated as the mean of the different developmental periods recorded. The number of progeny was obtained by counting the number of adults in each vial and taking the mean for each one- or six-day period.

For easy reference the measurements made are summarised below:

5.2.1 Measurements on Parents:

- body weight at emergence;
- developmental period – but unfortunately parents were selected on this basis and therefore cannot be analysed;
- productivity schedule.

5.2.2 Measurements on Offspring:

- body weight at emergence;
- developmental period
- productivity schedule.

Data obtained from the above measurements were used for the quantitative genetic analysis.

5.3 QUANTITATIVE ANALYSIS

5.3.1 Results

5.3.1.1 Phenotypic variation in body weight:

Tables 5.1 and5.2 describe body weight in parents and offspring respectively and Figures 1 to 7b show the distribution of body weight in parents and offspring. The distribution of body weight in insects has often been shown to be approximately normal (eg Holloway, 1984; Wrelton, 1987).

Female parents in set 1 and set 2 differed significantly in body weight ($P < 0.05$), set 2 being heavier. The experimental procedure resulted in the selection of early females (emerging first) in set 1 and of late females (emerging later) in set 2. Although female parents in set 1 and set 2 were from the same culture and the same generation with an overall mean developmental period of 26.0 days, the first 100 females used in set 1 had a mean developmental period of 25.2 days (SD = 0.39) while the next 100 females used in set 2 had a mean development period of 26.8 days (SD = 0.72). Mid-parent weights (the mean weights of the male and female pairs) were also significantly different in the two sets. Female adults were significantly heavier than male both in parents and offspring ($P < 0.05$ for all). Late offspring were significantly heavier than early offspring in set 2 ($P = 0.0007$) but not in set 1 ($P = 0.73$). Both early and late offspring in set 2 were heavier respectively than early and late offspring in set 1, but the difference between the two sets was significant only in late offspring ($P = 0.0002$).

5.3.1.2 Estimation of heritability of body weight:

Offspring-parent regression was used to estimate heritability (h^2) of body weight. The following regressions were performed:

- early offspring on mother (both sets);
- early offspring on father (both sets);
- early offspring on mid-parent (both sets);
- late male offspring on mother (both sets);
- late male offspring on father (both sets);

- late male offspring on mid-parent (both sets);
- late female offspring on mother (both sets);
- late female offspring on father (both sets);
- late female offspring on mid-parent (both sets);
- late mean offspring on mother (both sets);
- late mean offspring on father (both sets);
- late mean offspring on mid-parent (both sets).

The results are summarized in Tables 5.3 to 5.7.

Heritability estimates of body weight appear highest in female parents, presumably because of maternal effects as reported by various authors (eg Dawson, 1964b). Heritability is also higher in female offspring as compared to male offspring and higher in late offspring (except for male offspring in set 1) as compared to early offspring. Comparing heritability in set 1 to heritability in set 2 shows no consistent pattern. The estimated additive genetic variance (V_A) varied according to which parent offspring values were regressed on but remained consistently higher in female offspring as compared to male offspring and was, of course, always less than the phenotypic variance (V_P) (Tables5.4 and 5.5). The estimated genetic variance is higher in female parents (Table 5.4) as compared to male parents (Table 5.5). Table 5.7 gives the contribution of genetic components to variance in body weight, the highest genetic contribution (a genetic coefficient of variation of 7%) was found in female parents.

5.3.1.3 Correlation between body weight and developmental rate:

Figures 8a to 9b illustrate the negative correlations between offspring body weight and developmental rate. The coefficient of correlation is higher in late offspring in both sets. The negative correlation indicates a trade-off between body weight and development rate. However, these are phenotypic correlations and do not necessarily show a genetic trade-off, though if both characters had reasonably high heritabilities, the sign of the phenotypic correlation would be the same as the sign of the genetic correlation (Smith, 1991).

Table 5.1: Description of female parents and mid-parent weights in set 1 and set 2 and of male parent weight of strain N.21 of T. castaneum.

	Mean (mg) (± SE)	Variance	Number of observation (n)
Female parent in set 1	1.731 (± 0.014)	0.021	100
Female parent in set 2	1.871 (± 0.021)	0.044	100
Mid-parent in set 1	1.680 (± 0.010)	0.011	100
Mid-parent in set 2	1.750 (± 0.013)	0.017	100
Male parent	1.629 (± 0.012)	0.016	100

Table 5. 2: Description of body weight in early and late offspring of strain N.21 of T. castaneum in set 1 and set 2.

	Mean (mg) (± SE)	Variance	Number of observation (n)
Early offspring in set 1 (male and female)	1.852 (± 0.016)	0.020	83
Early offspring in set 2 (male and female)	1.880 (± 0.016)	0.023	94
Late male offspring in set 1	1.774 (± 0.021)	0.040	90
Late female offspring in set 1	1.949 (± 0.023)	0.047	89
Late mean offspring in set 1	1.860 (± 0.020)	0.036	90
Late male offspring in set 2	1.901 (± 0.021)	0.041	90
Late female offspring in set 2	2.034 (± 0.022)	0.044	90
Late mean offspring in set 2	1.967 (± 0.020)	0.036	90

Table 5.3: Estimates of heritability (± SE) of body weight in strain N.21 of T. castaneum using regression of offspring weight on female parent, male parent and mid-parent weight in set 1 and set 2.

	Parents in set 1			Parents in set 2		
	Female	Male	Mid-parent	Female	Male	Mid-parent
Early offspring (mean of both sexes)	0.616 (± 0.155)	0.516 (± 0.155)	0.492 (± 0.220)	0.326 (± 0.146)	0.460 (± 0.146)	0.318 (± 0.206)
Late male offspring	0.920 (± 0.149)	0.228 (± 0.149)	0.540 (± 0.211)	0.516 (± 0.149)	0.542 (± 0.149)	0.448 (± 0.211)
Late female offspring	0.992 (± 0.150)	0.286 (± 0.150)	0.598 (± 0.212)	0.876 (± 0.149)	0.706 (± 0.149)	0.718 (± 0.211)
Late offspring (mean of both sexes)	0.954 (± 0.149)	0.244 (± 0.149)	0.562 (± 0.211)	0.696 (± 0.149)	0.624 (± 0.149)	0.583 (± 0.211)

Table 5.4: Components of variance and heritability (with standard error) estimated by regression of offspring weight on female parent weight in set 1 and set 2.

		V_P	V_A	h^2
Set 1	Early mean offspring	0.020	0.012	0.616 (± 0.155)
	Late male offspring	0.039	0.036	0.920 (± 0.149)
	Late female offspring	0.047	0.047	0.992 (± 0.150)
	Late mean offspring	0.036	0.034	0.954 (± 0.149)
Set 2	Early mean offspring	0.023	0.007	0.326 (± 0.146)
	Late male offspring	0.040	0.021	0.516 (± 0.149)
	Late female offspring	0.044	0.038	0.876 (± 0.149)
	Late mean offspring	0.036	0.025	0.696 (± 0.149)

Table 5.5: Components of variance and heritability (with standard error) estimated by regression of offspring weight on male parent weight in set 1 and set 2.

		V_P	V_A	h^2
Set 1	Early mean offspring	0.020	0.010	0.516 (± 0.155)
	Late male offspring	0.039	0.009	0.228 (± 0.149)
	Late female offspring	0.047	0.013	0.286 (± 0.150)
	Late mean offspring	0.036	0.009	0.244 (± 0.149)
Set 2	Early mean offspring	0.023	0.011	0.460 (± 0.146)
	Late male offspring	0.040	0.022	0.542 (± 0.149)
	Late female offspring	0.044	0.031	0.706 (± 0.149)
	Late mean offspring	0.036	0.023	0.624 (± 0.149)

Table 5.6: Components of variance and heritability (with standard error) estimated by regression of offspring weight on mid-parent weight in set 1 and set 2.

		V_P	V_A	h^2
Set 1	Early mean offspring	0.020	0.009	0.492 (± 0.220)
	Late male offspring	0.039	0.021	0.540 (± 0.211)
	Late female offspring	0.047	0.028	0.598 (± 0.212)
	Late mean offspring	0.036	0.020	0.562 (± 0.211)
Set 2	Early mean offspring	0.023	0.007	0.318 (± 0.206)
	Late male offspring	0.040	0.018	0.448 (± 0.211)
	Late female offspring	0.044	0.032	0.718 (± 0.211)
	Late mean offspring	0.036	0.021	0.583 (± 0.211)

Table 5.7: Contribution of genetic components to the body weight variance.

	Genetic standard deviation $\sqrt{V_{A_1}}$	Genetic coefficient of variation $\frac{\sqrt{V_A}}{\bar{X}}$
Offspring on male parent	0.115	0.061 (6%)
Offspring on female parent	0.139	0.074 (7%)
Offspring on mid-parent	0.119	0.063 (6%)

5.3.2 Discussion

Darwin's (1859) theory of evolution by natural selection can be summarized through the following three principles:

- variation among individuals within a population in morphology (eg body weight), physiology and behaviour;
- heredity – offspring resemble their parents more than unrelated individuals; and,
- selection – some forms are more successful than others in a given environment.

The first two principles have been investigated in body weight in the present study. The phenotypic variances have been determined. The additive genetic variance is the variance among individuals in breeding values. Body weight in T. castaneum is affected by several genes: 14-15 according to Sokoloff (1974). Most quantitative works, however, have been performed on pupal weight rather than adult weight as here. Conner et al. (1992) reported significant additive genetic variance in pupa weight using quantitative analysis. As well as additive genetic variance, there may also be non-additive (interaction) effects such as dominance, which in its most extreme form is represented as heterosis. Different levels of heterosis were reported by various workers, eg Boylan et al. (1965c), Enfield et al. (1966).

The estimates of heritability reported here are higher than many estimates found in the literature; for example, Wilson et al. (1965) and Boylan et al. (1965c) both report an estimate of 0.33 for pupa weight heritability. High estimates of heritability for adult weight were found by Li et al. (in press) in a laboratory strain ($h^2 = 0.61 \pm 0.132$) as opposed to a field strain ($h^2 = 0.48 \pm 0.096$) of T. castaneum. Higher estimates of heritability in female parents are the result of maternal effects. Although Dawson (1964b), studying maternal effects on quantitative characters in development of T. castaneum, argues that maternal effects are pronounced for early stages of the development and diminish with increasing age of offspring,

here the maternal effects are higher in late offspring. Maternal effects on pupal weight were reported by Dawson (1975).

Heritability in a component of fitness indicates that selection can act to change the mean value of that character in a population, but a genetic correlation with another character affected by selection can constrain evolution; such a correlation is termed a genetic trade-off (Smith et al., 1987). Genetic correlations may be estimated by the methods of quantitative genetics, but it is often more satisfactory to measure correlated responses to selection (Smith, 1991).

A negative phenotypic correlation was found between body weight and developmental rate, which is an important component of fitness. Many other workers report genetic correlation between body weight and many other fitness components. Li et al. (in press) for example found that body weight is correlated with progeny production and developmental period. If, as shown by Møller et al. (1989) an increase in one component of fitness leads to a decrease in another, it is predicted that a selection for high and low development rate (respectively fast and slow development) will result in smaller body size in fast line and larger body size in slow line. The next chapter reports on the selection experiments for slow and fast development using culture regime.

Fig. 1 DISTRIBUTION OF BODY WEIGHT IN FEMALE PARENTS OF T. CASTANEUM FROM SET 1 (F.P.Wt.)

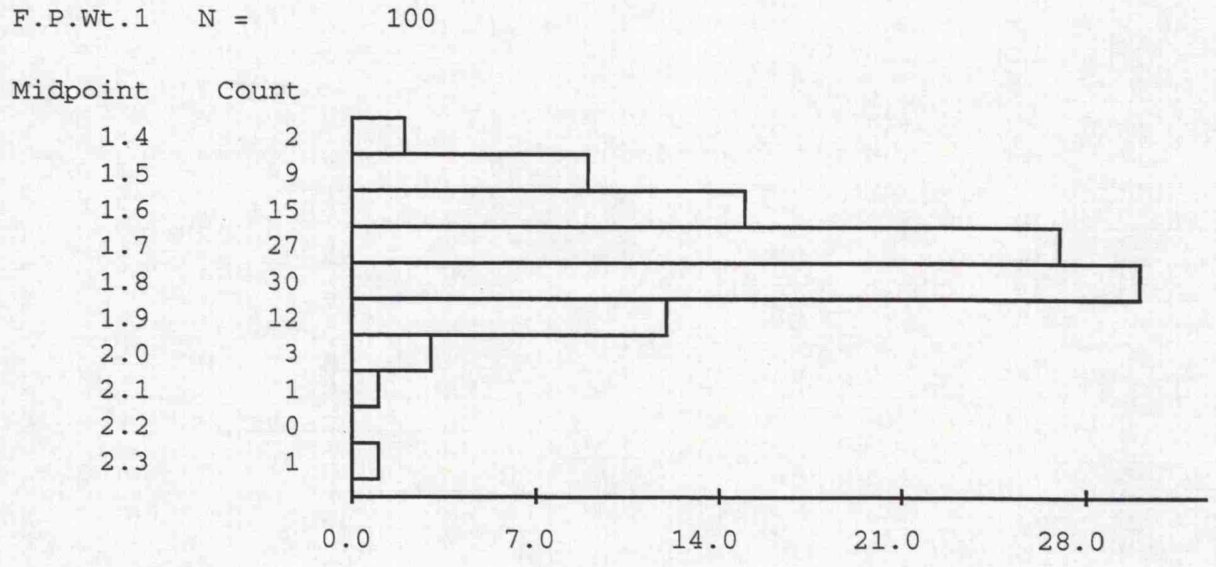


Fig. 2 DISTRIBUTION OF BODY WEIGHT IN FEMALE PARENTS OF T. CASTANEUM FROM SET 2 (F.P.Wt.2)

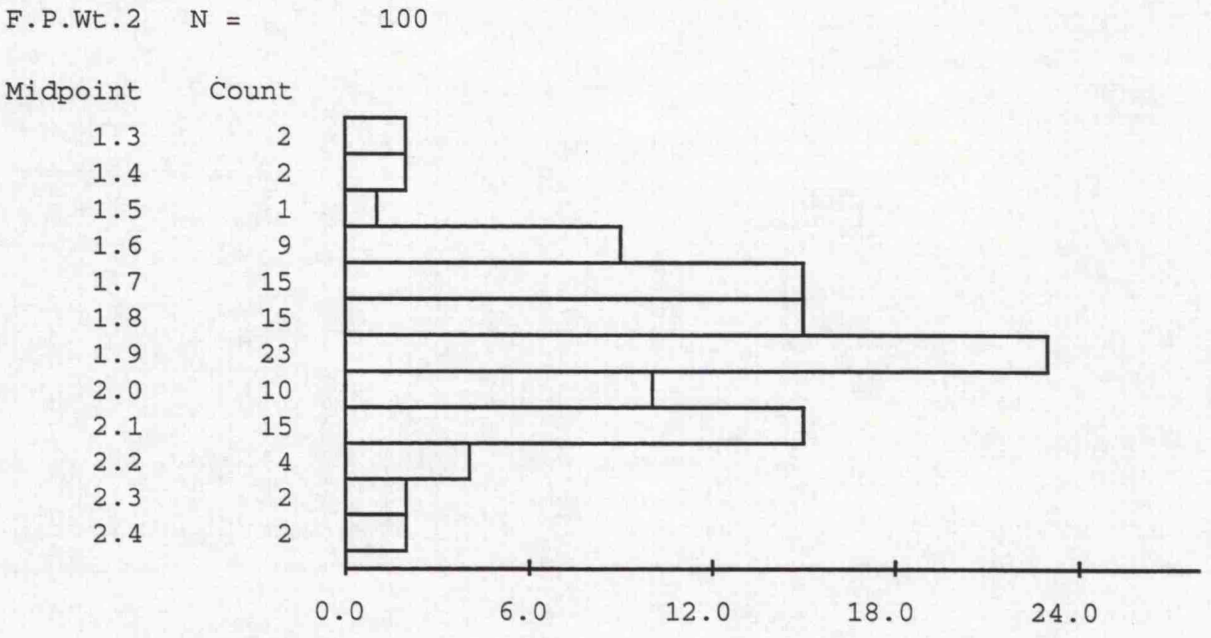


Fig. 3 DISTRIBUTION OF BODY WEIGHT IN MALE PARENTS OF T. CASTANEUM (M.P.Wt.) (USED IN BOTH SET 1 AND SET 2)

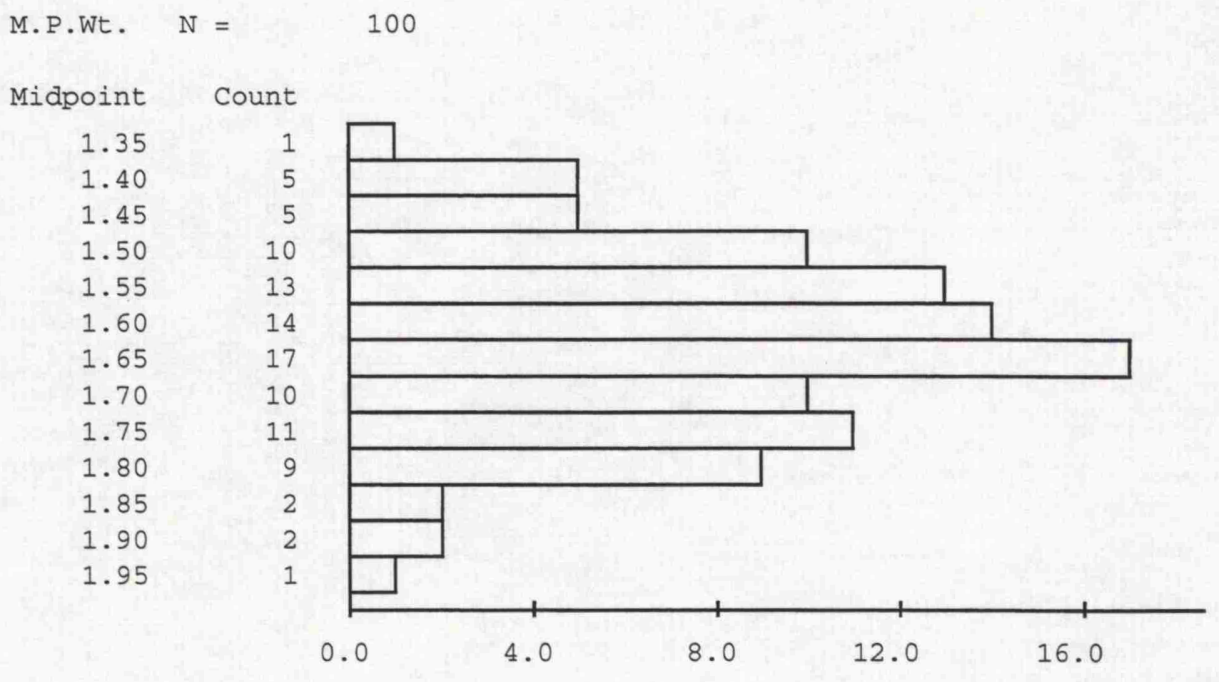


Fig. 4 DISTRIBUTION OF MID-PARENT WEIGHT IN T. CASTANEUM FROM SET 1 (midPW.1)

midPW.1 N = 100

Midpoint	Count
1.45	2
1.50	4
1.55	5
1.60	16
1.65	23
1.70	21
1.75	13
1.80	8
1.85	5
1.90	1
1.95	0
2.00	2

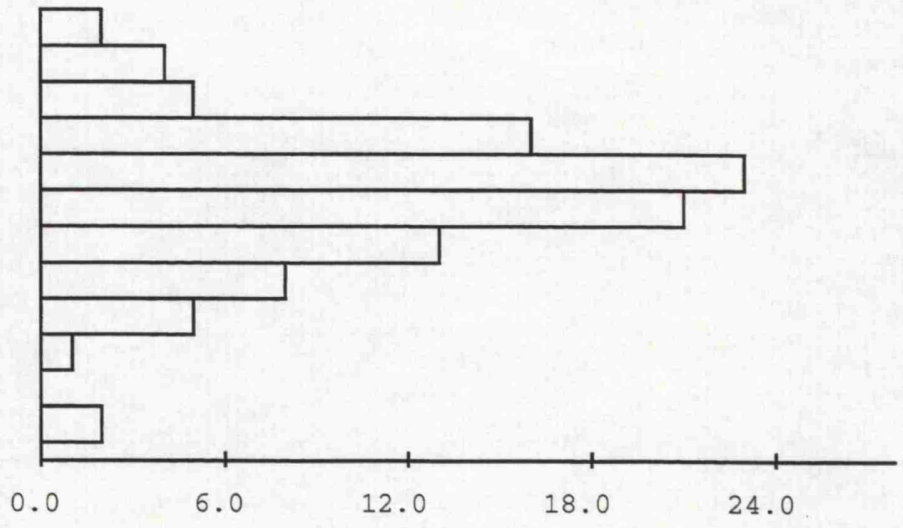


Fig. 5 DISTRIBUTION OF MID-PARENT WEIGHT IN T. CASTANEUM FROM SET 2 (midPW.2)

midPW.2 N = 100

Midpoint	Count
1.4	3
1.5	2
1.6	15
1.7	29
1.8	30
1.9	17
2.0	2
2.1	2

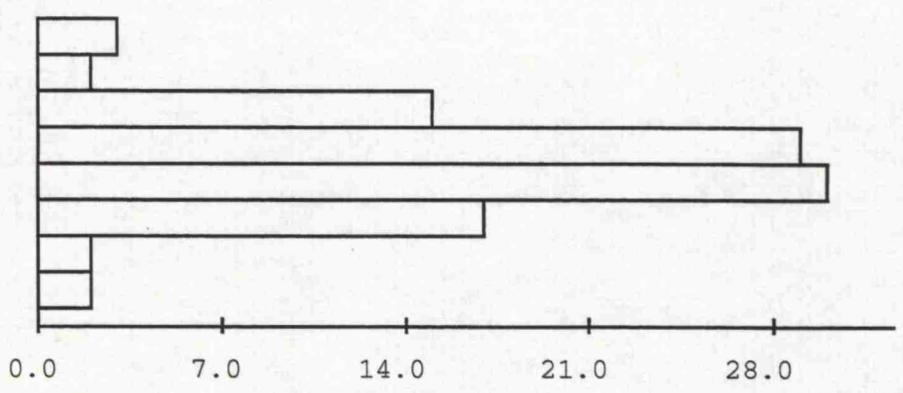


Fig. 6a DISTRIBUTION OF BODY WEIGHT IN EARLY OFFSPRING IN SET 1 (W.off.1e)

W.off.1e N = 83 N* = 17

Midpoint	Count
1.5	2
1.6	3
1.7	12
1.8	27
1.9	20
2.0	10
2.1	6
2.2	3

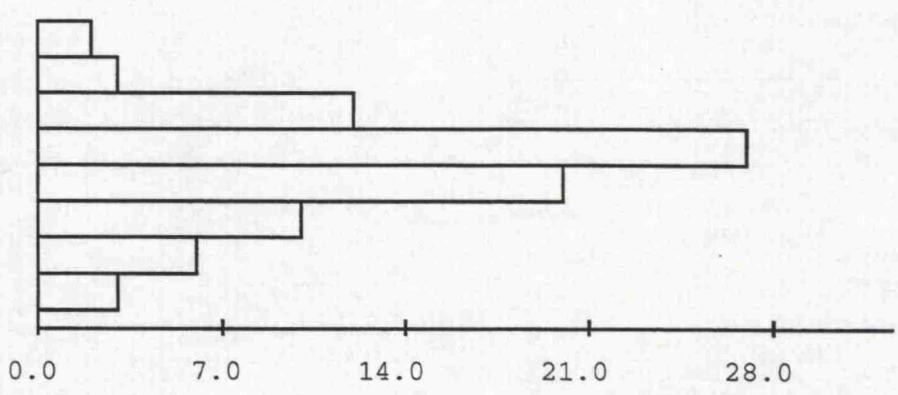


Fig. 6b DISTRIBUTION OF BODY WEIGHT IN LATE OFFSPRING IN SET 1 (MW.off1L)

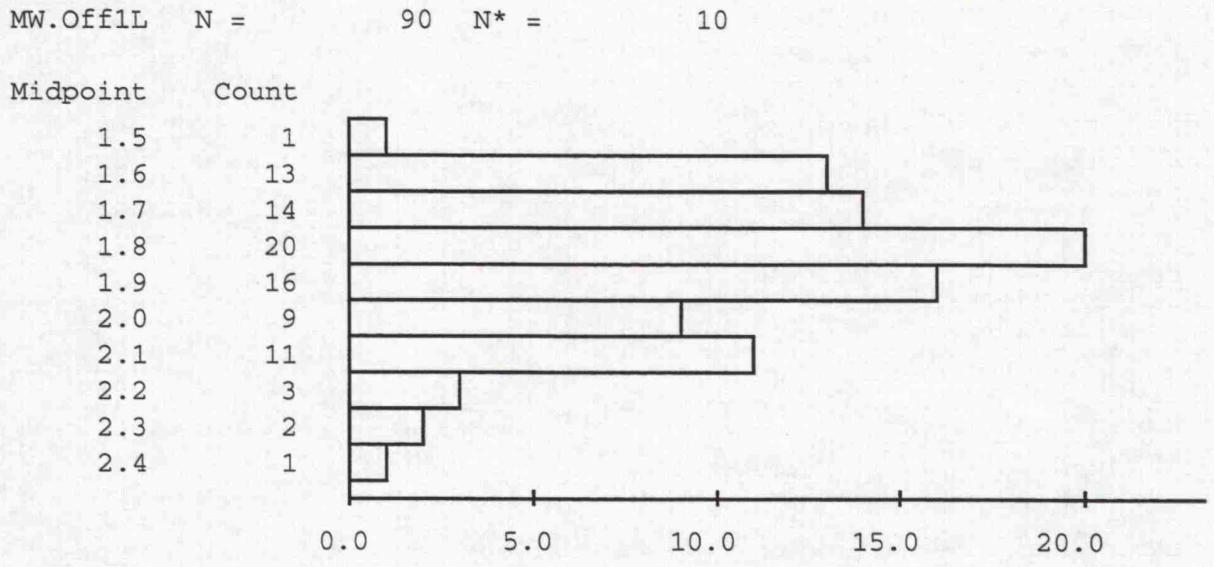


Fig. 7a DISTRIBUTION OF BODY WEIGHT IN EARLY OFFSPRING IN SET 2 (W.off.2e)

W.off.2e N = 94 N* = 6

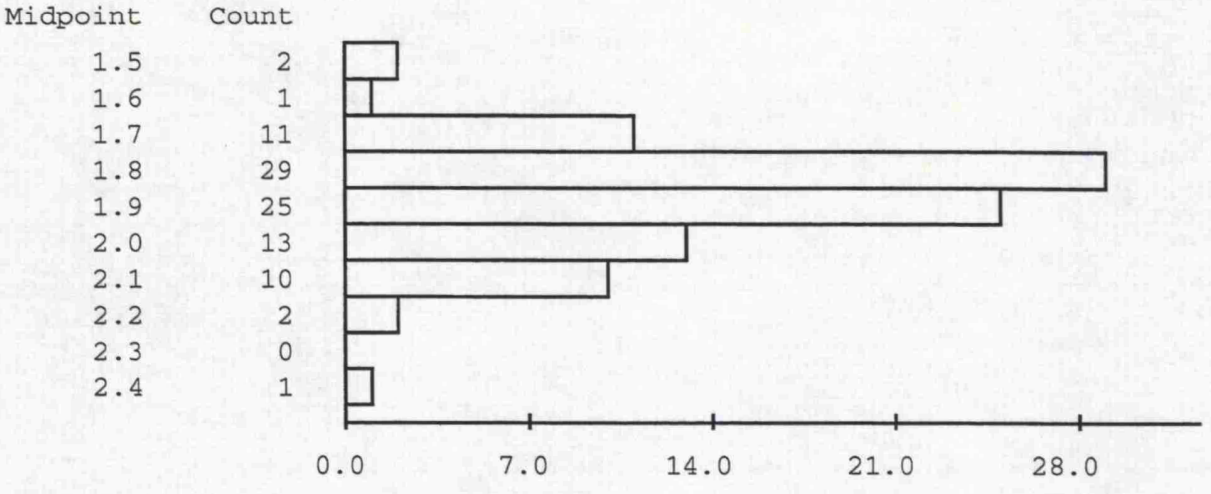


Fig. 7b DISTRIBUTION OF BODY WEIGHT IN LATE OFFSPRING IN SET 2 (MW.Off2L)

MW.Off2L N = 90 N* = 10

Midpoint	Count
1.5	1
1.6	3
1.7	9
1.8	16
1.9	14
2.0	12
2.1	20
2.2	10
2.3	4
2.4	1

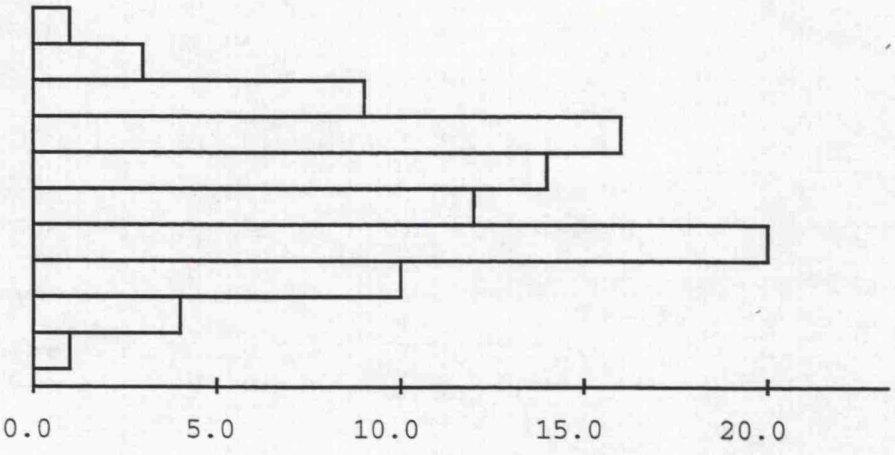


Fig.8a CORRELATION BETWEEN EARLY OFFSPRING BODY WEIGHT AND DEVELOPMENT RATE IN STRAIN N.21 OF T. CASTANEUM IN SET 1

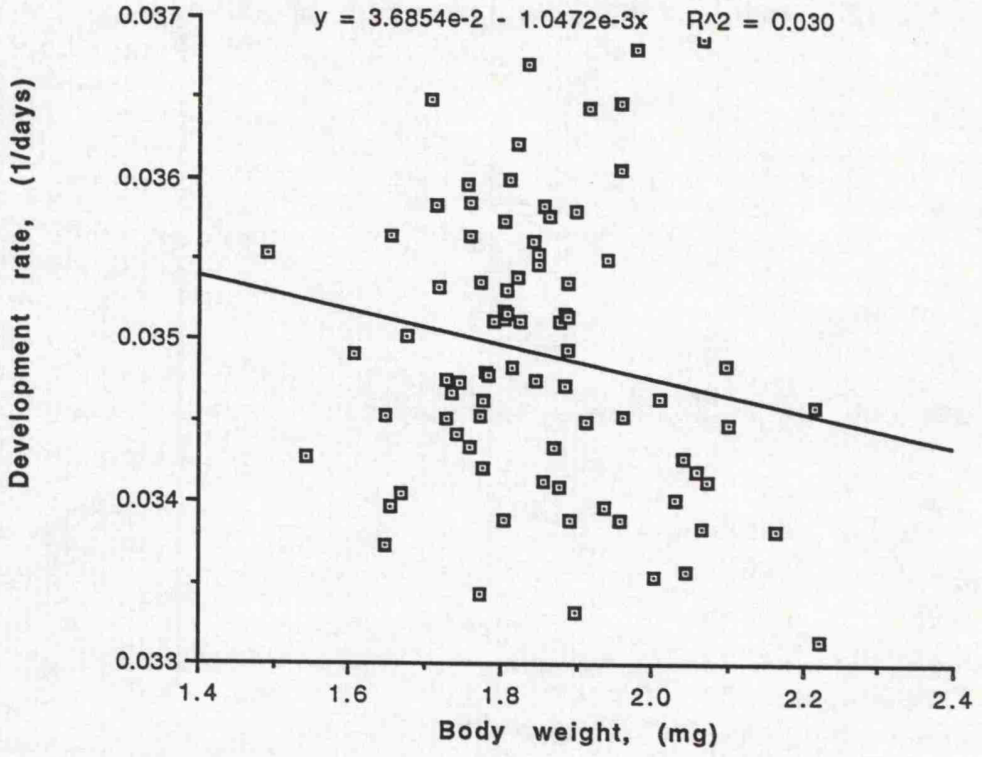


Fig8b CORRELATION BETWEEN LATE OFFSPRING BODY WEIGHT AND DEVELOPMENT RATE IN STRAIN N.21 OF T. CASTANEUM IN SET 1

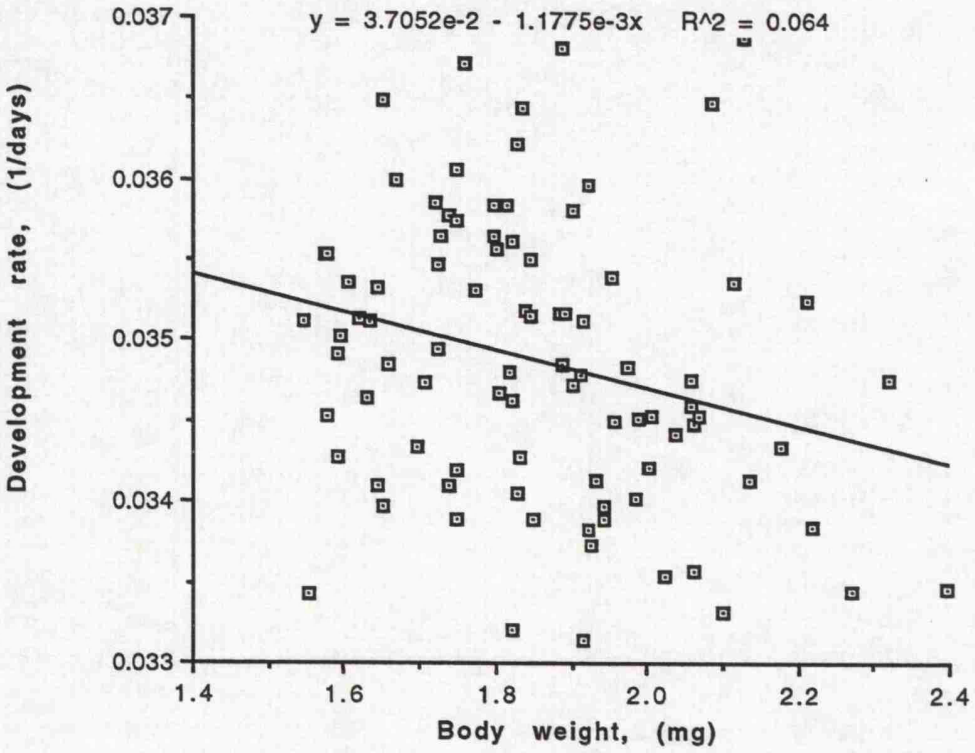


Fig.9a CORRELATION BETWEEN EARLY OFFSPRING BODY WEIGHT AND DEVELOPMENT RATE IN STRAIN N.21 OF T. CASTANEUM IN SET 2

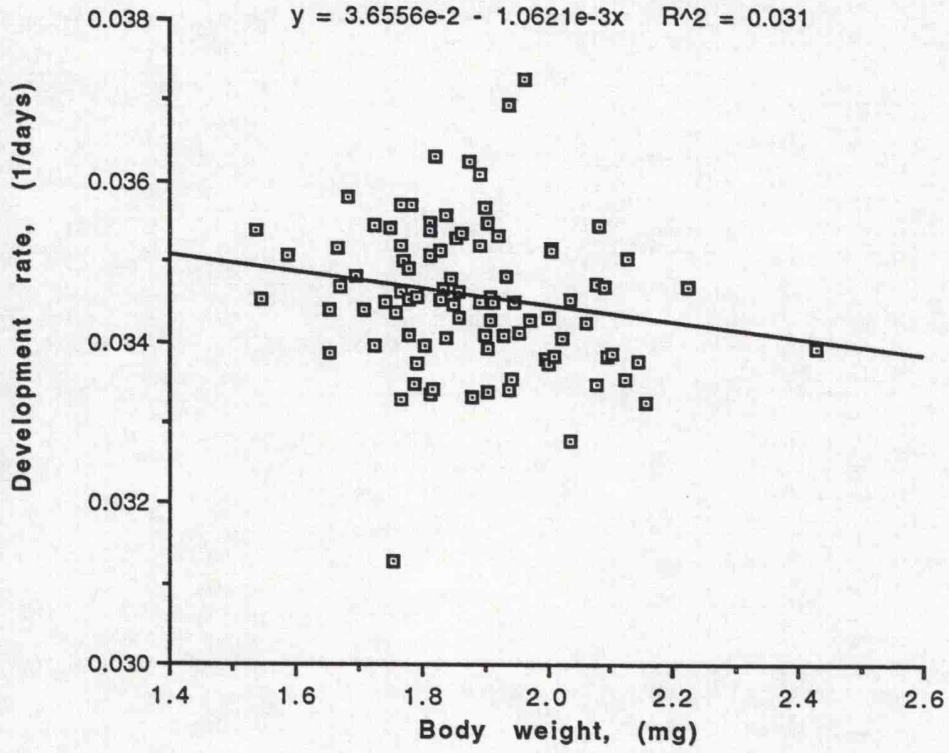
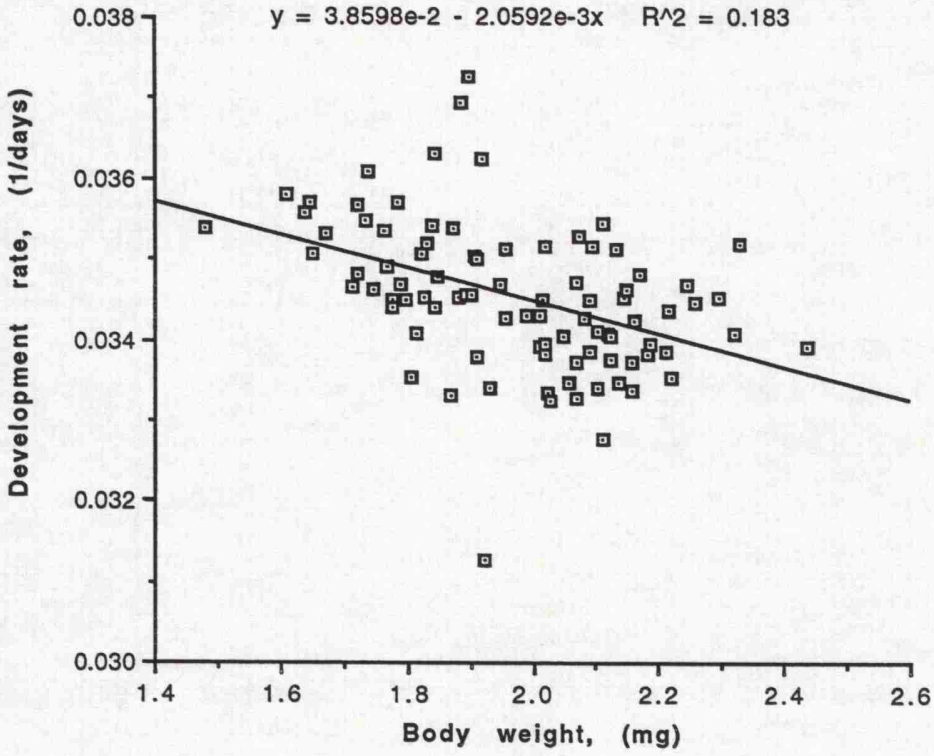


Fig.9b CORRELATION BETWEEN LATE OFFSPRING BODY WEIGHT AND DEVELOPMENT RATE IN STRAIN N.21 OF T. CASTANEUM IN SET 2



Chapter 6 SELECTION EXPERIMENT

6.1 INTRODUCTION

"Whatever the cause may be of each slight difference in the offspring from their parents - and a cause for each must exist - it is the steady accumulation, through natural selection, of such difference, when beneficial to the individual, that gives rise to all the more important modifications of structure, by which the innumerable beings on the face of this earth are enabled to struggle with each other and the best adapted to survive" (Darwin, 1859; cited in Flew, 1984).

In a population, there are individual differences which, as wrote Darwin, "afford materials for natural selection to act on and accumulate ..." (Falconer, 1989). From one generation to the next, characters are passed on, coded for by genes.

The genetic constitution of a population is described by the array of gene frequencies (Falconer, 1989). Gene frequencies can change due to the size of the population, differences of fertility and viability, migration and mutation, the mating system, and selection (Falconer, 1989). Here we are mostly interested in selection.

Artificial selection is the choice of some animals from a large number of animals on the basis of the differences they show for one or more criteria (Bowman, 1974).

Selection occurring in nature, usually referred to as natural selection, was first described by Darwin in "On the origin of species by means of Natural Selection" (1859). The citation above gives some idea of his approach. Natural selection is characterized by the fact that the choice of the gametes and the individuals surviving is determined by their suitability in a given environment (Bowman, 1974). If a particular characteristic is favoured in a given environment, the genes giving rise to it survive the selection process and are passed on to the next generation. In this way advantageous alleles increase in frequency at the expense

of less suitable ones and the genetic constitution of the population changes (Neo-Darwinism as quoted in Parkin, 1979). When the choice of the gametes and the individuals surviving is determined by the action of man, the selection is artificial (Bowman, 1974), or experimental. Selection is a useful tool in elucidating the genetic mechanisms involved in the inheritance of a quantitative character (Enfield et al., 1966). There are two ways in which the experimenter can change the genetic properties of the population: by the choice of the individuals to be used as parents or by control of the way in which the parents are mated (Falconer, 1989).

When a gene is subject to selection, its frequency in the offspring is not the same as in the parents, since parents of different genotypes pass on their genes unequally to the next generation (Falconer, 1989). The aim of experimental selection is to change over a period of several generations the gene frequencies in a population. The effect of artificial selection on a random breeding population will be most clearly seen in a change in the mean of the character under selection (Allan & Robertson, 1964). The difference between the mean performance of the selected individuals, $\overline{P_s}$ and the mean of the population before selection $\overline{P_u}$, is termed the Selection Differential, S and by dividing the selection differential by the phenotypic standard deviation, the standardized differential is obtained (Bowman, 1974).

Various life-history traits of T. castaneum have been the subject of selection experiments, in particular body weight (also referred to as body size), developmental period to adult emergence, and pupation time, eg Enfield et al. (1965, 1966); Englert and Bell (1970); Cardin and Minvielle (1986); Conner and Via (1992). For example, selection for heavier weight for 12 generations was effective in T. castaneum (Enfield et al, 1966), Conner and Via (1992) reported a significant directional selection for increased pupal weight, while Sokoloff (1972) states that selection can double body weight in four generations in T. castaneum. A comprehensive review of genetic studies on T. castaneum is provided by Sokoloff (1972, 1974, 1977). In most of these works, it is the pupal weight that is measured. The main reason for that is the convenience of working with an immobile stage

compared with the difficulties in using a very active adult stage, as well as the facility to sex pupae.

The aims of the present work were:

- to measure the short-term response to selection by two different culture-intervals (6 weeks and 8 weeks) and the mean developmental period of T. castaneum at 30°C;
- to record correlated responses in adult body weight at emergence, productivity and age-specific productivity schedule.

In the 6 week-lines, the culture interval was six weeks hence these lines were also referred to as the fast lines. The culture interval was eight weeks in the 8week-lines also called the slow lines (more detail is given in the materials and methods).

The two culture-interval regimes are expected to select for fast or slower development. Another difference between the two culture regimes is that the number of individuals (adults and larvae) present in the culture in the 6 week-line (fast line) will be lower than the number of individuals in the 8 week-line (slow line). Within each strain it can logically be assumed that the number of progeny in six weeks will be lower than the number of progeny produced in eight weeks. In other words, at reculturing, the density in the 6 week-line will be lower than the density in the 8 week-line and the possible consequence for that could be as follows:

Selection Line	6 Week-Line (Fast)	8 Week-Line (Slow)
Density	Low	High
Expected Results	Selection for rapid development and maybe also for fecundity. The outcome of the selection will depend on trade-offs.	Strong selection for competitive ability in larvae (ie larger larvae, larger and fewer eggs) or selection for rapid development to avoid cannibalism and if trade-offs rapid development will result in smaller individuals

6.2 MATERIALS AND EXPERIMENTAL DESIGN

Strain N.04 from Zangasso and strain N.21 from Gao were used in this experiment. The culture medium was as described in general materials.

The experiment was begun in Bamako, Mali and completed at the University of Leicester. In Mali cultures were held at 30°C and at a relative humidity of about 75%. These conditions were achieved using desiccators containing saturated common salt solution and an incubator as described in the previous chapter. At the University of Leicester, cultures were kept initially at 30°C and 55%RH and later at 30°C and 70%RH when the new humidified rooms of the Environmental Biology Laboratory were ready.

The basic principle of the experiment is that reculturings were done from constant initial density and at constant intervals for each of the two lines. The two lines were defined as follows:

- the fast or 6 week-line where reculturing was done every 6 weeks, this line was also referred to as the 6 week-culture regime;
- the slow or 8 week-line where reculturing was done every 8 weeks and was also called the 8 week-culture regime.

In Mali, the initial density for reculturing was two adults per gramme of culture medium; all the cultures were initiated with 100 adults in 50g of medium except for the initial culture. Due to insufficient numbers in strain N.04, the first culture was started with 70 adults in 35g of medium (the density still being two adults per gramme). At the University of Leicester the initial density was 100 adults in 100g of medium, that is one adult per gramme. The reason for the change in the initial density from two to one adult per gramme was that bigger jars could be used in Leicester whereas in Mali only small jars could be used so that a sufficient number of them could be placed in the two desiccators the incubator could hold. The desiccators containing the saturated salt solution achieved the desired relative humidity and the incubator was set at the appropriate temperature. The lines derived from the experiment in Mali were continued in Leicester. In addition a cross between strain N.04 and strain N.21 was set up only in Leicester, not in Bamako.

Two weeks after each reculture, adults were removed to ensure discrete, non-overlapping generations.

The following measurements were made at each reculture:

- the number of live and dead adult insects;
- the mean weight of adults with its standard deviation;
- the total weight of experimental jars with content (flour and insects): this allows determination of the weight loss over each generation as will be explained later;
- the weight of experimental jars after live and dead adult insects have been removed;

- the weight of jars of the next generation.

Two weeks after reculture when adults were removed, other measurements were made:

- the number of live and dead adult insects;
- the weight of jars after adults were removed: the difference between this weight and the weight of jars at reculture (after 4 weeks in the 6 week-regime and after 6 weeks in the 8 week-regime) constitutes the weight loss over one generation.

The raw data of the measurements are reported in Appendices 5 and 6.

The weight of adults was measured according to the facilities that were available. In Bamako five sets of 20 adults were weighed using an OHAUS Analytical balance; from the results of these measurements, the mean weight of one adult was deduced and the variance calculated. At Leicester a more sensitive balance, the CAHN Microbalance, allowed measurement of individual adults, so from each replicate, a minimum of 20 adult insects were weighed individually and the mean was calculated as well as the variance.

At reculturing, after all adults were counted, a random sample of 100 adults was retained from each replicate to start the new culture.

There were five replicates of each treatment in Bamako. The number was reduced to three in Leicester due to insufficient numbers of adults in some lines.

At the end of the selection experiment a full life-history study was carried out to measure the following traits: adult body weight at emergence, number of adult produced per female, and development period.

The selective pressure was first released by reculturing all the lines in the same conditions. This was done by allowing ten adult females from each selection

replicate to oviposit for one day on the same amount of flour (10g). Adult females were removed after one day and cultures were kept undisturbed until pupal emergence started. At emergence pupae were sexed and sexes were kept separate until adult emergence. As adults emerged three females and three males were selected from each replicate and paired at random. In this way there were nine pairs per strain per line, hence the total number of pairs formed was:

3 pairs X 3 selection replicates X 3 strains X 2 selection lines = 54 pairs.

Each pair was kept in a separate vial containing 2g of culture medium for three days (corresponding to the pre-oviposition period). At the end of this period males were removed and discarded and each female was transferred to a new vial containing fresh food after successive periods of **1-3-1-3-1 days**, hence females were transferred five times. The 1 day-vials contained 5g of flour and the 3 day-vials contained 15g. The total number of treatments was:

54 females X 5 transfers = 270 treatments (or 270 vials).

Vials were kept undisturbed until adult emergence started. Vials were then checked daily, and the number of adults emerged and the date were recorded. Number of adults produced per female (here referred to as productivity) was obtained by adding the number of adult recorded every day until emergence was completed. Development period was deduced from the dates (respective of the start of the experiment) on which emergences were recorded and the number of adult emerging on each date. Body weight at emergence was obtained by weighing four offspring per female per day of emergence, hence the total number of offspring measured per a particular female would depend on the pattern of emergence. For example when emergence was completed in three days, the number of offspring measured would be 12, assuming that at least four adults emerged every day, but when emergence was completed in five days, then the number of offspring measured would be 20 with the same assumption that at least four offspring emerged every day.

The following tables give the details of the timetable of the experiments in Bamako and in Leicester with the number of generations.

Reculturing timetable in Bamako:

	Dates on which the different operations were carried out	
	6 Week-regime	8 Week-regime
Initial culture	10.01.1992	10.01.1992
Removal of adults	24.01.1992	24.01.1992
1st Reculture	21.01.1992	06.03.1992
Removal of adults	06.03.1992	20.03.1992
2nd Reculture	03.04.1992	01.05.1992
Removal of adults	17.04.1992	15.05.1992
3rd Reculture	15.05.1992	26.06.1992
Removal of adults	29.05.1992	10.07.1992
4th Reculture	26.06.1992	
Removal of adults	10.07.1992	
End	07.08.1992	14.08.1992
Number of generations	5	4

Reculturing timetable at the University of Leicester:

Dates on which the different operations were carried out

	6 Week-regime	8 Week-regime
Initial culture	16.11.1992	16.11.1992
Removal of adults	30.11.1992	30.11.1992
1st Reculture	28.12.1992	11.01.1993
Removal of adults	11.01.1993	25.01.1993
2nd Reculture	08.02.1993	08.03.1993
Removal of adults	22.02.1993	22.03.1993
3rd Reculture	22.03.1993	
Removal of adults	05.04.1993	
Final measurements	03.05.1993	03.05.1993
Number of generations	4	3
Total number of generations	9	7

6.3 RESULTS

Mean body weight and the total number of adults were recorded every generation during selection (at each reculture). Additional data on development period and productivity were collected during a test at the end of selection. For the test, selection was relaxed in order to remove maternal (non-genetic) effects by keeping insects in the same conditions for one generation (eg same density) before measurements were taken. Data obtained from measurements taken during the selection experiment will be referred to as selection data and data obtained following relaxation of selection during the test will be referred to as test data.

Selection data are shown in Tables 6.1 to 6.5 and test data are reported in Tables 6.6 to 6.10.

For the selection data, Bamako-data and Leicester-data were analysed separately because the initial density at reculture was higher in Bamako where it was 2 adults per gramme whereas it was 1 adult per gramme in Leicester. Also for conveniance

the numbers of adults per generation were transformed to square root prior to statistical analysis.

6.3.1 Body Weight

The mean body weights during selection are shown in Table 6.2 (p120). The body weight at each reculture was not measured at emergence, but the mean body weight of live adults was recorded at reculture (see measurements at reculture in Appendices 5 and 6). The measurement of body weight at each reculture is explained in materials and methods. The analysis of variance for body weight using selection data is shown in Table 6.1a (p118) and shows a significant difference between strains but not between selection lines; the interaction between strain and selection line was not found to be significant.

The body weight test data are shown in Table 6.6a (p130). At emergence, four randomly-chosen offspring were weighed per female parent per day of emergence; when the number of offspring emerging was less than four, they were all weighed. The number of observations would then depend on the total number of offspring and on the pattern of emergence. The mean body weights in Table 6.6a were calculated across all the parental ages at mating. The analysis of variance using the test data also shows that the difference in body weight (part b of Table 6.6b, p131) was significant for strains but not for selection lines. While the interaction between strain and selection line was found to be not significant with the selection data, the test data indicated that it was significant, because the 8 week mean was higher than the 6 week mean in the N.04 X N.21 cross whereas 8 week was lower than 6 week in the within-strain lines. Both the selection data and the test data indicate that body weight averaged over strains did not respond significantly to different culture intervals, but the significant interaction effect shows that there was a response to selection.

The body weights in successive generations (for all the generations) are plotted against generation times (expressed in weeks) in Figs 1 to 4. In Fig 1 (strain N.04)

the expected pattern of the selection lines moving apart does not appear, the two lines remain close and cross over three times, indicating the lack of response to the culture intervals. In Fig 2 (strain N.21) the later stages (after 40 weeks) show that the lines have moved apart. Figs 3 and 4 compare respectively the 6 week-line and the 8 week-line in the two strains. Changes in body weight in the same selection line follow roughly the same pattern when compared in the two strains, possibly indicating a response to an unknown external, environmental variable rather than to selection regime or strain differences.

Table 6.1a: Analysis of variance for a) mean body weight per generation, b) mean final number of adults per generation and c) mean mortality rate (%) per generation (Bamako data).

a) Mean body weight

Source	df	SS	MS	F	P
Strain	1	0.1408	0.1408	4.24	0.057
Line	1	0.0074	0.0074	0.22	0.643
Error	15	0.4983	0.0332		
Total	17	0.6465			

b) Mean final number (Transformed)

Source	df	SS	MS	F	P
Strain	1	109.768	109.768	12.70	0.003
Line	1	0.093	0.093	0.01	0.919
Error	15	129.640	8.643		
Total	17	239.500			

c) Mean mortality rate

Source	df	SS	MS	F	P
Strain	1	4.52	4.52	0.08	0.787
Line	1	27.35	27.35	0.46	0.509
Error	15	898.51	59.90		
Total	17	930.38			

Table 6.1b: Analysis of variance for a) mean body weight per generation, b) mean final number of adults per generation and c) mean mortality rate (%) per generation (Leicester data).

a) Mean body weight

Source	df	SS	MS	F	P
Strain	2	0.1197	0.0599	2.01	0.165
Line	1	0.1104	0.1104	3.71	0.071
Error	17	0.5065	0.0298		
Total	20	0.7367			

b) Mean final number (Transformed)

Source	df	SS	MS	F	P
Strain	2	91.1	45.6	0.18	0.835
Line	1	409.9	409.9	1.64	0.218
Error	17	4250.6	250.0		
Total	20	4751.6			

c) Mean mortality rate

Source	df	SS	MS	F	P
Strain	2	464.5	232.3	0.54	0.590
Line	1	4194.5	4194.5	9.82	0.006
Error	17	7263.8	427.3		
Total	20	11922.9			

Table 6.2: Mean adult body weight (mg) for each generation in the two selection lines in strains N.04 and N.21 and their cross. The final row shows the mean body weights at emergence recorded in the test environment after one generation of relaxed selection.

Generation	Strain N.04		Strain N.21		Cross N.04 X N.21*	
	6 Week	8 Week	6 Week	8 Week	6 Week	8 Week
1	1.608	1.619	1.401	1.724		
2	1.210	1.297	1.613	1.720		
3	1.811	1.611	2.018	1.862		
4	1.592	1.687	1.724	1.762		
5	1.507	-	1.710	-		
Mean 1–5	1.546	1.554	1.693	1.767		
6	1.574	1.457	1.734	1.660	1.661	1.623
7	1.549	1.501	1.808	1.437	1.716	1.605
8	1.741	1.833	2.077	1.846	1.955	1.932
9	1.793	-	2.136	-	1.873	-
Mean 6–9	1.664	1.597	1.939	1.648	1.801	1.720
Test	1.790	1.763	2.263	2.144	1.861	2.148

1–5 = Bamako

6–9 = Leicester

\- = One generation less in 8 week-line

* = Cross was done after the 5th generation

Fig. 1:CHANGE IN BODY WEIGHT OVER GENERATIONS IN a-6WEEK-LINE AND b-8WEEK-LINE OF STRAIN N.04 OF T. CASTANEUM

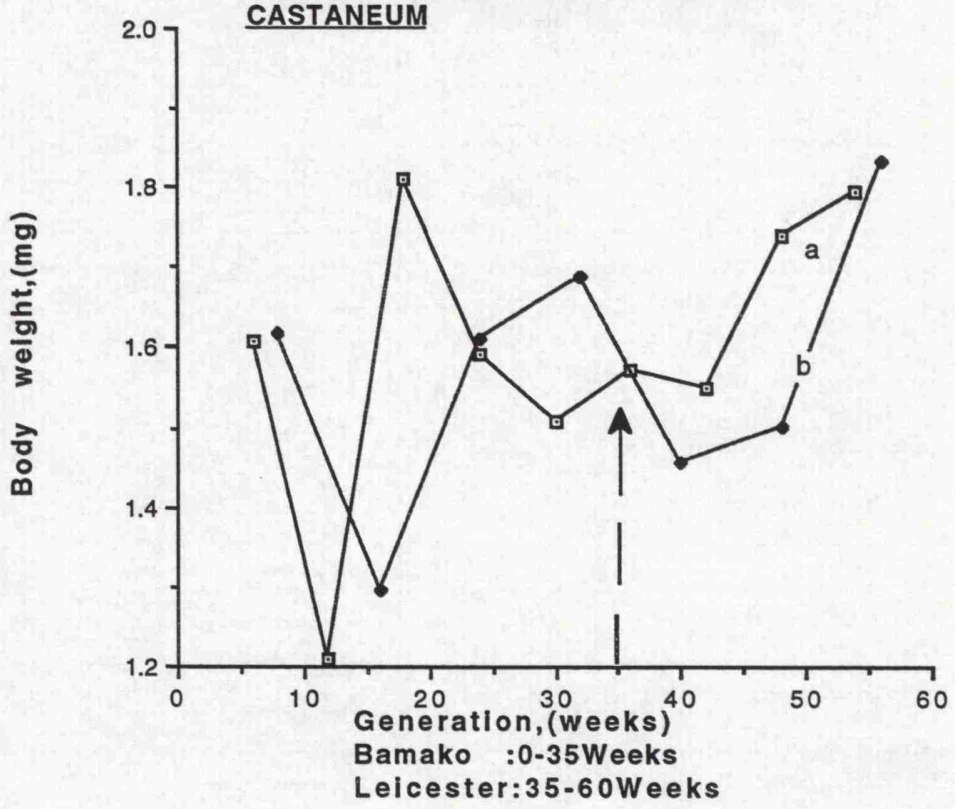


Fig.2: CHANGE IN BODY WEIGHT OVER GENERATIONS IN a-6WEEK-LINE AND b-8WEEK-LINE OF STRAIN.21OF T. CASTANEUM

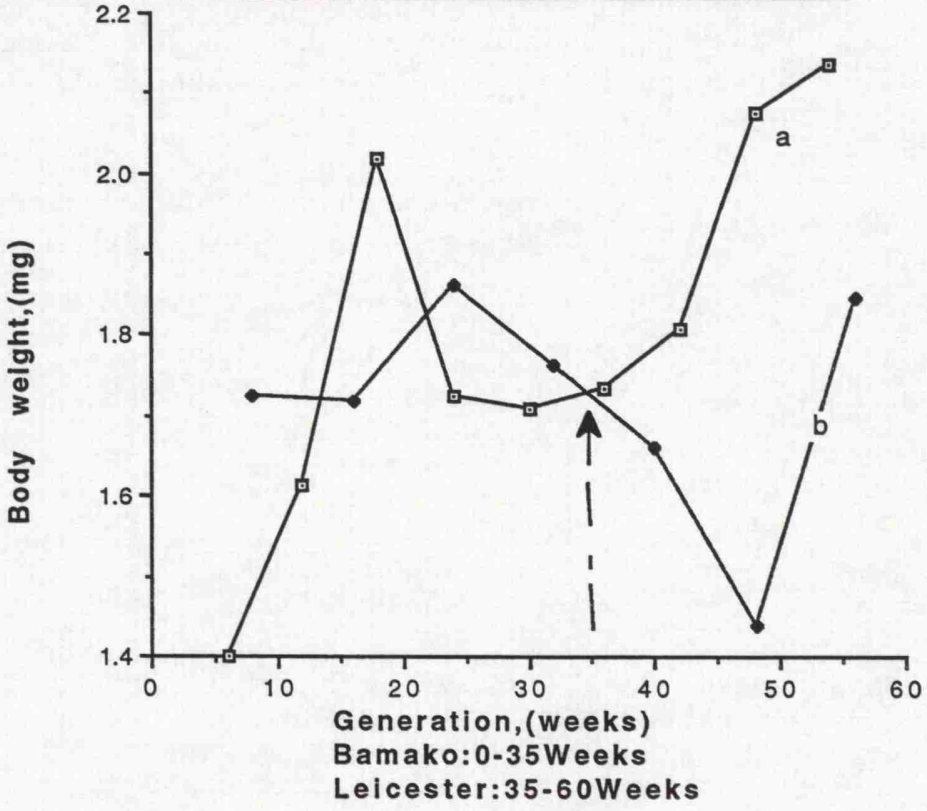


Fig.3:CHANGE IN BODY WEIGHT OVER GENERATIONS IN THE 6WEEK-LINES OF a-STRAIN N.04 AND b-STRAIN N.21 OF T.CASTANEUM

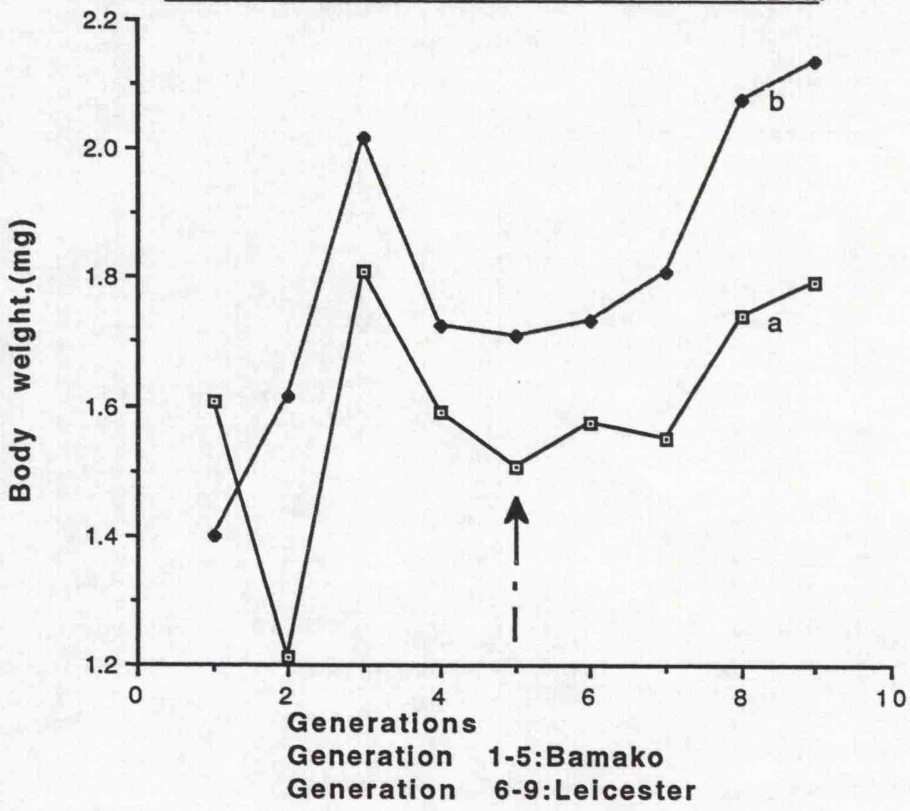
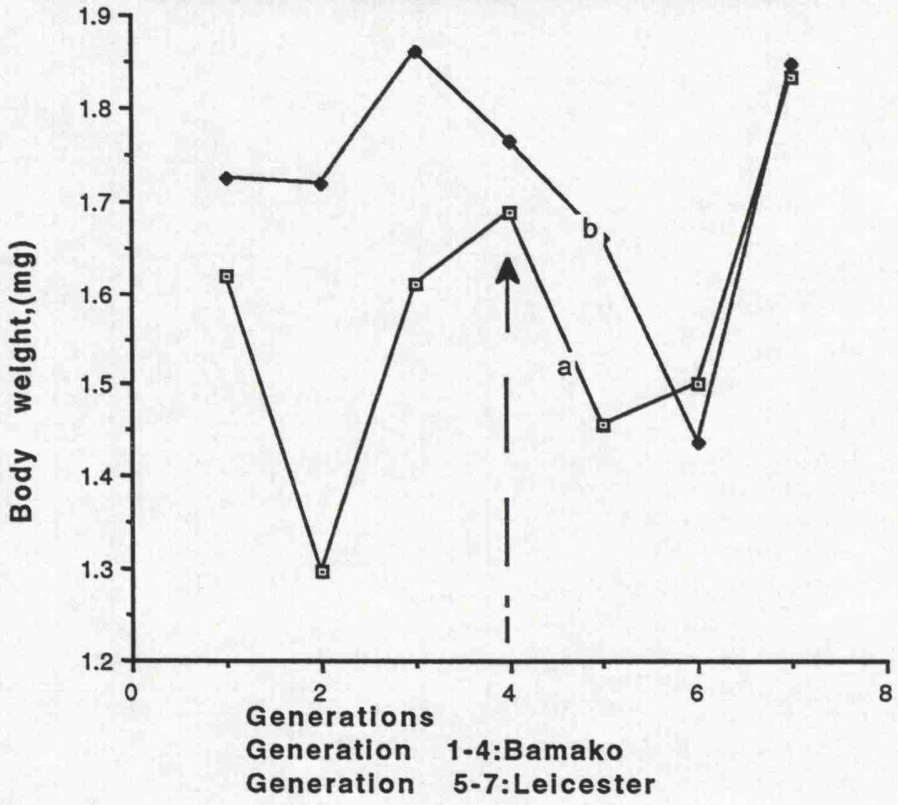


Fig.4: CHANGE IN BODY WEIGHT OVER GENERATIONS IN THE 8WEEK-LINES OF a-STRAIN N.04 AND b-STRAIN N.21 OF T.CASTANEUM



6.3.2 Productivity

Productivity as defined earlier was not directly measured in each generation. However, the total number of adults (live and dead) was measured, and will be used here as an index of productivity. Table 6.3 (p126) shows the mean adult number in each generation. The analysis of variance on the transformed Bamako data shown in Table 6.1a (part b) (p118) indicates that the difference in adult number per generation was significant between strains but not between selection lines. Table 6.1b (part b) (p119) shows that in Leicester, neither line or strain caused significant variation.

The test data for productivity are shown in Table 6.6a (p130). As explained in materials and methods, three females from each experimental replicate (nine females per selection line) were allowed to oviposit during three 1 day periods alternating with two 3 day periods. The mean 1 day productivity is the mean number of progeny produced during the three 1 day periods. The variation of number of observations is because some replicates were discontinued, as explained earlier. The analysis of variance in Table 6.6b (p131) shows that only strains were significantly different, with N.21 being more productive than either N.04 or the cross. Productivity also did not respond to the different culture intervals.

Two other parameters were measured at each reculture: number of dead adults (giving the mortality rate) and the density which was based on the total number of dead and live adults. Density and mortality rate in each generation are presented respectively in Tables 6.4 and 6.5 (p128 and 129). The analysis of variance in Table 6.1a (part c) (p118) shows that at high initial density (Bamako data), neither line or strain caused significant variation in mortality rate. But at low initial density (Leicester data) (Table 6.1b, part c, p119) selection lines were significantly different in mortality rate whereas strains were not.

Table 6.3: Mean number of adults (live and dead) in each generation at time of reculture in the two selection lines of strains N.04 and N.21 and their cross.

Generation	Strain N.04		Strain N.21		Cross N.04 X N.21*	
	6 Week	8 Week	6 Week	8 Week	6 Week	8 Week
1	164.60	200.40	166.60	390.60		
2	250.40	113.80	396.80	239.60		
3	137.80	294.40	402.20	549.20		
4	285.20	119	406.70	289.60		
5	133.20	-	303.40	-		
Mean 1–5	194.24	181.90	335.14	367.25		
6	1665	1720.33	1803.66	2121	1328.33	1569.66
7	35.66	66.67	50.33	743	118.66	499
8	14.66	52	14.66	292.33	20.66	85
9	108.67	-	89.67	-	69.67	-
Mean 6–9	455.9	612.9	489.58	1052.11	384.33	717.89

1–5 = Bamako

6–9 = Leicester

Sudden increase in Leicester then sharp drop in numbers.

* = Cross was done after the 5th generation

6.3.3 Development Period

Development period was not included in the measurements made at each reculture. The test data for development period are shown in Table 6.6a (p130). In this table the mean of the developmental period was calculated using all the observations within experimental and test replicates and across all the parental ages at mating (4, 8 and 12 days). The low number of observations (n) in the 8 week-lines in strain N.04 and the cross strain was due to the discontinuity of some experimental replicates, which had to be mixed following the important drop in productivity observed during the selection experiment at the 7th and 8th generations (see Table 6.3). Table 6.6a showing the analysis of variance for

development period indicates that the between line and between strain differences were not statistically significant and that their interaction also had no significant effect; therefore developmental period did not respond to the different culture intervals.

6.3.4 Effect of Parental Age

The effect of age of parent at mating on offspring development period, body weight and productivity was tested and is reported in Table 6.7a (p132). The parental ages tested were four, eight and twelve days post-emergence. Table 6.7b (p133) gives the different P values in analysis of variance for these traits. The effect of age of parents was found to be significant for development period in all strains and for productivity in strains N.04 and N.21 but not in the cross. The effect of parental age was not found to have a significant effect on offspring body weight except in the fast line of the cross strain. Thus older parents produced more young that developed more rapidly, on average.

6.3.5 Effect of Density on Body Weight

The correlation between density and body weight is shown in Figs 5 and 6 respectively for strain N.04 and strain N.21. The slopes are both negative, indicating that an increase in density tends to be associated with a decrease in body weight, though the trend is not significant. Thus variation in population density is not sufficient to account for the fluctuations in mean body weight seen during the selection experiment.

Table 6.4: Density of adults (number of dead and live adults per g of medium) at each reculture (or generation).

Generation	Strain N.04		Strain N.21		Cross N.04 X N.21	
	6 Week	8 Week	6 Week	8 Week	6 Week	8 Week
1	4.70	4.01	4.76	7.81		
2	5.00	2.28	7.94	4.79		
3	2.756	5.89	8.04	10.98		
4	5.70	2.38	8.13	5.79		
5	2.66	-	6.07	-		
Mean 1–5	4.16	3.64	6.99	7.34		
6	16.65	17.20	18.04	21.21	13.28	15.69
7	0.36	0.67	0.50	7.43	1.18	4.99
8	0.22	1.24	0.16	2.92	0.206	0.85
9	3.79	-	3.36	-	3.48	-
Mean 6–9	5.25	6.37	5.51	10.52	4.54	7.18

1–5 = Bamako

6–9 = Leicester

Table 6.5: Mortality (% dead adults) in each generation at time of reculturing in the two selection lines of strains N.04 and N.21 and their cross.

Generation	Strain N.04		Strain N.21		Cross N.04 X N.21	
	6 Week	8 Week	6 Week	8 Week	6 Week	8 Week
1	0.49	1.20	0.96	2.09		
2	21.09	20.74	11.40	18.78		
3	0.87	3.12	1.74	1.60		
4	0.56	2.02	1.46	2.00		
5	0.01	-	1.05	-		
Mean 1–5	4.60	6.77	3.32	6.12		
6	1.70	1.24	0.99	1.21	1.61	1.17
7	5.61	79	9.93	1.71	4.78	48.99
8	2.25	55.77	9.09	43.10	3.19	58.04
9	1.84	-	1.85	-	1.43	-
Mean 6–9	2.85	45.34	5.46	15.34	2.75	36.07

1–5 = Bamako

6–9 = Leicester

Table 6.6a: Means of a) egg to adult development period, b) body size at emergence and c) 1-day productivity of the two culture lines of strains N.04 and N.21 and their cross (data from the test)

	Strain N.04		Strain N.21		Cross N.04 X N.21	
	6 Week	8 Week	6 Week	8 Week	6 Week	8 Week
a)	Development period					
Mean ± SE	25.59 (±0.08)	25.67 (± 0.09)	25.15 (± 0.05)	25.11 (± 0.05)	25.75 (± 0.07)	25.65 (± 0.07)
St dev	1.303	0.630	1.028	0.974	1.315	1.417
n	254	48	378	367	306	100
b)	Body size at emergence					
Mean ± SE	1.790 (± 0.01)	1.763 (± 0.02)	2.263 (± 0.01)	2.144 (±0.01)	1.861 (± 0.01)	2.148 (± 0.03)
St dev	0.177	0.148	0.222	0.249	0.210	0.254
n	171	37	185	391	199	68
c)	1-day productivity					
Mean ± SE	11.48 (± 1.16)	10.60 (± 3.03)	15.62 (± 1.17)	15.91 (± 1.44)	10.84 (± 0.80)	8.42 (± 2.02)
St dev	5.33	6.77	5.71	6.91	4.09	7.01
n	21	5	24	23	26	12

Table 6.6b: Analysis of variance for a) mean development period, b) mean body weight and c) mean 1-day productivity: means used are means of test replicates.

a) Mean developmental period

Source	df	SS	MS	F	P
Line	1	0.0396	0.0396	0.07	0.787
Strain	2	2.0697	1.0348	1.93	0.158
Interaction	2	0.2491	0.1245	0.23	0.794
Error	41	22.0079	0.5348		
Total	46				

b) Mean body weight

Source	df	SS	MS	F	P
Line	1	0. 00002	0.00002	0.00	0.961
Strain	2	0.8658	0.4329	67.18	0.000
Interaction	2	0.6635	0.3317	51.48	0.000
Error	40	0.2577	0.0064		
Total	45				

c) Mean 1-day productivity

Source	df	SS	MS	F	P
Line	1	1.08	1.08	0.06	0.806
Strain	2	305.42	152.71	8.63	0.001
Interaction	2	37.21	18.61	1.05	0.359
Error	39	689.71	17.68		
Total	44				

Table 6.7a: Mean developmental period of a) body weight, b) 1-day productivity, and c) progeny produced by same parents mating at different ages in the two selection lines of the strains N.04, N.21 and their cross.

Parental age at mating	6 Week-Line			8 Week-Line		
	1 day	5 days	9 days	1 day	5 days	9 days
a) Development period						
N.04	26.06	26.21	24.73	-	-	-
N.21	25.13	25.97	24.36	25.41	25.62	24.49
Cross	25.63	26.50	24.78	25.20	26.27	24.82
Mean	25.61	26.23	24.62	25.30	25.94	24.65
b) Body weight						
N.04	1.745	1.821	1.792	1.701	1.789	1.743
N.21	2.291	2.279	-	1.981	2.047	1.999
Cross	1.806	1.875	1.899	1.998	2.201	2.138
Mean	1.947	1.992	1.846	1.893	2.012	1.960
c) 1-day productivity						
N.04	7.14	12	14	-	-	-
N.21	10.87	17.75	18.25	7.29	20.12	19.25
Cross	8.66	12.44	10.37	3.50	10.40	8.40
Mean	8.89	14.06	14.21	5.39	15.26	13.82

Table 6.7b: P values for the effect of the age of parents when mating on development period, body weight and productivity in the selection lines of strains N.04 and N.21 and their cross.

	Development Period	Body Weight	Productivity
N.04–6	0.000	0.09	0.02
N.04–8	-	0.610	-
N.21–6	0.000	0.165	0.009
N.21–8	0.000	0.280	0.000
Cross–6	0.000	0.03	0.34
Cross–8	0.000	0.163	0.55

Fig.5:CORRELATION BETWEEN BODY WEIGHT AND FINAL DENSITY IN a-6WEEK-LINE AND b-8WEEK-LINE OF STRAIN N.04 OF T.CASTANEUM

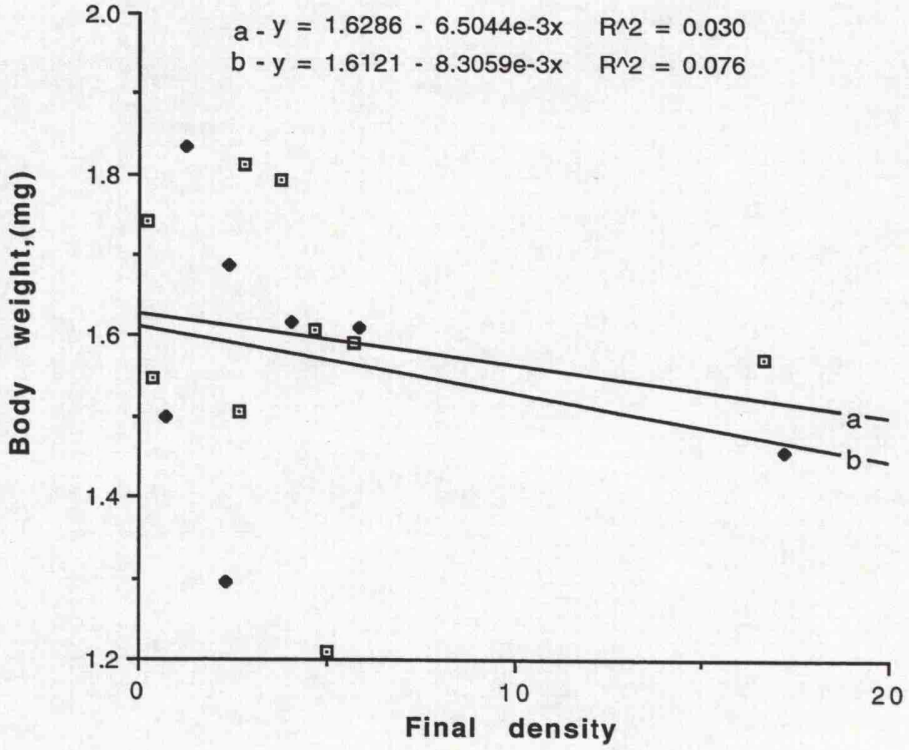


Fig.6: CORRELATION BETWEEN BODY WEIGHT AND FINAL DENSITY IN a-6WEEK-LINE AND b-8WEEK-LINE OF STRAIN N.21 OF T. CASTANEUM

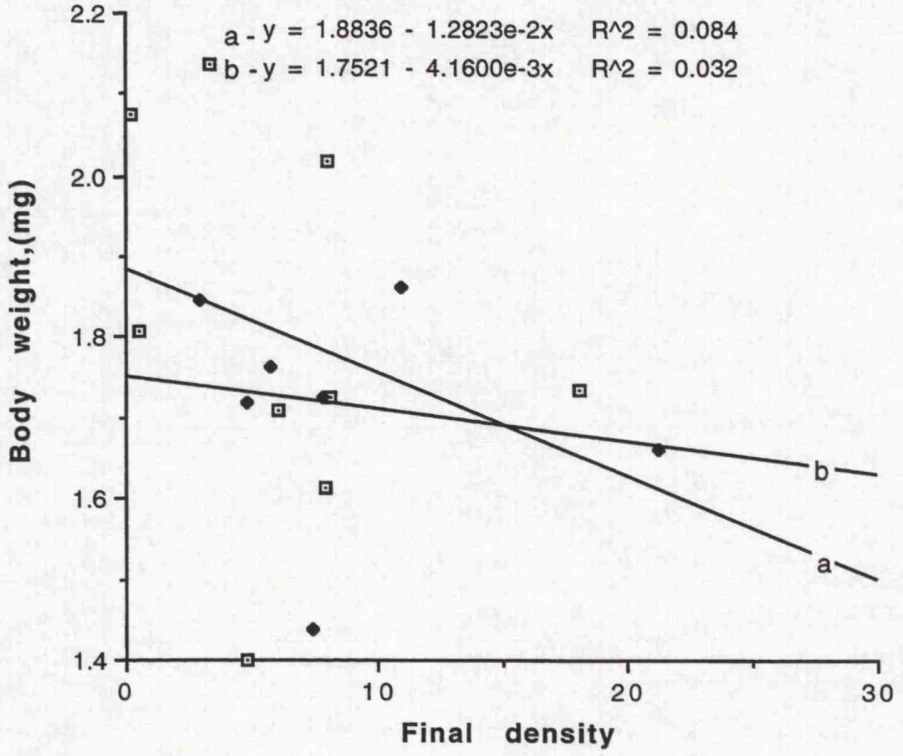


Fig.7a:CORRELATION BETWEEN DEVELOPMENT PERIOD AND BODY WEIGHT IN THE 6WEEK-LINE OF STRAIN N.04 OF T. CASTANEUM

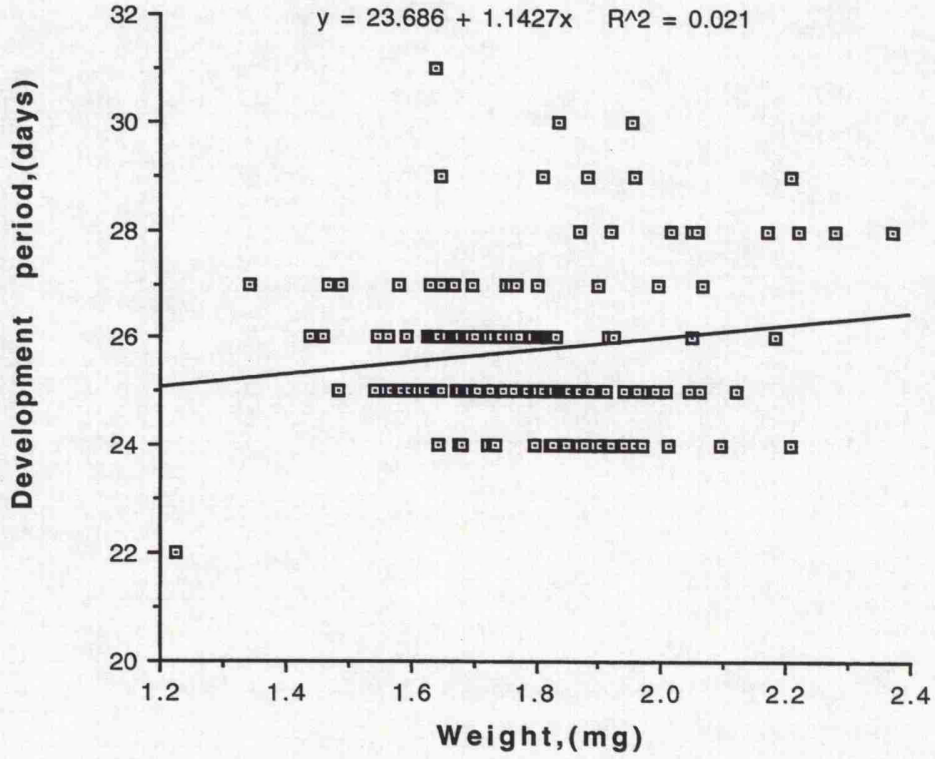


Fig.7b:CORRELATION BETWEEN DEVELOPMENT PERIOD AND BODY WEIGHT IN THE 8WEEK-LINE OF STRAIN N.04 OF T. CASTANEUM

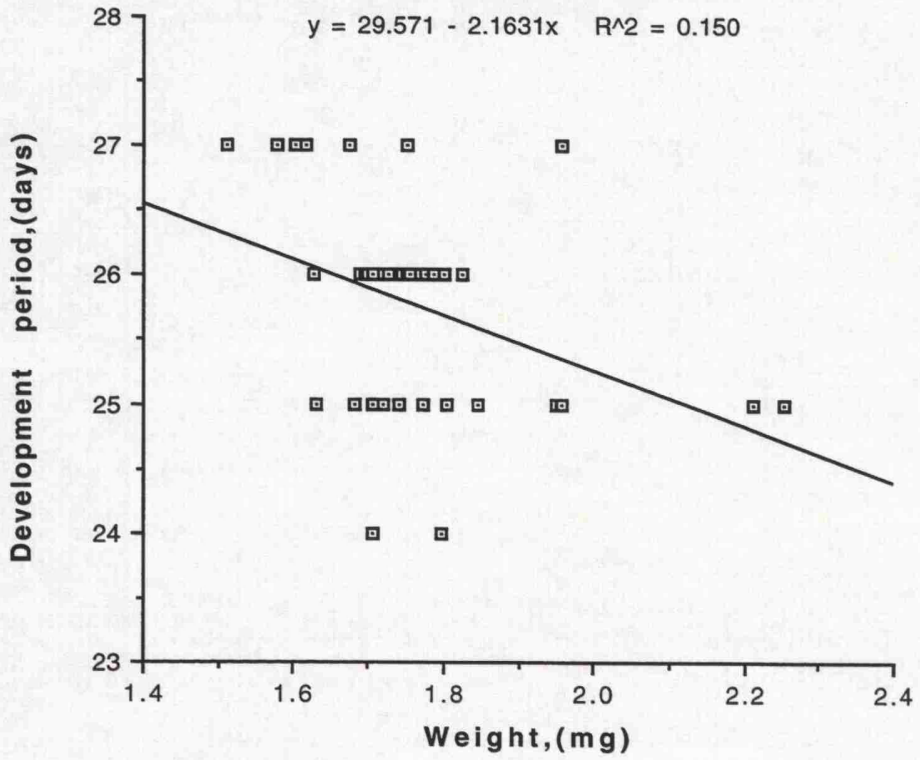


Fig.8a:CORRELATION BETWEEN DEVELOPMENT PERIOD AND BODY WEIGHT IN THE 6WEEK-LINE OF STRAIN N.21 OF T. CASTANEUM

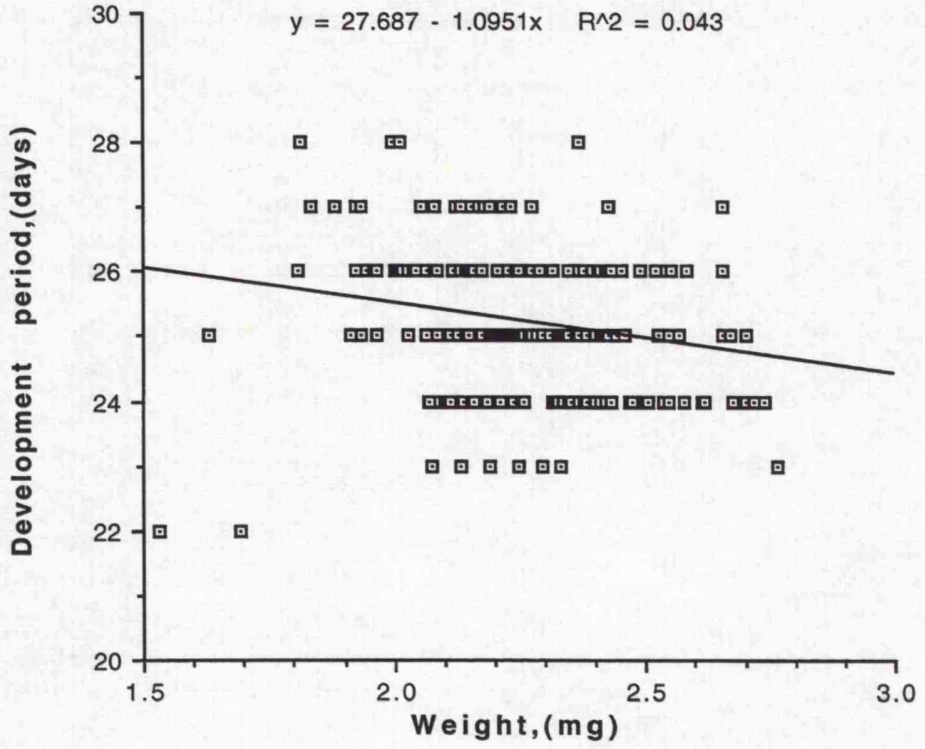


Fig.8b:CORRELATION BETWEEN DEVELOPMENT PERIOD AND BODY WEIGHT IN THE 8WEEK-LINE OF STRAIN N.21 OF T. CASTANEUM

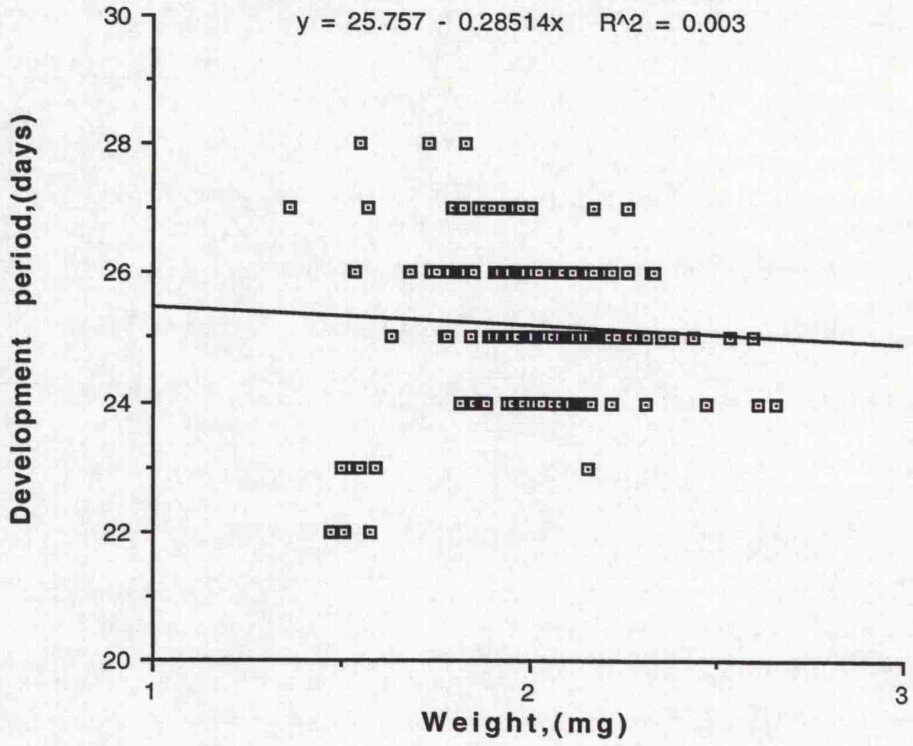


Fig.9a:CORRELATION BEWTEEN DEVELOPMENT PERIOD AND BODY WEIGHT IN THE 6WEEK-LINE OF THE CROSS-STRAIN

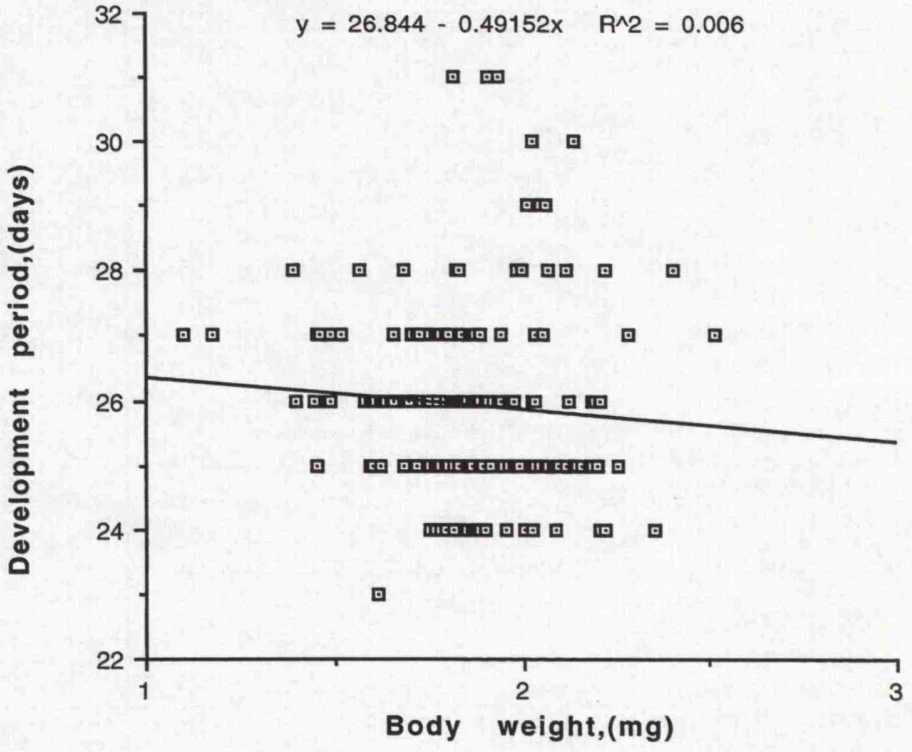
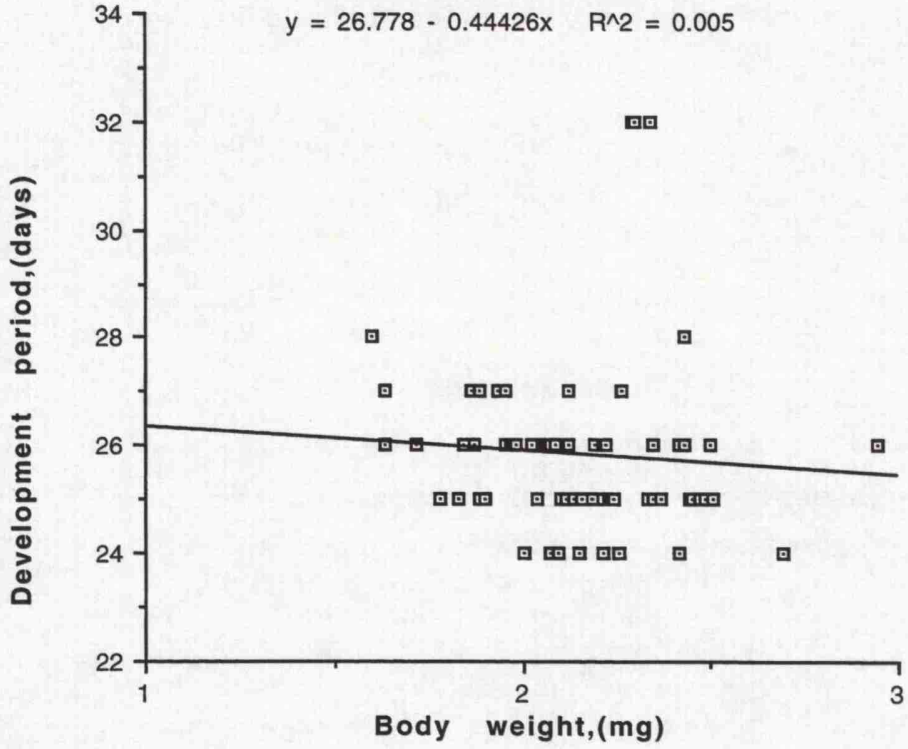


Fig.9b:CORRELATION BETWEEN DEVELOPMENT PERIOD AND BODY WEIGHT IN THE 8WEEK-LINE OF THE CROSS-STRAIN



6.3.6 Correlation Between Body Weight and Development Period

Following one generation of relaxed selection, test adults were weighed at emergence and the development period was also recorded. The phenotypic correlation between body weight and development period was shown by plotting individual values for development period against the corresponding values for body weight. Although the correlations had negative trends, the correlation was significant only in the 8 week-line of strain N.04 shown in Fig. 7b. The correlation had a positive trend in the 6 week-line of strain N.04 (Fig. 7a) due to two particularly small individuals weighing 1.10mg and 1.18mg and completing their development relatively fast in 27 days. Tables 6.8 to 6.10 (p143-145) show the regression of developmental period on body weight in the selection lines of the strains studied. The correlations were statistically significant only in the 8 week-line of strains N.04 and the 6 week-line of N.21. Although there is an indication that heavier insects tend to emerge earlier, the amount of variability means that no strong conclusions can be drawn.

Table 6.8: Regression of development period on body weight in a) 6 week-line and b) 8 week-line of strain N.04.

a) 6 week-line

Source	df	SS	MS	F	P
Regression	1	6.990	6.990	3.59	0.06
Error	169	328.636	1.945		
Total	170	335.626			

b) 8 week-line

Source	df	SS	MS	F	P
Regression	1	3.717	3.717	6.17	0.02
Error	35	21.094	0.603		
Total	36	24.811			

Table 6.9: Regression of development period on body weight in a) 6 week-line and b) 8 week-line of strain N.21.

a) 6 week-line

Source	df	SS	MS	F	P
Regression	1	10.630	10.630	9.06	0.003
Error	204	239.395	1.174		
Total	205	250.024			

b) 8 week-line

Source	df	SS	MS	F	P
Regression	1	0.742	0.742	0.60	0.438
Error	183	225.009	1.230		
Total	184	225.751			

Table 6.10: Regression of development period on body weight in a) 6 week-line and b) 8 week-line of the cross strain.

a) 6 week-line

Source	df	SS	MS	F	P
Regression	1	2.108	2.108	1.15	0.285
Error	107	360.907	1.832		
Total	198	363.015			

b) 8 week-line

Source	df	SS	MS	F	P
Regression	1	0.852	0.852	0.31	0.577
Error	66	179.030	2.713		
Total	67	179.882			

6.4 DISCUSSION

For cohesion in the discussion, development period will be considered first, then will follow body weight, productivity and related phenomena.

The inverse of development period is the development rate. Development rate is an important component of fitness because it affects the time to first breeding (Sibly and Calow, 1986) and hence the rate of increase.

Here it was predicted that the short culture interval (6 week-lines) would select directly for fast development by favouring fast developers as found by Møller et al. (1990) in Callosobruchus maculatus. In the slow lines (8 week-lines) densities of immature stages would be expected to increase to higher levels, maybe leading to indirect selection for strong competitive ability. As a consequence, longer culture intervals could result in larger larvae and eggs but in fewer numbers, or

alternatively in rapid development to avoid cannibalism, which is known to be an important factor regulating population size in T. castaneum (Lloyd, 1968). The results obtained in the present work show that development period did not respond to the different culture intervals. The reason might be that the mechanisms in force in the selection lines might have acted in the same directions: in the 6 week-line, the fast developers might be in increasing proportion from one generation to the next resulting in short development period; in the 8 week-line, higher density (with a higher degree of cannibalism) might lead to selection for fast development to escape cannibalism, although Dawson (1975) argued that cannibalism of early pupating individuals by large larvae may lead to selection for slower development.

Another reason for lack of response to selection may be the lack of additive genetic variance. Dawson (1975) argued also that selection for fast development is successful only in long-established laboratory populations of T. castaneum but not in strains recently derived from natural populations as was the case here for the strains used. The theoretical considerations leading to the prediction that colonizing organisms should be characterized by low amounts of additive genetic variation for development time and little if any response to artificial selection for fast development (Lewontin, 1965) support Dawson's (1975) statement. Soliman et al. (1972) also indicated a lack of genetic variance in development period in field populations of T. castaneum, but Møller et al. (1990) using quantitative genetic analysis found additive genetic variance in egg-to-adult development rate in Callosobruchus maculatus. Development period was not investigated in the quantitative genetics study (Chapter 5) and it is therefore not possible to determine whether the failure of selection to alter development rate is the result of lack of genetic variance within strains, or the action of different mechanisms operating in the different selection lines (eg fast developers favoured in the fast line and high density leading to fast development to avoid cannibalism in the slow line).

Another aim of the present study was to record the correlated responses in body weight at emergence and in productivity and age-specific productivity, as evidence of life-history trade-offs. When traits are genetically negatively correlated, artificial selection for an increase in one of the traits should cause a reduction in the other and vice versa (Sibly and Calow, 1986). Trade-offs are linkages between traits that constrain the simultaneous evolution of two or more traits (Stearns, 1992). For example, an increase in size potentially has two opposing effects on fitness; it usually requires longer development (negative effect) but might yield increased fecundity or decreased mortality rate (positive effect) (Sibly and Calow, 1986). Møller et al. (1989a) suggested that body size was the key character that links components of fitness in the cowpea weevil. The results obtained here show that the difference in body weight was significant only between strains but not between selection lines; the same conclusion was obtained with the selection line data as well as the test data. It was shown in Chapter 5 that body weight is heritable in strains used here. Thus the absence of a response in body weight to selection could indicate either that there is a trade-off between body weight and another variable that over-rode the effects of selection, or that body weight was selectively neutral in the selection regimes used here. Failures to find evidence of genetic trade-offs between development rate and body weight are reported in the literature. Wrelton (1987) for example, could not conclude that there was such a genetic correlation in Sitophilus oryzae although body weight was found to be subject to selection pressure. Here, the mean body weights from generations 6 to 9 (Leicester data only) show that individuals in the slow lines are lighter than those in the fast lines, possibly due to a density effect although the difference between the lines was not quite statistically significant ($P = 0.075$). Data from generations 1 to 5 (Bamako data) do not lead to the same conclusion, maybe because experimental conditions were less stable. The negative slopes in Figs 7 to 9 indicate that an increase in density tends to be associated with a decrease in body weight, but the fact that the correlations they describe were not significant suggests that the variation in population density was not sufficient to account for the variation in mean body weight seen during the selection experiment.

Body size was found also to be negatively phenotypically correlated with development period although the trend was not always significant. The negative correlation suggests that heavier insects tend to emerge earlier but the amount of variability mean that no strong conclusion can be drawn. The unexpected positive phenotypic correlation in the 6 week-line of strain N.04 (Zangasso) (Fig 7a) might have been caused by two individuals that were particularly small (1.100mg and 1.180mg) and completed their development relatively fast (in 27 days).

The results reported here show that there was no significant difference between selection lines in productivity (number of adult produced per female) but the test data show that the difference between the strains was significant. If body size is correlated with productivity as reported by Holloway (1984), the absence of significant differences in body weight between selection lines might explain the above result. Productivity is directly related to fecundity, which is reported by various authors to be correlated with body weight in most insects (eg Sibly et al. 1991). Smith et al. (1987) stated that trade-offs between body weight and fecundity and development rate are thought to influence responses to selection pressures and Sokoloff (1977) suggested that the negative correlation between productivity and development time indicates that different mechanisms may act independently in determining maximum population fitness. Conner et al. (1992) stated that other studies have not found evidence for constraint through genetic correlation, due either to a lack of correlation or to selection acting in the same direction on positively correlated traits. More investigations are still required in order to understand all the correlations involved in trade-offs and, as rightly pointed out by Conner et al. (1992), unmeasured characters (eg larval and pupal mortality) might affect the outcome, hence making difficult the interpretation of results.

The significant between-strain difference in productivity as well as in body weight suggests that the two strains are genetically different. Strain N.21 from Gao is heavier and more productive than strain N.04 from Zangasso. References

reporting differences between strains of T. castaneum are abundant in the literature (eg Sokoloff et al., 1965; Soliman et al., 1972). Thus the cross between the strains should have been genetically variable, though again there was no response to selection for culture interval.

The effect of parental age at time of mating (or more precisely at time of oviposition) was tested on body weight, development period and productivity and was found to be significant for development period and productivity but not for body weight. The results suggest that older individuals (12 days old) produced more young which developed faster; the reason might simply be that, although egg laying begins 72 hours after emergence (Dawson 1964b), sexual maturity is not complete. Older parents may lay eggs that need less time to develop. The reason why the parental age was not significant in the cross line is not understood.

From the seventh generation (Table 6.3), productivity dropped drastically in all strains. There was no evidence of an environmental effect, such as a sporozoan infection, and a possible explanation is an inbreeding effect. Dawson (1965) observed a similar phenomenon and had to change his selection method. In the present work also some replicates within selection lines had to be mixed for the following generation to avoid possible extinction.

Experimental conditions might have affected variation between generations. In Mali in particular it was difficult to maintain stable and uniform experimental conditions throughout. Desiccators had to be opened regularly for aeration and during measurements causing temporary changes in humidity. Cultures were kept in the incubator set at the experimental temperature but frequent power cuts occurred resulting also in temperature variations.

6.5 SUMMARY OF RESULTS

The main results of the work reported here can be summarized as follows:

6.5.1 Body Weight

- Significant difference between strains.
- No significant difference between selection lines.
- Significant strain x selection line interaction.

6.5.2 Development Period

- No significant difference between strains nor between selection lines.

6.5.3 Productivity

- Significant difference between strains.
- No significant difference between selection lines.

6.5.4 Trade-off

There was no evidence for or against trade-off between body weight and development period and productivity.

6.5.5 Effect of Parental Age

- Significant effect on development period and productivity.
- No significant effect on body weight.

Chapter 7 GENERAL DISCUSSION

T. castaneum is an economically important, secondary-storage insect pest attacking a wide range of stored commodities and is found throughout tropical and temperate regions. The presence of T. castaneum under temperate and tropical conditions may be an indication of its high evolutionary potential. Evolution takes place through natural selection which is defined as a fitness-maximizing process (Smith et al., 1987). Various life-history traits contribute to the fitness of an individual (Sibly and Calow, 1983). Combinations of traits that characterize different strains of a species may be sufficiently distinct to be recognised as biotypes. The present work was focused on biotype variation in T. castaneum from Mali.

To characterize the different biotypes, insect strains collected from various geographical locations were tested for differences in their ability to damage grain, in their resistance status, and in some life-history parameters that contribute to fitness. The results show that the strains studied were significantly different, in particular the two strains (N.04 and N.21) that were subject to detailed investigations. Despite some inconsistencies in the results reported in Chapter 4, essentially due to experimental conditions in Bamako, strain N.21 (Gao) was found to be significantly heavier than strain N.04 (Zangasso) (Chapters 5 and 6). The difference in body weight between the two strains was associated with the difference in oxygen consumption reported in Chapter 3. This result agrees with the findings of other authors showing that key metabolic processes depend on body size (eg Schmidt-Nielsen, 1984; Sibly and Calow, 1986; Reiss, 1989).

The weight loss caused by the different strains was also found to be significantly different at constant temperature. Selection lines caused significantly different weight loss (Table 3b, Chapter 3) suggesting that the ability to damage grain was affected by selection. The prediction that strains causing higher levels of damage would have higher metabolic rates was not supported by the results. Instead,

productivity was found to be the critical factor as it was positively correlated with weight loss. Productivity is the net result of the effect of the various parameters related to reproduction, ie fecundity, fertility, mortality rate at the different stages of development. Although productivity did not respond to the selection regimes using different culture intervals (Chapter 6), selection for culture interval seems to have affected ability to damage grain (Chapter 3), presumably through a change in one or more parameters that were not measured during the experiment; this response to selection indicates that one or more of the parameters involved is under genetic control.

The strains tested were found to be significantly different in their ability to tolerate insecticides (resistance status) as well as in some components of fitness measured. Conflicting findings concerning the fitness of resistant phenotypes are reported in the literature (eg Muggleton, 1983; Beeman and Nanis, 1986). Although very little is known about the initial frequency of resistant alleles (Roush and Croft, 1986) it is argued that frequency of resistant genes is expected to decrease in the absence of insecticide, because these genes are initially extremely rare in natural populations before the pesticide is used; this sort of inferential argument shows that there is the need for a better understanding of the true pleiotropic effects of resistant genes (Dyte, 1990). Pesticide resistance has long been recognised as an evolutionary change (Dobzhansky, 1937), and can be used as a tool to study the evolutionary process. However, in this study, resistance status was not correlated with life-history variation.

The ability to damage grain was found to be correlated with productivity (a fitness trait). But, as there was no evidence of correlation between resistance and fitness, weight loss could not be correlated with resistance. McFarlane et al. (1990), working with strains of R. dominica and Sitophilus oryzae resistant to phosphine, found similar results and stated that resistant strains were not necessarily characterized by a greater or lesser capacity to damage grain.

Having established the variation between strains, it was of practical interest to try to understand how variations (within and between strains) are maintained under natural selection and how insect populations respond to environmental factors including pest management practices. Several mechanisms by which genetic variation may be maintained have been described by various authors (eg Falconer, 1989; Sibly et al., 1990). Genetic correlations between strains, referred to as genetic trade-offs, have been described by Smith et al. (1987) as a process maintaining genetic variation under the influence of natural selection. Environmental variability may also contribute to the maintenance of genetic variability (Falconer, 1989).

In the present work, the phenotypic and genotypic variance and the heritability (h^2) of body weight were estimated (Chapter 5). In the selection experiment reported in Chapter 6, there was no evidence of genetic trade-offs between body weight, development period and productivity. The lack of evidence does not necessarily indicate the absence of trade-off, but it does show the need for further and detailed investigations because the mechanisms involved seem to be very complex. In particular, as many traits as possible should be measured together (Smith, 1991) as unmeasured traits can affect the outcome of the selection experiments (Conner et al., 1992). Holloway (1984) provided a review of some evolutionary theories and argued that "genetic revolutions" (Stanley, 1970 cited in Holloway, 1984) involving population bottlenecks could lead to the formation of distinct geographical strains in S. oryzae; his suggestion that other stored products insect species might be differentiated in the same manner remains to be supported with further data.

The effects of man's storage techniques and pest management practices are very important for the evolution of storage pests. For example, pressure from insecticide use results in selection for resistance (Fest et al., 1973), and Dyte (1990) for various reasons argued that resistant and susceptible strains might differ in properties other than their ability to tolerate particular pesticides. The climatic

conditions in Mali and the pest control practices outlined in Chapter 1, essentially characterized by contrasting climatic conditions (hot and dry versus cooler and wetter) and use of contact insecticides over a long period of time, can be expected to contribute to the genetic variations observed.

The present work and similar studies have great practical implications. A better understanding of how insect pests respond to environmental factors with respect to their genetic background can help to make more precise predictions, and as a result pest management strategies can be improved. This is particularly important as modern pest management practices are aiming at minimizing the use of pesticides. The reduction in pesticide use is especially urgent for developing countries where the lack of awareness of pesticide hazard leading to the abuse and misuse of toxic chemicals increases the risks attached to pesticides. For this reason, any studies that present original data concerning life-histories (Wrelton, 1987) or concerning the responses of insect pests to environmental factors may be of value.

Appendix 1 SUMMARY OF MEETING TO DISCUSS RESEARCH PROGRAMME FOR CHEICK OUMAR COULIBALY

(C O COULIBALY, J A MCFARLANE, R H SMITH)

MONDAY 15 OCTOBER 1990

PROGRAMME

1 **Initial Period in Reading**

Reduce to one term ie October to December 1990 (there will be a corresponding increase in the final period).

2 **Work in Mali (from January 1991)**

(i) collect strains/biotypes from different parts of Mali

(ii) carry out a preliminary sweep to identify major differences in ability to damage grain

(iii) examine in detail the life-histories of three or four strains (including resistance status)

(iv) do some quantitative genetics and selection experiments to reveal underlying genetic and environmental correlations between life-history characters.

3 **Final Period in Reading (from October 1992 or May 1992 if possible)**

Increase to 9 months (minimum) or 12 to 15 months (preferable).

Reason: controlled environment and computing facilities are available in Reading but not in Mali.

MATERIALS IN MALI

Pest Species: Rhyzopertha dominica and Tribolium castaneum.

Commodity:

- either millet or sorghum
- need to establish a stock of 50 (minimum) or 100 (preferable) kg early in 1991 for use in all experimental work in Mali
- require to purchase airtight drums for disinfestation and storage.

Equipment available:

microscope
oven
analytical balance
jars.

Equipment required:

small glass/plastic tubes
statistical package for microcomputer (eg SAS pc)
resistance test kits (from NRI)
pheromone traps (flight trap with DOMINICALURE).

AIMS

1 Biotype characterisation and fitness estimation.

2 Estimation of genetical and environmental correlations between life-history characters.

3 Estimation of direct and correlated responses to selection eg for fast/slow development.

4 Relationship of resistance (contact insecticides – Mali, maybe phosphine – in the UK) to biotype characteristics and genetic linkage.

5 Prediction of effect of insect migration on biotypes and fitness.

6 Development of model of pests in stored grain, based on areas in Mali which are good producers (always export) or poor producers (always import).

IMMEDIATE REQUIREMENTS (to December 1990)

1 Arrange for Mr Coulibaly to visit NRI, Chatham for two or three days (British Council to support?).

2 Attendance at appropriate courses:

(i) Statistics

(ii) Population Biology (Evolutionary Ecology).

3 Gain familiarity with laboratory techniques:

(i) rapid characterisation of apparent weight loss in 8 to 10 weeks

(ii) detailed examination of life-history characters.

4 Familiarity with SAS pc for data analysis.

5 Literature review.

Appendix 2 GILSON RESPIROMETER OPERATING INSTRUCTIONS

Each respirometer unit is separate from its neighbour consisting of a reference flask, a digital micrometer and a reaction flask connected by constant bore micro tubing. The system is set up in the following way.

1 Make sure the water bath is filled with distilled water to about 4cm from the top.

2 The air space volume of the reference flask is adjusted by adding distilled water to equal that of the reaction flask.

3 Turn the screw at the top of the contact thermometer so that the top of the moveable marker is at the temperature required. Put the main heater, refrigerator and stirrer on whilst you prepare the system (operating in a constant temperature room is advisable).

4 The coloured dye in the "U" tubes should be at about the halfway point up the tube, the moveable meniscusbar should be positioned to a fixed point on all units (say the bottom of the dye's meniscus). Set all the micrometer readings to 100 (this allows for + and - movement). NEVER go above 500 or below 000 on the micrometer as this will break the seal.

5 During all preparative work the Valve must be open (lever up as you look from the front).

6 Lightly grease all joints, put a small amount of water (5% KOH for animals) into the centre well and attach the reaction flasks using spring clips or elastic bands.

7 Allow about 1 hour for equilibration of the system.

8 To commence measurements close the Valve (lever down). Take readings at regular intervals, this is done by turning the micrometer so that the meniscus of the dye is level with the pre-set meniscusbar again. Values are in microlitres.

9 When finished open all valves (lever up) and remove the reaction flasks. Wipe all grease off and switch off stirrer, heater and refrigeration units.

To convert to STP use the following equation:

$$\frac{(273)(P_b - 3 - P_w)}{(273 + t)(760)}$$

t = temperature of water bath (°C)

P_b - 3 = operating pressure (barometric pressure minus three to compensate for mercury specific gravity at room temperature

P_w = vapour pressure of water.

Before using the apparatus for animals you need to pressure test the system if this has not been done for some time. This requires no experimental animals. You set the system as above and after equilibration you close the Valve and turn the contact thermometer one full turn clockwise, this increases the temperature of the bath a fraction of a degree. If no leaks are present a minimal change of ± 0-5µl will occur. If a unit decreases by more than this then there is a leak on the reference side, if a unit increases then there is a leak on the reaction side.

For each reading session take the mean of the controls and correct each experimental flask by subtracting this value to that recorded. This corrected value (µl Oxygen) can then be plotted against time for all readings. Using Cricket Graph you can plot this and fit a curve.

Narcotise animals with Carbon Dioxide and kill with Ethyl Acetate, record fresh then dry weights. Also plot Oxygen uptake against dry weight. Calculate µl/hour/mg dry weight.

Appendix 3 NRI INSECTICIDE RESISTANCE TEST KIT

3.1 INSTRUCTIONS FOR USE

The NRI test kit permits the identification of resistant insects by exposing them to a single discriminating dose of insecticide on treated filter papers. There are separate discriminating doses for different insecticides and for different insect species. The kit currently permits identification of resistance only for malathion, pirimiphos methyl and fenitrothion, and for the insects Tribolium castaneum and Sitophilus spp. The kit comprises test papers treated with insecticide, control papers treated only with solvent mixture, plastic rings, perforated covers, fluon and brushes. Data recording sheets (parts 1 and 2) prepared by the Insecticide Resistance Action Committee (IRAC) and intended for a wide range of uses are also provided. Some of the detail on the sheet is not relevant, eg 3 of the 6 replications can be ignored.

Test papers are supplied in packets, labelled with the insecticide and insect species for which they are to be used, each packet contains 9 papers. All control papers are identical and may be used for any test. Papers should be kept in a refrigerator (5°C), and not opened until they are to be used. Under these conditions papers can be expected to remain effective for six months from the date of preparation (expiry dates are marked on packets). Papers in packets which have been opened, can also be expected to remain effective for six months, provided they are refrigerated, and the foil wrapping re-closed securely. It may be preferable to keep all packets, particularly those which have been opened, in wide-mouthed jars with screwlids.

Where sufficient insects are available, it is usual practice in a discriminating dose test, to replicate the test using three rings on insecticide-treated papers, and to have one untreated control. Forty insects are used for each ring, a total of 160 insects being necessary for a full test. If a rapid result is required and only a few insects are available, two rings (or even one) plus a control can be used, and the full test

repeated later. Repeating the test is particularly necessary if resistance appears to be indicated from the preliminary test, because of the survival of some insects.

Unaged, unsexed, adult insects should be counted out in groups of 40 at random, and held without food for at least one hour, in clear escape proof containers, before application into the rings. Where Sitophilus spp. are to be tested, rings should be given a thin coating of fluon on the inside surface (and dried thoroughly), to prevent insect escape by crawling. Perforated lids are used to cover rings and prevent escape of flying insects during tests. Rings and papers should be placed on a clean smooth surface, preferably glass, for the test, during which, the temperature should be not less than 25°C or more than 30°C.

The exposure period on papers for T. castaneum is 5 hours, and for Sitophilus spp. is 6 hours, this time period must be strictly followed in order to obtain worthwhile results. At the end of the test period, the numbers of insects alive and dead are counted, and recorded on part 2 of the data sheets. For the purposes of the test, the numbers of insects dead and "knocked down" (moribund) are combined and assessed as susceptible. "Knocked down" insects cannot walk straight, or right themselves easily when lying on their backs. A gentle prodding should reveal whether insects are alive or dead which is particularly important with T. castaneum which pretends death. For Sitophilus spp., there is usually a much clearer indication between dead and living insects.

Provided the test procedure has been correctly followed, all control insects should be alive at the end of the test period. Any mortality in control insects (which is more likely with insects tested direct from the field than when an F_1 generation is used), will need to be corrected for in test insect counts. This can be done using Abbott's formula, which appears at the bottom of part 2 of the data recording sheet. If there is more than 10% control mortality in a test, that test should be regarded as unsatisfactory and repeated. The appearance of any live insects on insecticide-treated test papers at the end of the exposure period indicates that those insects

may be resistant to the insecticide. All data obtained in the monitoring survey should be returned to NRI at Chatham, and these data will be forwarded to the IRAC organisation in due course. Where any instances of serious resistance are revealed, NRI may be able to assist with further detailed investigation.

Note: Plastic rings are more likely to absorb insecticides from test papers than those made of glass. In order to ensure that no contamination occurs when rings are used in subsequent tests, they should be well scrubbed after use with detergent, rinsed and soaked overnight, or longer, in clear water with at least one water change during the soaking period.

NRI Chatham
March 1991

3.2 DATA RECORDING SHEETS (Three specimens)

INSECTICIDE RESISTANCE ACTION COMMITTEE

INSECTICIDE/ACARICIDE SUSCEPTIBILITY TESTS
DATA RECORDING SHEET, PART 1

1 General Information

IRAC Test Method No:

Investigator: CHEICK-OUMAR COULIBALY

Address: OPAM
BP 132 BAMAKO
REP MALI (WEST AFRICA)

2 Details of insects/mites collected

Species/stage: TRIBOLIUM CASTANEUM/ADULT STRAIN N.09

Crop/cultivar (where applicable):

Country/Location/date: MALI/MOPTI/23.7.1990

Brief history of recent insecticide/acaricide use: UNKNOWN

3 Test details

Treatment date/assessment date: 12.8.1991

Insect/mite stage used: ADULT

Holding conditions (min/max °C, humidity): T = 24°C-38°C: RH = 30-95% (RAINY SEASON)

Type of container: JARS

Plant material (crop, cultivar, size etc): SORGHUM

4 Test insecticide/acaricide

Active ingredient/formulation: FENITROTHION/E.C.

Origin/batch no./date:

Additional wetter if used; specify:

5 Remarks

STRAIN COLLECTED IN PRIVATE STORE IN MOPTI (MALI)

INSECTICIDE RESISTANCE ACTION COMMITTEE

INSECTICIDE/ACARICIDE SUSCEPTIBILITY TESTS

DATA RECORDING SHEET, PART 2*

Compound	(%ai) dose	Replicate 1		Replicate 2		Replicate 3		Replicate 4		Replicate 5		Replicate 6**	Overall Total			
		Dead/ moribund	Total	Dead/ moribund	Total	Dead/ moribund	Total	Dead/ moribund	Total	Dead/ moribund	Total	Dead/ moribund	Dead/ moribund	Total	Mortality %	Correction Mortality %
Fenitrothion	0.8	40	40	40	40	40	40	—		—		—	120	120	100	100
Fenitrothion	0.5	40	40	40	40	40	40	—		—		—	120	120	100	100
Malathion	0.5	0	40	0	40	1	40	—		—		—	1	120	0.83	0.83
Pirimiphos-methyl	2.0	40	40	40	40	40	40	—		—		—	120	120	100	100
Pirimiphos-methyl	0.7	40	40	40	40	40	40	—		—		—	120	120	100	100
Untreated control		0	40										0	40	0	

(Abbotts formula) Corrected mortality = $\frac{P-C}{100-C}$ X100 where P =% mortality in treatment, C =% mortality in controls

* Must be accompanied by Part 1.

** If more than 6 replicates use second line

INSECTICIDE RESISTANCE ACTION COMMITTEE

INSECTICIDE/ACARICIDE SUSCEPTIBILITY TESTS
DATA RECORDING SHEET, PART 1

1 General Information

IRAC Test Method No:

Investigator: CHEICK-OUMAR COULIBALY

Address: OPAM
BP 132 BAMAKO
REP MALI (WEST AFRICA)

2 Details of insects/mites collected

Species/stage: TRIBOLIUM CASTANEUM STRAIN N.09/ ADULT

Crop/cultivar (where applicable):

Country/Location/date: MALI/NIAFUNKE/20.6.1990

Brief history of recent insecticide/acaricide use: STRAIN COLLECTED IN A STORE
REGULARLY TREATED WITH
FENITROTHION AND PHOSTOXIN

3 Test details

Treatment date/assessment date: 14.8.1991

Insect/mite stage used: ADULT

Holding conditions (min/max °C, humidity):

Type of container: JARS

Plant material (crop, cultivar, size etc): GRAIN OF SORGHUM

4 Test insecticide/acaricide

Active ingredient/formulation:

Origin/batch no./date:

Additional wetter if used; specify:

5 Remarks

INSECTICIDE RESISTANCE ACTION COMMITTEE

INSECTICIDE / ACARICIDE SUSCEPTIBILITY TESTS

DATA RECORDING SHEET, PART 2*

Compound	(%ai) dose	Replicate 1		Replicate 2		Replicate 3		Replicate 4		Replicate 5		Replicate 6**		Overall Total			
		Dead/ moribund	Total	Dead/ moribund	Total	Dead/ moribund	Total	Dead/ moribund	Total	Dead/ moribund	Total	Dead/ moribund		Dead/ moribund	Total	Mortality %	Correction Mortality %
Fenitrothion	0.8	39	40	39	40	40	40	—		—		—		118	120	98.33	98.33
Fenitrothion	0.5	11	40	15	40	9	40	—		—		—		35	120	29.16	29.16
Pirimiphos-methyl	2	40	40	40	40	40	40	—		—		—		120	120	100	100
Pirimiphos-methyl	0.7	40	40	39	40	40	40	—		—		—		119	120	99.16	99.16
Malathion	0.5	2	40	0	40	2	40	—		—		—		4	120	3.33	3.33
Untreated control		0	40											0	40	0	

(Abbotts formula) Corrected mortality = $\frac{P-C}{100-C}$ X100 where P =% mortality in treatment, C =% mortality in controls

* Must be accompanied by Part 1.

** If more than 6 replicates use second line

INSECTICIDE RESISTANCE ACTION COMMITTEE

INSECTICIDE/ACARICIDE SUSCEPTIBILITY TESTS
DATA RECORDING SHEET, PART 1

1 General Information

IRAC Test Method No:

Investigator: CHEICK-OUMAR COULIBALY

Address: OPAM
BP 132 BAMAKO
REP MALI (WEST AFRICA)

2 Details of insects/mites collected

Species/stage: TRIBOLIUM CASTANEUM STRAIN N.17

Crop/cultivar (where applicable):

Country/Location/date: MALI/SEVARE (MOPTI)/13.2.1991

Brief history of recent insecticide/acaricide use: REGULAR TREATMENT WITH FENITROTHION AND PH_3

3 Test details

Treatment date/assessment date: 20.8.1991

Insect/mite stage used: ADULT

Holding conditions (min/max °C, humidity): LABORATORY CONDITIONS

Type of container: JARS

Plant material (crop, cultivar, size etc): GRAIN SORGHUM

4 Test insecticide/acaricide

Active ingredient/formulation: FENITROTHION/E.C.

Origin/batch no./date:

Additional wetter if used; specify:

5 Remarks

INSECTICIDE RESISTANCE ACTION COMMITTEE

INSECTICIDE/ACARICIDE SUSCEPTIBILITY TESTS

DATA RECORDING SHEET, PART 2*

		Replicate 1		Replicate 2		Replicate 3		Replicate 4		Replicate 5		Replicate 6**	Overall Total			
Compound	(%ai) dose	Dead/ moribund	Total	Dead/ moribund	Total	Dead/ moribund	Total	Dead/ moribund	Total	Dead/ moribund	Total	Dead/ moribund	Dead/ moribund	Total	Mortality %	Correction Mortality %
Pirimiphos-methyl	2	39	40	39	40	40	40	—		—		—	118	120	98.33	98.33
Pirimiphos-methyl	0.7	30	40	34	40	30	40	—		—		—	94	120	78.33	78.33
Untreated control		0	40										0	40	0	

(Abbotts formula) Corrected mortality = $\frac{P-C}{100-C}$ X100 where $\begin{matrix} P \\ C \end{matrix}$ =% mortality in treatment
=% mortality in controls

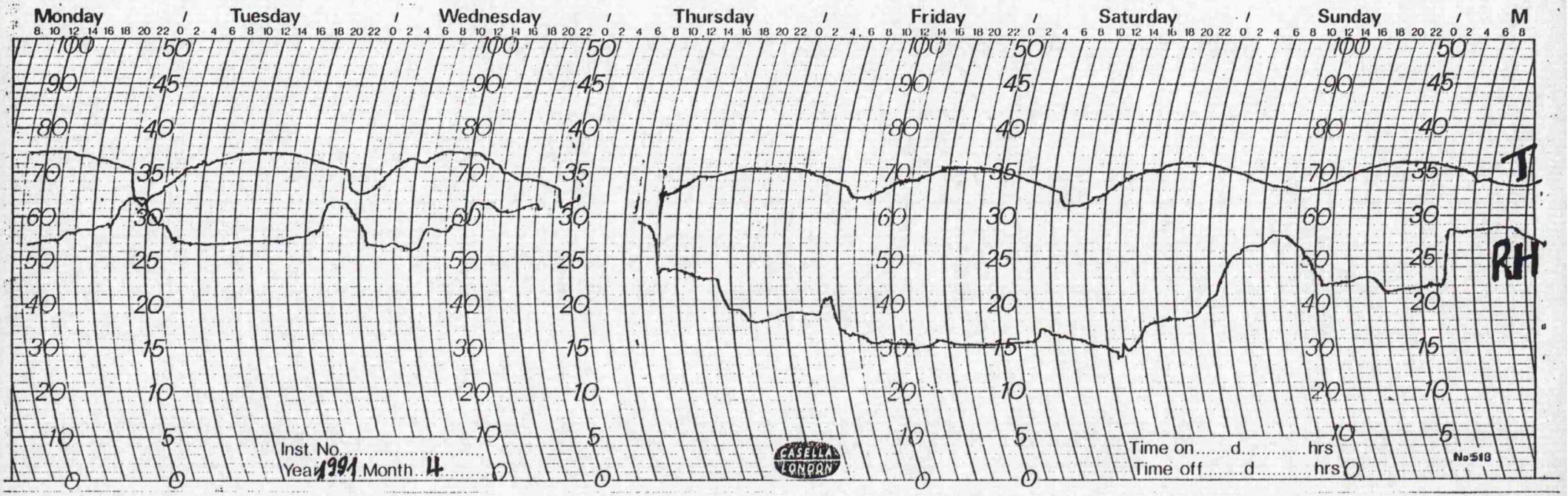
* Must be accompanied by Part 1.

** If more than 6 replicates use second line

Appendix 4 GRAPHS FROM THE THERMO-HYGROGRAPH

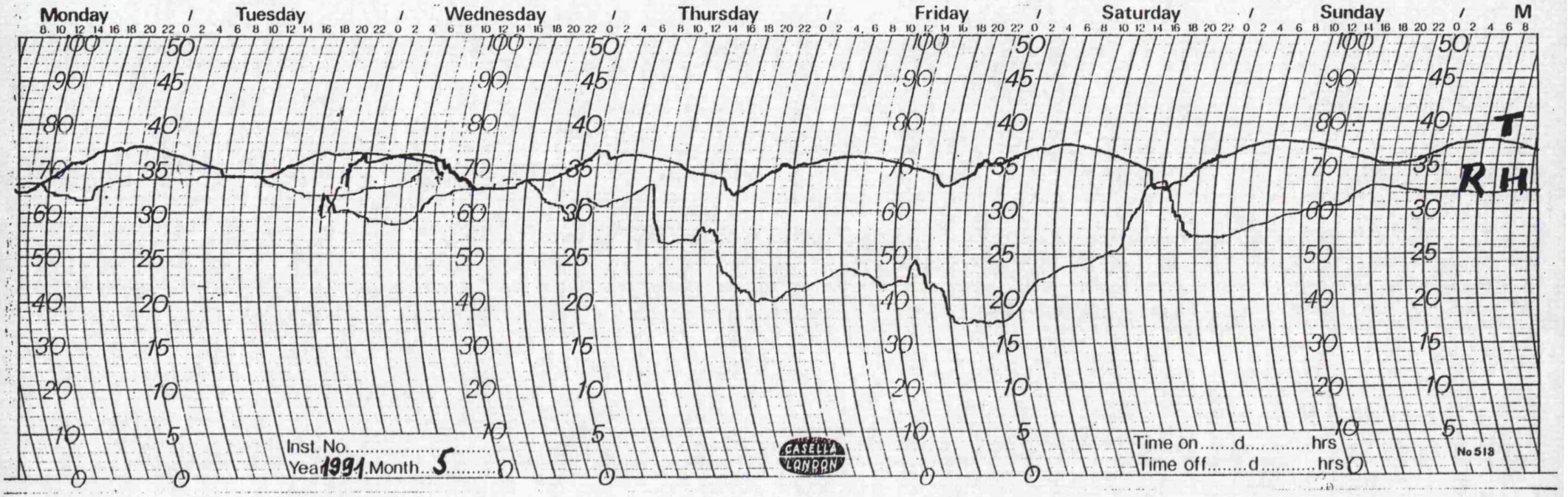
4.1 Temperature (T) and relative humidity (RH) variations recorded by thermo-hygrograph from 11 to 18 April 1991 in Bamako

	Minimum	Maximum
T	31°C	36°C
RH	29%	62%



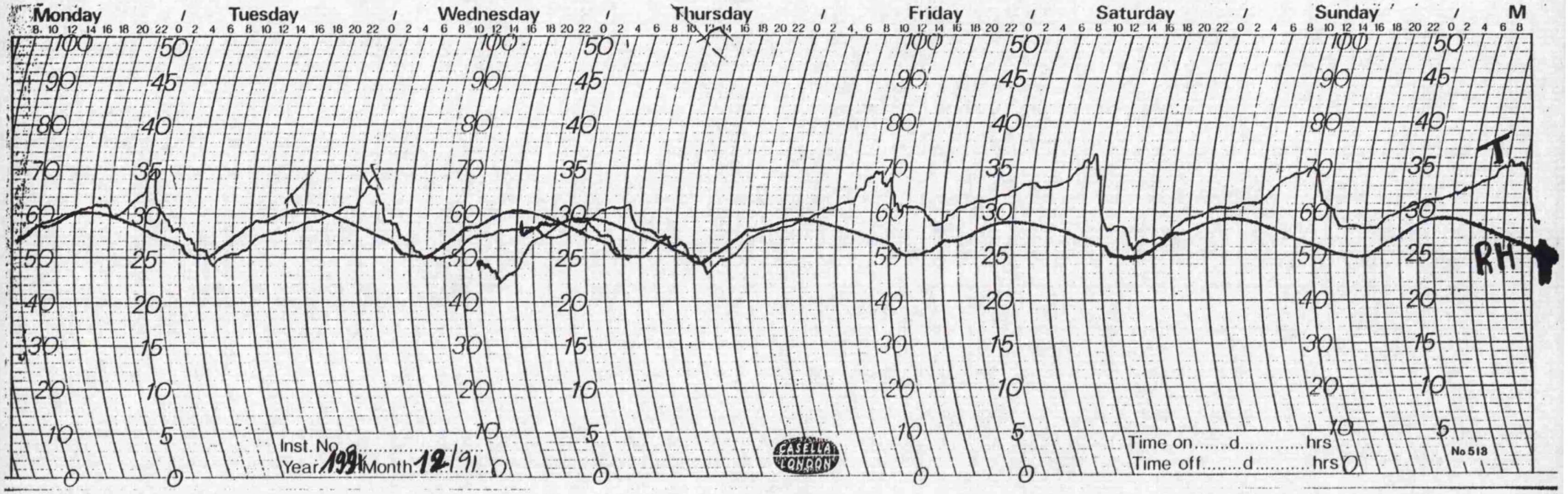
4.2 Temperature (T) and relative humidity (RH) variations recorded by thermo-hygrograph from 30 April to 8 May 1991 in Bamako

	Minimum	Maximum
T	32.5°C	38°C
RH	34.5%	66%



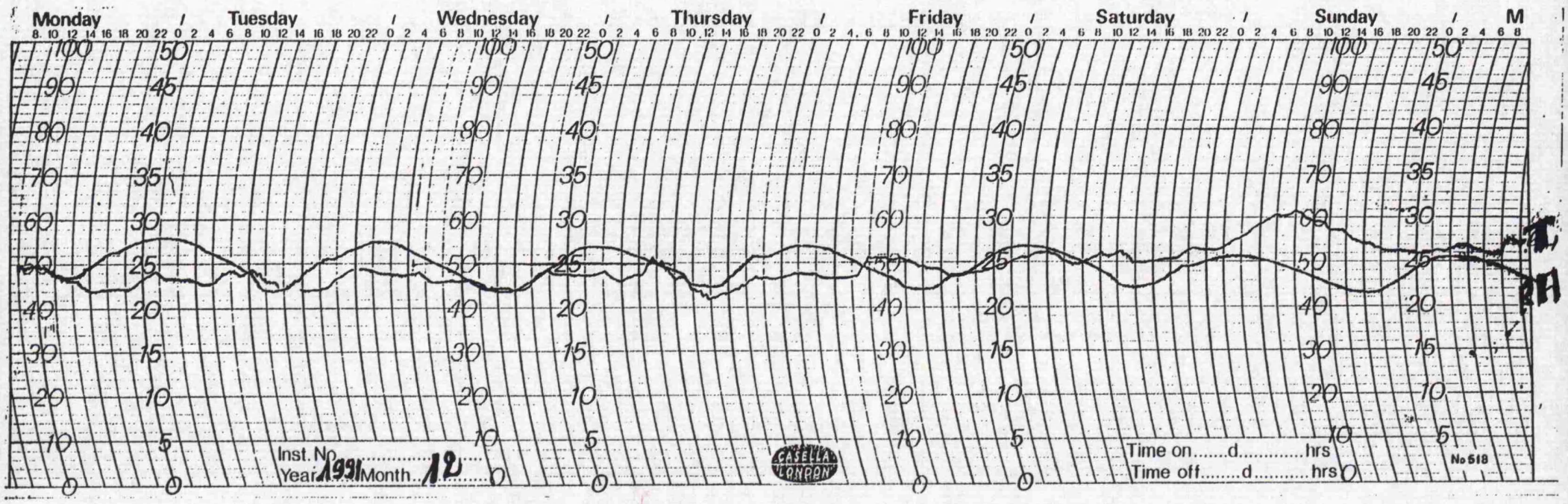
4.3 Temperature (T) and relative humidity (RH) variations recorded by thermo-hygrograph from 12 to 20 December 1991 in Bamako

	Minimum	Maximum
T	24°C	31°C
RH	50%	70%



4.4 Temperature (T) and relative humidity (RH) variations recorded by thermo-hygrograph from 23 to 30 December 1991 in Bamako

	Minimum	Maximum
T	22°C	28°C
RH	42%	60%



Appendix 5

Table 1: Measurement at 1st reculture of 6 week-regime in Bamako

- Date of initial culture 10.01.1992
- Date adults removed 24.01.1992
- Date of reculture 21.02.1992

ZANGASSO N.04

	Replicate Numbers					
	1	2	3	4	5	MEAN
Number of adults	124	207	267	90	135	164.6
Mean weight of adults (mg)	1.568	1.662	1.611	1.573	1.629	1.608
Standard deviation (mg)	0.884	0.872	0.892	0.590	1.119	0.071
Wt of jars with content (g)	168.407	169.257	168.764	169.905	170.016	169.409
Adults alive/dead	124/0	207/0	267/0	88/2	133/2	163.8/08
Wt of jars after adults removed (g)	167.726	168.918	167.740	169.279	169.205	168.573
Wt of jars at reculturing (g)	180.363	180.691	182.351	182.288	175.698	180.278

GAO N.21

	Replicate Numbers					
	11	12	13	14	15	MEAN
Number of adults	178	235	104	151	165	166.6
Mean weight of adults (mg)	1.363	1.338	1.397	1.469	1.440	1.401
Standard deviation (mg)	1.105	1.091	1.569	1.252	2.080	1.419
Wt of jars with content (g)	165.965	165.179	168.107	166.512	168.180	166.788
Adults alive/dead	172/6	235/0	104/0	150/1	164/1	165/1.6
Wt of jars after adults removed (g)	164.972	164.133	167.554	165.663	167.198	165.904
Wt of jars at reculturing (g)	180.828	180.084	181.181	180.253	182.362	180.941

Table 2: Measurement at 2nd reculture of 6 week-regime in Bamako

- Date of initial culture 10.01.1992
- Date adults removed 07.03.1992
- Date of reculture 03.04.1992

ZANGASSO N.04

	Replicate Numbers					
	1*	2	3	4	5	MEAN
Number of adults	98	219	245	416	274	250.4
Mean weight of adults (mg)	1.188	1.216	1.181	1.173	1.289	1.210
Standard deviation (mg)	0.46	1.85	1.01	1.27	1.87	1.29
Wt of jars with content (g)	182.333	-	182.407	181.000	176.317	180.514
Adults alive/dead	67/31	198/84	761/84	349/47	256/18	206.2/52.8
Wt of jars after adults removed (g)	181.651	179.523	181.491	179.908	175.276	179.569
Wt of jars at reculturing (g)	176.037	181.927	184.535	182.986	182.065	181.510

* For reculturing was completed with n2

GAO N.21

	Replicate Numbers					
	11	12	13	14	15	MEAN
Number of adults	536	449	246	450	303	396.8
Mean weight of adults (mg)	1.459	1.449	1.394	1.895	1.866	1.613
Standard deviation (mg)	1.64	1.19	1.68	1.34	1.36	1.44
Wt of jars with content (g)	178.685	178.477	181.244	178.354	181.839	179.719
Adults alive/dead	482/54	420/29	215/31	400/50	246/57	352.6/44.2
Wt of jars after adults removed (g)	177.241	177.366	180.166	176.976	180.870	178.518
Wt of jars at reculturing (g)	182.771	182.689	182.425	184.720	178.613	182.244

Table 3: Measurement at 3rd reculture of 6 week-regime in Bamako

- Date of initial culture 10.01.1992
- Date adults removed 17.04.1992
- Date of reculture 15.05.1992

ZANGASSO N.04

	Replicate Numbers					
	1	2	3	4	5	MEAN
Number of adults	115	106	107	133	228	137.8
Mean weight of adults (mg)	1.738	1.913	1.779	1.834	1.792	1.811
Standard deviation (mg)	1.54	0.81	0.67	1.60	1.10	1.144
Wt of jars with content (g)	177.450	182.782	185.476	183.759	182.077	182.309
Adults alive/dead	113/2	106/0	107/0	132/1	225/3	136.6/1.2
Wt of jars after adults removed (g)	177.033	182.445	185.128	183.375	181.479	181.892
Wt of jars at reculturing (g)	185.458	178.343	182.921	185.018	184.414	183.223

GAO N.21

	Replicate Numbers					
	11	12	13	14	15	MEAN
Number of adults	396	417	397	359	442	402.2
Mean weight of adults (mg)	1.993	1.982	2.002	2.080	2.030	2.018
Standard deviation (mg)	1.28	1.50	0.70	1.12	1.44	1.21
Wt of jars with content (g)	181.458	180.566	180.817	183.090	176.219	180.430
Adults alive/dead	390/6	406/11	387/10	355/4	438/4	395.2/7
Wt of jars after adults removed (g)	180.249	179.312	179.556	181.837	174.803	179.151
Wt of jars at reculturing (g)	182.875	178.072	184.432	184.107	183.207	182.537

Table 4: Measurement at 4th reculture of 6 week-regime in Bamako

- Date of initial culture 10.01.1992
- Date adults removed 29.05.1992
- Date of reculture 26.06.1992

ZANGASSO N.04

	Replicate Numbers					
	1	2	3	4	5	MEAN
Number of adults	279	377	219	322	229	285.2
Mean weight of adults (mg)	1.58	1.69	1.518	1.619	1.551	1.592
Standard deviation (mg)	1.02	1.42	1.17	0.99	1.35	1.19
Wt of jars with content (g)	183.643	175.971	182.005	182.861	180.119	180.919
Adults alive/dead	279/0	375/2	218/1	319/3	227/2	283.6/1.6
Wt of jars after adults removed (g)	182.879	175.046	181.446	182.102	182.677	180.830
Wt of jars at reculturing (g)	184.084	183.582	182.510	181.916	183.649	183.148

GAO N.21

	Replicate Numbers					
	11	12	13	14	15	MEAN
Number of adults	348	106*	481	260	538	406.75
Mean weight of adults (mg)	1.768	1.683	1.789	1.652	1.730	1.724
Standard deviation (mg)	1.02	1.11	1.15	1.39	0.92	1.12
Wt of jars with content (g)	-	-	-	-	-	-
Adults alive/dead	345/3	106/0	467/14	259/1	532/6	400.7/5.95
Wt of jars after adults removed (g)	178.549	174.167	179.779	178.093	178.323	177.782
Wt of jars at reculturing (g)	182.856	182.413	183.944	182.524	180.899	182.527

* omitted in mean calculation

Table 5: Measurement at 5th reculture of 6 week-regime in Bamako

- Date of initial culture 10.01.1992
- Date adults removed 10.07.1992
- Date of reculture 08.08.1992

ZANGASSO N.04

	Replicate Numbers					
	1	2	3	4	5	MEAN
Number of adults	149	127	94	201	95	133.200
Mean weight of adults (mg)	1.550	1.657	1.460	1.538	1.517	1.507
Standard deviation (mg)						
Wt of jars with content (g)	-	-	-	-	-	-
Adults alive/dead	147/2	125/2	92/2	199/2	95/0	131.6/1.6
Wt of jars after adults removed (g)	-	-	-	-	-	-
Wt of jars at reculturing (g)	-	-	-	-	-	-

GAO N.21

	Replicate Numbers					
	11	12	13	14	15	MEAN
Number of adults	296	231	279	359	352	303.450
Mean weight of adults (mg)	1.865	1.636	1.638	1.688	1.573	1.710
Standard deviation (mg)						
Wt of jars with content (g)	-	-	-	-	-	-
Adults alive/dead	290/6	230/1	278/1	358/1	355/7	302.20/3.2
Wt of jars after adults removed (g)	-	-	-	-	-	-
Wt of jars at reculturing (g)	-	-	-	-	-	-

- Not determined because end of experiment in Bamako

Table 6: Measurement at 1st reculture of 8 week-regime in Bamako

- Date of initial culture 10.01.1992
- Date adults removed 24.01.1992
- Date of reculture 06.03.1992

ZANGASSO N.04

	Replicate Numbers					
	6	7	8	9	10	MEAN
Number of adults	276	183	244	147	152	200.4
Mean weight of adults (mg)	1.656	1.561	1.558	1.680	1.641	1.619
Standard deviation (mg)	1.004	0.578	1.058	2.617	1.210	1.293
Wt of jars with content (g)	167.664	167.727	167.510	168.020	163.829	166.95
Adults alive/dead	272/4	182/1	242/2	145/2	149/3	198/2.4
Wt of jars after adults removed (g)	166.681	167.018	166.273	167.171	162.752	165.959
Wt of jars at reculturing (g)	179.964	181.501	176.998	183.059	181.393	180.583

GAO N.21

	Replicate Numbers					
	16	17	18	19	20	MEAN
Number of adults	177	375	351	582	468	390.6
Mean weight of adults (mg)	1.722	1.788	1.767	1.641	1.702	1.724
Standard deviation (mg)	1.894	1.297	1.827	2.365	2.359	1.948
Wt of jars with content (g)	167.037	165.620	165.219	167.129	168.218	166.644
Adults alive/dead	173/4	369/6	343/8	570/12	458/10	382.6/8
Wt of jars after adults removed (g)	165.979	164.092	163.673	165.156	166.202	165.020
Wt of jars at reculturing (g)	182.933	183.706	182.007	181.494	181.313	182.290

Table 7: Measurement at 2nd reculture of 8 week-regime in Bamako

- Date of initial culture 06.03.1992
- Date adults removed 20.03.1992
- Date of reculture 01.05.1992

ZANGASSO N.04

	Replicate Numbers					
	6	7	8	9	10	MEAN
Number of adults	136	97	93	141	102	113.8
Mean weight of adults (mg)	1.282	1.267	1.306	1.331	1.298	1.297
Standard deviation (mg)	2.78	2.15	1.73	2.44	0.51	1.94
Wt of jars with content (g)	178.029	180.271	175.613	181.111	180.725	179.150
Adults alive/dead	104/32	80/17	80/13	104/37	83/19	90.2/23.6
Wt of jars after adults removed (g)	177.551	179.799	175.039	180.583	180.087	178.612
Wt of jars at reculturing (g)	184.569	183.533	184.262	185.750	179.172	183.457

GAO N.21

	Replicate Numbers					
	16	17	18	19	20	MEAN
Number of adults	254	307	220	214	203	239.6
Mean weight of adults (mg)	1.626	1.628	1.77	1.71	1.86	1.72
Standard deviation (mg)	2.26	2.81	2.49	2.61	3.27	2.68
Wt of jars with content (g)	176.413	177.278	176.842	176.320	176.867	176.744
Adults alive/dead	201/53	258/49	169/51	168/49	177/26	194/45.6
Wt of jars after adults removed (g)	175.480	175.647	176.153	174.807	175.420	175.501
Wt of jars at reculturing (g)	182.097	183.722	183.343	183.294	183.626	183.216

Table 8: Measurement at 3rd reculture of 8 week-regime in Bamako

- Date of initial culture 10.01.1992
- Date adults removed 15.05.1992
- Date of reculture 26.06.1992

ZANGASSO N.04

	Replicate Numbers					
	6	7	8	9	10	MEAN
Number of adults	321	271	352	281	247	294.4
Mean weight of adults (mg)	1.599	1.551	1.604	1.650	1.653	1.611
Standard deviation (mg)	1.31	1.19	1.37	1.13	0.78	1.16
Wt of jars with content (g)	181.119	181.029	180.872	182.925	177.588	180.706
Adults alive/dead	318/3	259/12	346/6	264/17	239/8	285.2/9.2
Wt of jars after adults removed (g)	180.107	180.201	179.913	182.143	176.697	179.812
Wt of jars at reculturing (g)	182.338	183.501	183.217	182.127	177.912	181.819

GAO N.21

	Replicate Numbers					
	16	17	18	19	20	MEAN
Number of adults	547	550	545	594	510	549.2
Mean weight of adults (mg)	1.807	1.869	1.838	1.882	1.916	1.862
Standard deviation (mg)	1.37	0.93	0.92	1.18	1.02	1.08
Wt of jars with content (g)	175.155	178.555	177.816	177.657	178.743	177.585
Adults alive/dead	542/5	534/16	542/3	583/11	501/9	540.4/8.8
Wt of jars after adults removed (g)	173.420	177.068	176.307	176.022	177.223	176.008
Wt of jars at reculturing (g)	183.467	182.117	183.502	181.191	183.786	182.813

Table 9: Measurement at 4th reculture of 8 week-regime in Bamako

- Date of initial culture 10.01.1992
- Date adults removed 10.07.1992
- Date of reculture 21.08.1992

ZANGASSO N.04

	Replicate Numbers					
	6	7	8	9	10	MEAN
Number of adults	153	81	142	122	97	119
Mean weight of adults (mg)	1.707	1.628	1.665	1.695	1.741	1.687
Standard deviation (mg)	1.66	1.33	0.90	1.74	0.55	1.24
Wt of jars with content (g)	181.275	183.033	182.451	181.413	177.338	181.102
Adults alive/dead	147/6	81/0	139/3	120/2	96/1	116.6/2.4
Wt of jars after adults removed (g)	180.945	182.662	182.097	181.095	177.104	180.781
Wt of jars at reculturing (g)	-	-	-	-	-	-

GAO N.21

	Replicate Numbers					
	16	17	18	19	20	MEAN
Number of adults	243	284	276	353	292	289.6
Mean weight of adults (mg)	1.709	1.716	1.751	1.788	1.848	1.762
Standard deviation (mg)	1.66	0.98	0.88	1.18	0.91	1.12
Wt of jars with content (g)	181.891	180.105	181.258	177.809	181.225	180.457
Adults alive/dead	239/4	276/8	269/7	349/4	286/6	283.8/5.8
Wt of jars after adults removed (g)	181.035	179.185	180.405	176.845	180.003	179.494
Wt of jars at reculturing (g)	-	-	-	-	-	-

- End of experiments in Bamako

Appendix 6

Table 1: Body size measurements

- Date 21.02.1992
- Culture regime 6 weeks
- Species T. castaneum

	Weight of 20 adults X (5) (mg)					Mean of (i)-(v)	St Dev	Mean of (i)-(v)/20 Wt of 1 ad
	(i)	(ii)	(iii)	(iv)	(v)			
1	32.5	32.1	31.0	31.1	30.0	31.34	0.986	1.567
2	32.0	32.8	34.7	33.6	33.6	33.34	1.008	1.667
3	32.6	32.1	34.5	32.5	32.0	32.74	1.016	1.637
4	32.1	30.7	31.4	31.7	-	31.47	0.591	1.573
5	33.7	31.7	34.0	32.4	31.1	32.58	1.251	1.629
11	27.0	26.6	27.5	28.2	27.8	27.42	0.634	1.371
12	25.9	25.7	27.3	28.3	28.2	27.01	1.233	1.354
13	27.8	25.9	29.0	29.9	27.1	27.94	1.569	1.397
14	28.3	28.9	28.1	29.9	29.6	28.96	0.078	1.448
15	27.7	26.2	26.6	29.6	30.5	28.12	0.187	1.406

1 - 5 = Strain N.04 (Zangasso)
11 - 15 = Strain N.21 (Gao)

Table 2: Body size measurements

- Date 03.04.1992
- Culture regime 6 weeks
- Species T. castaneum

	Weight of 20 adults X (5) (mg)					Mean of (i)-(v)	St Dev	Mean of (i)-(v)/20 Wt of 1 ad
	(i)	(ii)	(iii)	(iv)	(v)			
1	23.5	24.3	23.5	-	-	23.76	0.462	1.188
2	26.5	26.2	23.8	23.3	27.2	25.40	1.736	1.270
3	23.7	24.2	23.2	23.0	25.3	23.88	0.920	1.194
4	24.1	22.7	22.1	23.7	22.0	22.92	0.944	1.146
5	23.6	24.4	23.7	25.5	26.3	24.70	1.172	1.235
11	28.1	26.4	28.0	29.6	27.8	27.98	1.136	1.399
12	28.7	28.8	30.2	26.9	28.2	28.56	1.188	1.428
13	28.8	28.8	29.8	30.4	27.4	29.04	1.143	1.452
14	36.6	38.3	35.8	38.3	38.3	37.46	1.184	1.873
15	36.0	35.1	36.6	37.5	39.0	36.84	1.491	1.842

1 - 5 = Strain N.04 (Zangasso)
11 - 15 = Strain N.21 (Gao)

Table 3: Body size measurements

- Date 15.05.1992
- Culture regime 6 weeks
- Species T. castaneum

	Weight of 20 adults X (5) (mg)					Mean of (i)-(v)	St Dev	Mean of (i)-(v)/20 Wt of 1 ad
	(i)	(ii)	(iii)	(iv)	(v)			
1	34.4	34.5	36.0	32.5	36.4	34.7	1.543	1.738
2	37.8	39.6	38.1	38.3	37.5	38.2	0.808	1.913
3	36.3	35.3	35.1	36.3	34.9	35.6	0.672	1.779
4	36.5	37.8	36.0	35.0	35.5	36.1	1.073	1.808
5	37.7	36.8	34.1	35.3	36.9	36.1	1.441	1.808
11	38.4	41.2	40.5	40.8	39.1	40.0	1.193	2.000
12	39.8	40.5	40.2	38.1	37.4	39.2	1.369	1.960
13	40.6	40.6	40.0	40.5	40.1	40.4	0.288	2.018
14	42.8	41.1	41.0	40.7	41.0	41.3	0.841	2.066
15	42.4	40.7	40.2	40.5	38.5	40.4	0.139	2.023

1 - 5 = Strain N.04 (Zangasso)
11 - 15 = Strain N.21 (Gao)

Table 4: Body size measurements

- Date 26.06.1992
- Culture regime 6 weeks
- Species T. castaneum

	Weight of 20 adults X (5) (mg)					Mean of (i)-(v)	St Dev	Mean of (i)-(v)/20 Wt of 1 ad
	(i)	(ii)	(iii)	(iv)	(v)			
1	31.5	33.3	31.6	31.7	32.4	32.10	0.758	1.605
2	35.7	33.8	32.4	33.7	31.4	33.40	1.623	1.670
3	29.9	30.1	30.0	32.0	30.2	30.44	0.879	1.522
4	33.4	38.8	32.3	33.7	33.4	32.72	1.198	1.636
5	31.5	29.3	33.2	28.9	31.6	30.90	0.178	1.545
11	35.3	35.1	37.1	33.7	34.4	35.12	1.273	1.756
12	33.9	32.2	35.0	32.9	34.3	33.66	1.115	1.683
13	34.7	36.6	37.4	36.6	37.1	36.48	1.052	1.824
14	34.9	31.7	33.5	32.4	31.7	32.84	1.366	1.642
15	35.0	33.8	34.7	33.4	35.8	34.54	0.958	1.727

1 - 5 = Strain N.04 (Zangasso)
11 - 15 = Strain N.21 (Gao)

Table 5: Detail of adult weight measurement in generation one of 8 week-regime.

Strain	Replicate	Mean Wt of sets of 20 adults	Mean Wt of 1 adult	Number of Observations	St Dev
	6	33.138	1.656	13	1.004
	7	31.233	1.561	9	0.57
	8	31.166	1.558	9	1.06
	9	33.600	1.680	7	2.62
	10	32.828	1.641	7	1.21
	16	34.450	1.722	8	1.89
	17	35.766	1.788	18	1.29
	18	35.347	1.767	17	1.83
	19	32.828	1.641	28	2.36
	20	34.054	1.702	22	2.36

NOTE: Weight in mg

Table 6: Body size measurements

- Date 06.03.1992
- Culture regime 8 weeks
- Species T. castaneum

	Weight of 20 adults X (5) (mg)					Mean of (i)-(v)	St Dev	Mean of (i)-(v)/20 Wt of 1 ad
	(i)	(ii)	(iii)	(iv)	(v)			
6	32.6	32.9	33.1	32.9	35.3	33.36	1.099	1.668
7	35.4	36.0	35.2	36.2	34.1	35.38	0.825	1.769
8	29.8	32.2	30.3	32.1	32.1	31.30	1.155	1.565
9	32.0	32.4	30.7	37.0	37.0	33.82	2.970	1.691
10	33.5	34.7	32.0	31.5	33.6	33.06	1.297	1.653
16	36.5	33.7	32.2	34.9	32.3	34.52	1.591	1.726
17	31.0	30.4	31.7	32.0	31.7	31.36	0.650	1.568
18	36.9	32.8	35.8	36.1	35.2	35.36	1.556	1.768
19	33.9	32.0	32.8	27.8	33.3	31.96	2.427	1.598
20	36.6	34.0	33.8	35.5	35.6	35.10	1.178	1.755

6 - 10 = Strain N.04 (Zangasso)
16 - 20 = Strain N.21 (Gao)

Table 7: Body size measurements

- Date 01.05.1992
- Culture regime 8 weeks
- Species T. castaneum

	Weight of 20 adults X (5) (mg)					Mean of (i)-(v)	St Dev	Mean of (i)-(v)/20 Wt of 1 ad
	(i)	(ii)	(iii)	(iv)	(v)			
6	30.4	24.3	22.8	25.7	25.7	25.64	2.869	1.282
7	26.8	22.5	27.2	24.9	-	25.35	2.148	1.267
8	26.9	24.8	28.2	24.6	-	26.12	1.730	1.306
9	27.7	23.6	28.9	24.4	28.5	26.62	2.446	1.331
10	25.8	25.9	25.5	26.7	-	25.97	0.512	1.298
16	30.0	33.5	35.3	35.0	32.0	33.16	2.202	1.658
17	34.8	30.7	34.6	33.6	34.6	33.66	1.719	1.683
18	32.3	38.0	38.6	38.3	35.6	36.56	2.661	1.828
19	36.4	36.2	36.3	31.7	33.1	34.74	2.193	1.737
20	41.1	32.7	37.9	39.4	34.0	37.02	3.566	1.851

6 - 10 = Strain N.04 (Zangasso)
16 - 20 = Strain N.21 (Gao)

Table 8: Body size measurements

- Date 26.06.1992
- Culture regime 8 weeks
- Species T. castaneum

	Weight of 20 adults X (5) (mg)					Mean of (i)-(v)	St Dev	Mean of (i)-(v)/20 Wt of 1 ad
	(i)	(ii)	(iii)	(iv)	(v)			
6	30.2	32.2	32.1	34.4	30.6	31.90	1.655	1.595
7	32.4	31.7	32.6	30.1	31.1	31.58	1.018	1.579
8	32.5	32.5	32.5	32.6	32.1	32.44	1.194	1.622
9	33.7	33.1	31.5	32.7	33.1	32.82	0.819	1.641
10	33.2	32.6	33.2	31.9	32.4	32.66	0.554	1.633
16	39.3	38.9	36.8	36.9	35.0	37.38	1.748	1.869
17	36.1	37.0	35.2	37.9	36.9	36.62	1.018	1.831
18	38.2	34.7	36.9	35.3	37.2	36.46	1.432	1.823
19	40.9	37.5	39.7	37.0	38.2	38.66	1.613	1.933
20	39.9	39.1	39.8	38.4	38.3	39.10	0.751	1.955

6 - 10 = Strain N.04 (Zangasso)
16 - 20 = Strain N.21 (Gao)

Table 9: Body size measurements

- Date 21.08.1992
- Culture regime 8 weeks
- Species T. castaneum

	Weight of 20 adults X (5) (mg)					Mean of (i)-(v)	St Dev	Mean of (i)-(v)/20 Wt of 1 ad
	(i)	(ii)	(iii)	(iv)	(v)			
6	33.3	34.3	35.2	33.4	33.1	33.86	0.879	1.693
7	33.2	33.9	30.8	32.4	-	32.57	1.332	1.628
8	32.4	33.5	32.5	34.9	33.1	33.28	1.011	1.664
9	31.9	35.4	33.2	32.0	35.3	33.56	1.712	1.678
10	35.6	34.6	34.8	34.3	-	34.82	0.550	1.741
16	34.2	33.8	33.4	33.2	36.3	34.18	1.246	1.709
17	34.1	35.8	33.5	36.0	35.9	35.06	1.172	1.753
18	33.7	35.6	34.3	35.4	33.1	34.42	1.075	1.721
19	35.2	34.1	35.3	36.6	34.9	35.22	0.903	1.761
20	35.1	37.2	36.5	37.2	36.6	36.52	0.858	1.826

6 - 10 = Strain N.04 (Zangasso)
16 - 20 = Strain N.21 (Gao)

Table 10: Weight-loss estimate over the first generation of the 6 week-regime

Strain	Replicate	Initial Weight*	Final Weight**	Loss	
				Weight (g)	%
	1	167.734	167.726	0.008	0.02
	2	169.612	168.918	0.694	1.92
N.04	3	168.193	167.740	0.453	1.31
	4	169.916	169.272	0.644	1.77
	5	169.441	169.205	0.236	0.65
Mean		168.979	168.571	0.407	1.13
St Dev		0.95	0.77	0.28	0.79
	11	167.917	167.972	2.945	8.55
	12	167.511	164.133	3.378	9.93
N.21	13	169.076	167.554	1.522	4.28
	14	167.880	165.663	2.217	6.45
	15	169.553	167.198	2.355	6.53
Mean		168.387	166.504	2.483	7.15
St Dev		0.87	1.58	0.71	2.17

* Initial Weight: weight of jars at adult removal

** Final Weight: weight of jars at reculturing after adults removed

Duration: 4 weeks (24.01.1992 to 21.02.1992)

Table 11: Weight-loss estimate over the first generation of the 8 week-regime

Strain	Replicate	Initial Weight*	Final Weight**	Loss	
				Weight (g)	%
	6	168.688	166.581	2.107	5.98
	7	168.513	167.018	1.495	4.27
N.04	8	168.052	166.273	1.779	5.15
	9	168.573	167.171	1.402	3.99
	10	164.128	162.752	1.376	4.49
Mean		167.590	165.959	1.632	4.77
St Dev		1.95	1.82	0.31	0.79
	16	168.049	165.979	2.070	5.99
	17	168.105	164.092	4.013	11.59
N.21	18	167.789	163.673	4.116	12.00
	19	171.420	165.156	6.264	16.52
	20	171.588	166.202	5.386	14.14
Mean		169.390	165.020	4.369	12.05
St Dev		1.93	1.12	1.59	3.91

* Initial Weight: weight of jars at adult removal

** Final Weight: weight of jars at reculturing after adults removed

Duration: 4 weeks (24.01.1992 to 06.03.1992)

REFERENCES

ABDELSAMAD, R. M., ELHAG, E. A. & ELTAYE, B. (1988). Studies on the phenology of Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) in the Sudan Gezira. J. Stored Prod. Res., 24(2), 101-105.

ADAMS, J. M. (1977). A bibliography on post-harvest losses in cereals and pulses with particular reference to tropical and subtropical countries. Tropical Science, 19(1), 1-27.

ALAN, J. S. & ROBERTSON, A. (1964). The effect of initial reverse selection upon total selection response. Genet. Res., 5, 68-79.

ANSELL, M. R. (1992) The mode of inheritance of resistance to phosphine in two species of stored product beetles. PhD Thesis, University of Reading.

ARTHUR, F. H. & ZETTLER, J. L. (1992). Malathion resistance in Tribolium confusum Duv. (Coleoptera: Tenebrionidae): Correlation results from topical applications with residual mortality on treated surfaces. J. stored Prod. Res., 28(1), 55-58.

BEEMAN, R. W. & NANIS, S. M. (1986). Malathion resistance alleles and their fitness in the red flour beetle (Coleoptera: Tenebrionidae). J. Econ. Ent., 79, 580-587.

BERTHE, S. (1990) Estimation des pertes occasionnees par les insectes ravageurs des stocks de sorgho au niveau de villages du Mali. Essai de mise au point d'une technique de criblage pour la resistance varietale du sorgho au Rhyzopertha dominica (F.) (Coleoptera : Bostrychidae). Memoire de fin d'Etude, Institut Polytechnique de Katibougou.

BHATIA, S. K. & PRADHAN, S. (1972). Studies on resistance to insecticides in Tribolium castaneum (Herbst). I I I. Selection of a strain resistant to lindane and its biological characteristics. J. Stored Prod. Res., 16, 331-337.

BIRCH, L. C. (1944). The influence of temperature on the development of the different stages of Sitophilus oryzae and Rhyzopertha dominica. Aust. J. Exp. Biol., 23, 29-35.

BIRCH, L. C. (1945). The influence of temperature, humidity and density on the oviposition of small strains of Calandra oryzae (L.)and Rhyzopertha dominica(Fab.)(Coleoptera). Aust. J. Exp. Biol., 23, 197-203.

BIRCH, L. C. (1945c). A contribution to the ecology of Calandra oryzae L. and Rhyzopertha dominica Fab. (Coleoptera) in stored wheat. Trans. R. Soc. Aust., 69, 140.

BOWKER (1974). Bioenergetics in Tribolium populations. Tribolium Information Bulletin, 17, 88.

BOWMAN, J. C. (1974). An Introduction to Animal Breeding. Edward Arnold Limited, London. 76 pp.

BOXAL, R. A. (1986). A critical review of the methodology for assessing farm level grain losses after harvest. Tropical Development and Research Institut, 137 Clerkenwell Road,London, EC1 5DB.

BOYLAN, W. J. & WONG, W. C. (1965c). Heterosis in Tribolium castaneum. Can. J. Genet. Cytol., 7, 84-87.

BRADSHAW, A. D. (1984). The importance of evolutionary ideas in ecology and vice versa. In B. Shorrocks (Eds.), Evolutionary Ecology (pp. 1-25). Blackwell Scientific Publications.

BREDON, R.M. (1961). Chemical composition of some foods and feeding stuffs in Uganda. Scientific Commettee on Human Nutrition Protein Supplies Conference, Uganda Gvt. Vet. Sci. and Industries.(Cited in Doggett,1970).

CARDIN, S. & MINVIELLE, F. (1986). Selection on phenotypic variation of pupa weight in Tribolium castaneum. Can. J. Genet. Cytol., 28, 856-861.

CHAMP, B.R.(1986). Occurence of resistance to pesticides in grain storage pests. In Champ, B.R. and Highley, E. 1986, Proceedings of an International seminar, Manilla, Phillipines, 27-30 May 1985. ACIAR Proceedings N.14, 346pp,Canberra.

CHAMP, B. R. & CAMPBELL-BROWN, M. T. (1970a). Insecticide resistance in Australian Tribolium castaneum (Herbst)--A test method for detecting insecticide resistance. J. stored Prod. Res., 6, 53-70.

CHAMP, B. R. & DYTE, C. E. (1977). FAO Global survey of pesticide susceptibility of stored grain pests. FAO Plant Prot. Bull., 25(2), 49-67.

CHITTENDEN, F. H. (1911). The lesser grain borer and the larger grain borer. Bull. U.S. Bur. ent., 96, 29-47.

CLAYTON, W. D. (1961). Proposal to conserve the generic name Sorghum Moench (Graminae) versus Sorghum Adans.(Graminae). Taxon., 10(8), 242.

CONNER, J. & VIA, S. (1992). Natural selection on body size in Tribolium castaneum: possible genetic constraints on adaptive evolution. Heredity, 69, 73-83.

COULIBALY, C. O. (1986) Temperature toxicity relationships of two organophosphorous insecticides in several stored product insects and effect of tolerance to insecticide on competitive ability. MSc Thesis, University of Reading.

DANIEL, S. H. (1991). The use of neem (Azadirachta indica A juss) and some plant oils as protectants of cowpeas (Vigna unguiculata (L.) Walp.) against the cowpea weevil Callosobruchus maculatus (Fab.). PhD Thesis, University of Reading, U.K.

DARWIN, C. (1859). The origin of species. Philadelphia: University of Pennsylvania Press.

DAWSON, P. S. (1964a). Age at sexual maturity in female flour beetles, Tribolium castaneum and Tribolium confusum. Ann. Entom. Soc. Amer. ., 57, 1-3.

DAWSON, P. S. (1964b). Maternal effects on quantitative characters in the development of Tribolium castaneum(Herbst.). Genetics, 50, 244.

DAWSON, P. S. (1965). Estimation of components of phenotypes variance for developmental rate in Tribolium. Heredity, 20, 403-417.

DAWSON, P. S. (1965). Genetic homeostasis and developmental rate in Tribolium. Genetics, 51, 873-885.

DAWSON, P. S. (1966). Correlated response to selection for developmental rate in Tribolium

Genetica, 37, 63-77.

DAWSON, P.S. (1975). Directional versus stabilizing selection for developmental time in natural and laboratory populations of flour beetles. Genetics, 80,773-783

DAWSON, P. S. (1977). Life history strategy and evolutionary history of Tribolium flour beetles. Evolution, 31, 226-228.

DOBIE, P., HAINES, C.P., HODGES, R.J., PREVETT, P.F. AND REES, D.P. (1991). Insects and arachnids of tropical stored products: their biology and identification. NRI Training manual.

DOBZHANSKY,T. (1937). Genetics and origin of species. New York : Columbia University Press.(Cited in Roush et al., 1986)

DOGGETT, H. (1970). Sorghum. Longmans Green and Co. Ltd.

DYTE, C. E. (1990). Living with resistant strains of storage pests. In F. Fleurat-Lessard and P. Ducom (Eds.), The fifth International Working Conference on Stored-product Protection, Bordeaux, France:

DYTE, C. & DALY, J. A. (1970). Contrasting susceptibilities to DDT in different flour beetles. Pest Infest. Res. 1969, 45-46.

ENFIELD, F. D., COMSTOCK, R. E. & BRASKERUD, O. (1965). Linkage and the degree of dominance of genes affecting pupal weight in Tribolium castaneum. Genetics., 52, 442.

ENFIELD, F. D., COMSTOCK, R. E. & BRASKERUD, O. (1966). Selection for pupal weight in Tribolium castaneum. I.Parameters in base populations. Genetics., 54, 523-533.

ENGLERT, D. C. & BELL, A. E. (1970). Selection for time of pupation in Tribolium castaneum. Genetica, 64, 541-552.

FALCONER, D. S. (1981). Quantitative genetics. (2 ed.). Longman, London and New York.

FAO (1969). FAO International collaborative programme for the development of standardized tests for resistance of agricultural pests to pesticides. FAO Plant Protection Bulletin., 17(4), 73-75.

FAO (1969a). Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. FAO Plant Protection Bulletin, 17(4), 76-82.

FAO (1977). Control of post-harvest food losses. FAO Plant Protection Bulletin, 25, 184-188.

FAO (1992). Cereal production. Quarterly Bulletin of Statistics, 5(3).

FEST, C. & SCHMIDT, K. J. (1973). The chemistry of organophosphorous pesticides: Reactivity, Synthesis, Mode of Action, Toxicology. Bayer A. G., Wissenschaftliches Laboratorium für Pflanzenschutz Wuppertal, Elberfeld.

FLEW, A. (1984). Darwinian evolution. Granada Publishing.

FRAENKEL, G. S. & HERFORD, G. V. B. (1940). The physiological action of abnormally high temperatures on poikilotherm animals.II-The respiration at high sublethal and lethal temperatures. J. Exp. Biol., 17, 386.

GARBER, E. D. (1950). Cytotaxonomic studies in the genus Sorghum. I I I.The polyploid species of the sub-genera Para-sorghum and Stipo-sorghum. Bot. Gaz., 115, 336.(Cited in Doggett,1970)

GILSON, W. E. (1963). Differential respirometer of simplified and improved design. Science, 141, 531-532.

GRAHAM, W. M. (1970d). Warehouse ecology studies of bagged maize in Kenya. IV. Reinfestation following fumigation with methyl bromide gas. J. Stored Prod. Res., 6, 177-180.

HALSTEAD, D.G.H. (1968). External sex differences in stored product Coleoptera, Bull. Ent. Res., 54:119-134.

HARRIS, K. L. & LINDBLAD, C. J. (1976). Post-harvest grain loss assessment methods. The American Association of Cereal Chemists in collaboration with LIFE,TPI,FAO and GASGA.

HASSALL, K.A. (1982). The chemistry of pesticides : Their metabolism, modes of action and uses in crop protection. The Macmillan Press Ltd.,London.

HINTON, H. E. (1942). Secondary sexual characters of Tribolium. Nature, 149, 500-501.

HINTON, H. E. (1948). A synopsis of the genus Tribolium macleay with some remarks on the evolution of this species groups. Bull. Ent. Res. 39, 13-55.

HOHENBOKEN, W. D., KOCHERA, Y. & DAWSON, P. S. (1991). Variability among families of Tribolium castaneum in inbreeding depression for fitness traits. Journal for Animal Breeding and Genetics, 108(6), 446-454.

HOLLOWAY, G.T. (1984). Genetic differentiation and life history variation in the rice weevil Sitophilus oryzae. PhD Thesis , University of Reading.

HOWE, R. W. (1950). The development of Rhyzopertha dominica under constant conditions. Entomol. mon. Mag., 86, 1-5.

HOWE, R. W. (1956). The effect of temperature and humidity on the rate of development of Tribolium castaneum. Ann. appl. Biol., 48(2), 363-376.

HOWE, R. W. (1962). The effect of temperature and humidity on the oviposition rate of Tribolium castaneum (Hbst.)(Coleoptera: Tenebrionidae). Bull. Entomol. Res., 53, 301-310.

HOWE, R. W. (1965). A summary of estimates of optimal and minimal conditions for population increase of some stored product insects. J. Stored Prod. Res., 1, 177-184.

HUGHES, A. M. (1976). The mites of stored food and houses. MAFF Technical Bulletin 9.

ICRISAT (1984). Agroclimatology of West Africa: Mali. Information Bulletin, 19.

KAMEL, A.H. AND FAM, R. (1973). Evaluation of two organophosphorous compounds against certain insects infesting stored grain. Agric. Res. Rev., Cairo, 51:21-28, Tropical Abstract, 1974, 29.

KENNINGTON, G.S. (1957). Influence of altitude and temperature upon rate of oxygen consumption of Tribolium confusum Duval and Componotus pennsylvanicus Modoc Wheeler.In Physiol. Zool. 30, 305.

LESNE, P. (1910-11). Liste des Bostrychides et Lyctides de Java. Notes Leyden Mus., 33, 71.

LESNE, P. (1924b). Les coleopteres bostrychides de l'Afrique Tropicale Française. Encyclop. ent., A3, 49-50.

LEWONTIN, R. C. (1965). Selection for colonizing ability. In: The genetics of colonizing species. Ed. H. G. Baker and G. L Stebbins. Academic Press, New York.

LEWONTIN, R. C. (1974). The genetic basis of evolutionary change. New York: Colombia University.

LI, L., ARBOGAST, R. T. & SMITH, R. H. (1993). Control of size and fecundity in Tribolium castaneum: indirect stabilizing selection for body size . Submitted to Journal of Animal Ecology

LLOYD, M. (1968). Self-regulation of adult numbers by cannibalism in two laboratory studies of flour beetles (Tribolium castaneum). Ecology 49, 245-259.

MAUNY, R. (1953). Principales plantes cultivées d' Afrique Occidentales. Bull. Inst. Franç. Afr. Noire, 15, 684.

MAY, J. M. (1968). The ecology of malnutrition in the French speaking countries of West Africa and Madagascar. Hafner Publishing Company Inc.,New York.

McFARLANE, J. A., CHRISTINE, S. & WALKER, A. K. (1990). Differences between some strains of stored grain beetles in their capacity to cause grain damage: possible implications for the management of pesticide resistance. Tropical Science, 30, 357-371.

MØLLER, H., SMITH, R. H. & SIBLY, R. M. (1989). Evolutionary demography of a bruchid beetle.I. Quantitative genetical analysis of the female life history. Functional Ecology, 3, 673-681.

MØLLER, H., SMITH, R. H. & SIBLY, R. M. (1990). Evolutionary demography of bruchid beetles.III Correlated responses to selection and phenotypic plasticity. Functional Ecology, 4.

MUGGLETON, J. (1983). Relative fitness of malathion-resistance phenotypes of Oryzaephilus surrinamensis(L.) (Coleoptera: Silvanidae). J. appl. Ecol., 20, 245-254.

PARK, T. & FRANK, M. B. (1948). The fecundity and development of the flour beetles, Tribolium confusum and Tribolium castaneum at three constant temperatures. Ecology, 29, 368-374.

PARK, T., MERTZ, D. B., GRODZINSKI, W. & PRUS, T. (1965). Cannibalistic predation in populations of flour beetles. Phisiol. Zool., 38, 289-321.

PARKIN, D. T. (Ed.). (1979). An introduction to evolutionary genetics. Edward Arnold Ltd.,New York.

PARKING, E. A. (1965). The onset of insecticide resistance among field populations of stored product insects. J. Stored Prod. Res. 1, 38.

PETERS, R. H. (1983). The ecological implications of body size. Cambridge University Press., Cambridge.

POINTEL, J. G. & COQUARD, J. Le pourcentage de perte en poids et la perte spécifique, criteres d'évaluation des degats causés par les insectes dans les céréales et les légumineuses stockées. Agronomie Tropicale, XXXIV(4).

POTTER, C. (1935). The biology and distribution of R. dominica (Fab.) Trans. R. Ent. Soc. Lon. 83(4), 449-82.

PREVETT, P.F. (1964). The distribution of insects in stacks of bagged groundnut in Northen Nigeria. Bulletin of Entomological Research, 54, 689-713.

PROCTOR, D.L. AND ROWLEY, J.Q. (1983). The Thousand Grain Mass (TGM) method. A basis for a better assessment of weight loss in stored grain. Tropical Stored Products Information 45, 19-23.

PRUS, T., BIJOK, P. & PRUS, M. (1988). Variation of fecundity and hatchability in strains: Tribolium castaneum Herbst. cI and Tribolium confusum Duval bIV. Tribolium Information Bulletin, 28, 67-70.

RAWLEY, J. (1969). Crop storage. A report to the government of Ghana. (Technical report No. 7). FAO/UNDP SF/GHA.

REED, C. (1987). The precision and accuracy of the standard volume weight method of estimating dry weight losses in wheat grain, sorghum and maize, and a comparison with the Thousand grain mass method in wheat containing fine material. J. Stored Prod. Res., 23, 223-237.

REISS, M. J. (1989). The allometry of growth and reproduction. Cambridge University Press, Cambridge.

REUBEN, K. U. (1978). A comprehensive geography of West Africa. Heinemann Educational Books Ltd.,Ibadan.

ROCKSTEIN, M. (1974). The physiology of insecta. 2nd Edition, Academic press, New York and London.

ROEDER, D. K. (1953). Insect physiology.John Wiley and Sons Inc., New York.

ROUSCH, R. T. & CROFT, B. C. (1986). Experimental population genetics and ecological studies of pesticide resistance in insects and mites. In Pesticide resistance: Strategies and Tactics for Management (pp. 471). Washington, D.C.: National Academy Press.

ROWLEY, J. Q. (1984). An assessment of losses during handling and storage of millet in Mali. Trop. stored Prod. Info., 47.

SAXENA, R. C. & BARRION, A. A. (1987). Biotypes of insect pest of agricultural crops. Insect. Sc. Applic., 8, 453-458.

SCHMIDT-NIELSEN, K. (1984). Scaling: why is animal size so important? Cambridge: Cambridge University press.

SHAZALI, M. E. H. & SMITH, R. H. (1986). Life history studies of externally feeding pests of stored sorghum: Corcyra cephalonica(Staint.) and Tribolium castaneum(Herbst). J. stored Prod. Res., 22, 55-61.

SIBLY, R. M. & CALOW, P. (1986). Physiological ecology of animals: an evolutionary approach. Blackwell Scientific Publications.

SIBLY, R.M.; SMITH, R.H. AND MØLLER, H. (1991). Evolutionary demography of a bruchid beetle. iv. Genetic trade-off, stabilizing selection and a model of optimal body size. Funct. Ecol., 5, 1-8.

SIBLY, R. M. & ANTONOVICS, J. (1992). Life history evolution. In T. J. Crowford and G. M. Hewitt (Eds.), Genes in Ecology. Blackwell Scientific Publications, Oxford.

SINCLAIR, E. R. (1981). Sexing live adult Rhyzopertha dominica (F.) (Coleopera: Bostrychidae). J. Stored Prod. Res., 17, 143-145.

SMITH, R.H. (1987). Oviposition, competition and population dynamics in storage insects. In Donahaye, E. and Navarro, S. (eds). Fourth International Working Conference on Stored-product Protection, pp426-433.

SMITH, R. H. (1991). Genetic and phenotypic aspects of life history evolution in animals. Advances in Ecological Research, 21, 63-120.

SMITH, R.H., SIBLY, R.M. AND MØLLER, H. (1987). Control of size and fecundity in Pieris rapae : towards a theory of butterfly life cycles. Journal of Animal Ecology, 56, 341-350.

SMITH, J. MAYNARD (1989). Evolutionary genetics. Oxford University Press. Oxford.

SOKOLOFF, A. (1972). The biology of Tribolium with special emphasis on genetic aspects. Vol.1. Oxford University Press, Oxford.

SOKOLOFF, A. (1974). The biology of Tribolium with special emphasis on genetic aspects. Vol.2.Oxford University Press, Oxford.

SOKOLOFF, A. (1977). The biology of Tribolium with special emphasis on genetic aspects. Vol.3.Oxford University Press, Oxford.

SOKOLOFF, A., SHRODE, R. R. & BYWATTERS, J. H. (1965). Productivity in Tribolium castaneum. Phisiol. Zool., 38, 165-173.

SOLIMAN, M. H. (1968) Studies of geographical populations of Tribolium castaneum. MSc Dissertation, University of Alberta, Edmonton.

SOLIMAN, M. H. & HARDIN, R. T. (1971). Variation in population of Tribolium castaneum(Herbst) (Coleoptera: Tenebrionidae). I. Body weights. J.Stored Prod. Res., 7, 35-43.

SOLIMAN, H. M. & HARDIN, R. T. (1972). Variation in population of Tribolium castaneum (Herbst)(Coleoptera: Tenebrionidae). II. Developmental rate and productivity. J. Stored Prod. Res., 8, 1-10.

SOLOMON, M. E. (1951). Control of humidity with potassium hydroxide, sulfuric acid or other solutions. Bull. ent. Res., 42, 543-554.

SONLEITNER, F. J. & GUTHRIE, P. S. (1991). Factors affecting oviposition rate in the flour beetle Tribolium castaneum and the origin of population regulation mechanism. Researches on Population Ecology, 33(1), 1-11.

STEARNS, S.C. (1992). The evolution of life histories. Oxford University Press, Oxford, New York.

TAYLOR, R. W. D. (1990). Field detection of resistance in beetle pests to contact insecticides. In: Proceedings of the 5th International Working Conference on Stored-Product Protection. Vol II, Bordeaux, France, September 9-14. Ed. F. Fleurat-Lessard and P. Ducom.

TAYLOR, R. W. & HALLIDAY, D. (1986). The geographical spread of resistance to phosphine (in) Coleopterous pests of stored products. In Proc. British Crop Protection Conf., (pp. 607-613).

TOURE, S. (1975) Agronomie des différentes variétés de sorgho (Sorghum bicolor) cultivées au Mali. Mémoire de fin d'étude, Institut Polytechnique Rurale, Katibougou.

TYLER, P. S. & BINNS, T. J. (1977). The toxicity of seven organophosphorous insecticides and lindane to eight species of stored product beetles. J. Stored Prod. Res., 13, 39-43.

TYLER, P. S. & BINNS, T. J. (1982). The influence of temperature on the susceptibility to eight organophosphorous insecticides of susceptible and resistance strains of Tribolium castaneum, Oryzaphilus surinamensis and Sitophilus granarius. J. Stored Prod. Res., 18, 13-19.

UMBREIT, W. W., BURRIS, R. H. & STAUFFER, J. F. (1972). Manometric biochemical techniques. Minneapolis: Burgess Minneapolis.

WEAST, R. C. (1979-80). Handbook of chemistry and physics. (60 ed.). CRC Press Inc.

WEAVING, A. J. S. (1975). Grain protectants for use under tribal storage conditions in Rhodesia. Comparative toxicity of some insecticides on maize and sorghum. J. stored Prod. Res., 11(2), 65-70.

WHITE, G. D. (1953). Weight loss in stored wheat caused by insect feeding. J. Econ. Entomol., 46(4), 609-610.

WHITE, G. G. (1984). Variation between field and laboratory populations of Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae). Aust. J. Ecol., 9, 153-155.

WHITE, G. G. (1987). Effects of temperature and humidity on the rust-red flour beetles Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae), in wheat grain. Aust. J. Zool., 35, 43-59.

WHITE, G. G. & LAMBKIN, T. A. (1988). Damage to wheat grain by larvae of Tribolium castaneum (Herbst)(Coleoptera: Tenebrionidae). J. Stored Prod. Res., 24(2), 61-65.

WHITE, N. D. G. & BELL, R. R. J. (1990). Relative fitness of malathion resistance strain of Cryptolestes ferrugineus. J. Stored Prod. Res., 26(1), 23-27.

WIGGLESWORTH, V. B. (1966). Insect physiology. London: Methuen and Co. Ltd. and Science Paperbacks.

WILSON, S. P., KYLE, W. H. & BELL, A. E. (1965). The effects of mating systems and selection on pupal weight in Tribolium castaneum. Genet. Res., 6(3), 341-351.

WOOL, D., BROWER, J. H. & KAMIN-BELSKY, N. (1992). Reduction of malathion resistance in caged almond moth, Cadra cautela (Walker) (Lepidoptera: Pyralidae), populations by the introduction of susceptible males. J. stored Prod. Res., 28(1), 59-65.

WRELTON, A. E. (1987) The influence of adult body size weight on the population biology of the rice weevil, Sitophilus oryzae (L.) and the grain weevil, Sitophilus granarius (L.). PhD Thesis, University of Reading.

ZEUTHEN, E. (1953). Oxygen uptake as related to body size in organisms. Quart. Rev. Biol., 28, 1-12.

ADDITIONAL REFERENCES

Relevant to the study but not referred to in the text.

ALVAREZFUSTERA, A., JUAN, C. & PETITPIERRE, E. (1991). Genome size in Tribolium flour beetles. Interspecific and intraspecific variations. Genetical Research, 58(1), 1-5.

ANDRES, A. (1931). Catalogue of the Egyptian Tenebrionidae. Bull. Soc. Entom. Egypte, 15, 74-125.

ANNETTE, M. P. (1979). An analysis of grain storage in three interior Sahelian Countries. Ann Arbor.: Centre for Research on Economic Development.

ANON (1975). Recommended methods for the detection and measurements of resistance of agricultural pests to pesticides. 16 Tentative methods for adults of some major pest species of stored cereals with methyl bromide and phosphine. FAO Plant Protection Bulletin, 23, 12-25.

BAINS, S. S. (1971). Effect of temperature on the biology of Rhyzopertha dominica (Fabricius) (Bostrychidae: Coleoptera). Bull. Grain Technol., 9, 257.

BARNES, J. N. & GROVE, A. H. (1916). The insects attacking stored wheat in the Punjab and the methods of combating them including a chapter on the chemistry of respiration. Mem. Dep. Agric. India Chem., 4(6), 165-280d.

BARTHOLOMEW, G. A. & CASEY, T. M. (1977). Body temperature and oxygen consumption during rest and activity in relation to body size in some tropical beetles. Journal of Thermal Biology, 2, 173-176.

BELL, A. E. (1969). The nature of selection responses in Tribolium. Jap. J. Genet., 44, 299-309.

BELL, R. J. & WATTERS, F. L. (1982). Environmental factors influencing the development and the rate of increase of Prostephanus truncatus (Horn)(Coleoptera:Bostrychidae) on stored maize. J. Stored Prod. Res., 18, 131.

BOARD OF SCIENCE AND TECHNOLOGY FOR INTERNATIONAL DEVELOPMENT, COMMISSION ON INTERNATIONAL RELATION S, NATIONAL RESEARCH COUNCIL (1978). Post-harvest Food Losses in Developing Countries. National Academy of Sciences, Washington DC.

BOYLAN, W. J. & WONG, W. C. (1965a). Response to selection and combining ability in Tribolium castaneum. Genetics, 51, 429-430.

BRAY, D. F., BELL, A. E. & KING, S. C. (1962). The importance of genotype by environment interaction with reference to control population. Genetical Research, 3, 282-302.

BREESE, M. H. (1962). Studies on the oviposition of Rhyzopertha dominica (F.) in rice and paddy. Bull. Ent. Res., 53, 621-637.

BROWER, J. H. & TILTON, E. W. (1973). Weight loss of wheat infested with gamma radiated Sitophilus oryzae and Rhyzopertha dominica. J. Stored Prod. Res., 9, 37-41.

BURGEON, L. (1930). Les bostryches. Rev. Zool. Bot. Af., 20, 20-24.

CALDER, W. A. (1984). Size, function and life history. Harward University Press, Cambridge.

CAMPBELL, A. & SINHA, R. N. (1976). Damage of wheat by feeding of some stored product beetles. J. Econ. Entomol., 61(1), 11-13.

CAMPO, J. L. & DELAFUENTE, M. B. (1991). Comparison of 1-stage and 2-stage selection on adult weight and egg number in Tribolium castaneum. Journal of Animal Breeding and Genetics., 108(1), 17-22.

CHAPMAN, R. N. (1928). The quantitative analysis of environmental factors. Ecology, 9, 111-122.

CHARLESWORTH, B. (Ed.). (1984). The evolutionary genetics of life histories. Blackwell Scientific Publications, London, Oxford.

CREDLAND, P. F. (1987). Effect of host change on fecundity and development of an unusual strain of Callosobruchus maculatus(F.) (Coleoptera: Bruchidae). J. Stored Prod. Res., 23(2), 91-98.

CREDLAND, P. F. (1989). Biotype variation and host change in Bruchids: causes and effects in the evolution of Bruchid pests. In K. ;. Fujii A.M.R.; Jonhson, C.D.; Mitchel, R. and Yoshida, T. (Eds.), Bruchids and Legumes: Economics, Ecology and Coevolution

CROMBIE, A. C. (1941). On oviposition, olfaction conditioning and host selection in Rhyzopertha dominica Fab.(Insecta, Coleoptera). J. exp. Biol., 18, 62-79.

CROMBIE, A. C. (1942). The effect of crowding upon the oviposition of grain-infesting insects. J. Exp. Biol., 19, 311.

CROW, J. F. & KIMURA, M. (1970). An introduction to population genetics theory. Harper and Row Publishers,New York.

CURNOW, R. N. (1964). The effect of continued selection of phenotypic intermediates on gene frequency. Genet. Res., 5, 341-353.

DANIEL, S. H. & SMITH, R. H.(1993). Functional anatomy of egg pore. Submitted to Physiological Entomology.

DIEL, S. R. & BUSH, G. L. (1984). An evolutionary and applied perspective of insect biotypes. Ann. Rev. Entomol., 29, 471-504.

ERDMAN, H. E. (1964b). Age and temperature effects on reproductive onset and productivity of Tribolium castaneum Herbst. Tribolium Inf. Bull., 7, 58-59.

EVANS, D. E. (1987). The survival of immature grain beetles at low temperatures. J. Stored Prod, Res., 23(2), 79-83.

EZEKIEL, M. & FOX, K. A. (1959). Methods of correlation and regression analysis: linear and curvilinear. (3 ed.). John Wiley and Son, Inc.,London and Sydney.

FAIRFULL, R. W., FRIARS, G. W. & NAYAK, B. N. (1974). Sire, dam, and age effects on offspring number in Tribolium castaneum. Tribolium. Inf. Bull. 17, 91-94.

FALCONER, D. S. (1963). Quantitative inheritance. In Methodology in Mammalian Genetics. Edited by Walter, J. and Burdette, A. B.. Holden-Day, Inc., San Francisco, 646 pp.

FISHER, R. A. (1958). Statistical methods for research workers. Oliver and Boyd Ltd., Edinburgh.

FISHER, R. A. (1958). The Genetical Theory of Natural Selection. Dover Publications Inc., New York.

GHORPADE, K. D. & THYAGARAJAN, K. S. (1980). A reliable character for sexing live or dead Rhyzopertha dominica (F.) (Coleoptera: Bostrychidae). J. Stored Prod. Res., 16, 151-153.

GIDDINGS, L. V., KANESHIRO, K. Y. & ANDERSON, W. W. (1989). Genetic, speciation, and the founder principle. Oxford University Press, New York, Oxford.

GIGA, D. P., KADZERE, I. & CANHAO, J. (1993). Bionomics of four strains of Callosobruchus rhodesianus (PIC)(Coleoptera: Bruchidae) infesting different food legumes. J. stored Prod. Res., 29(1), 19-26.

GILMOUR, D. (1961). Biochemistry of Insects. Academic Press Inc., New York, 343 pp.

GOODENOUGH, U. (1984). Genetics (3 ed.). Saunders College Publishing, Philadelphia.

GRINGH, B. G., NYIRENDA, B. & VOHRA, P. (1989). Growth response of Tribolium larvae on different cultivars of sorghums. Tribolium Information Bulletin, 29, 60-62.

GUGGENHEIM, H. & DIALLO, H. H. (1977). Grain storage in the fifth region of Mali: problems and solutions. No. USAID;Wunderman Fondation, New York.

HAGSTRUM, D. W. & LEACH, C. E. (1973). Infestation of flour by Tribolium castaneum: rate of adult dispersal in relationships to sex, mated conditions and other factors. Ann. ent. Soc. Am., 66, 384-387.

HUTCHINSON, G. E. (1978). An introduction to population ecology. Yale University Press, London.

JUAN, C., SEGARRAA, C. & PETITPIERRE, E. (1989). Nuclear DNA content of Tribolium castaneum and Tenebrio molitor, (Coleoptera: Tenebrionidae). Tribolium Information Bulletin, 29, 66-68.

KARTEN, I. (1965). Genetic differences and conditioning in Tribolium castaneum. Phisiol. Zool., 38, 69-79.

KAYSER, C. & HEUSNER, A. (1964). Etude comparative du metabolism energetique dans la serie animale. Journal de Physiologie (Paris) 56, 489-524.

KEISTER, M. & BUCK, J. (1974). Respiration: some exogenous and endogenous effects on rate of respiration. In: Rockstein M. (ed.) The physiology of Insecta, 2nd ed. pp. 469-509. Academic Press, New York.

KENTON, L. H. & CARL, J. L. Post-harvest grain loss assessment methods. American Association of Cereal Chemists.

KIDWELL, J. F., FREEMAN, A. E., HAVERLAND, L. N. & ROLFES, G. M. H. (1964). Genotype X environment interaction in Tribolium castaneum. Genetical Research, 5, 335-340.

KING, C. E. & DAWSON, P. S. (1972). Population biology and the Tribolium model. Evol. Bio., 5, 133-227.

LESLIE, P. H. & PARK, T. (1949). The intrinsic rate of natural increase of Tribolium castaneum Herbst. Ecology, 30, 469-477.

LORD, K. A. (1950). Effect of insecticides on respiration in Tribolium (Coleoptera). Ann. Appl. Biol., 37, 105-122.

MATHER, K. (1973). Genetical structure of populations. Chapman and Hall Ltd.London.

PARK, T., MERTZ, D. B. & PETRUSEWICZ, K. (1961). Genetic strains of Tribolium: their primary characteristics. Phisiol. Zool., 34, 62-80.

POTER, J. (1986). Some studies on the life history and oviposition of Carpophilus dimidatus (F.) (Coleoptera: Nitidulidae) at various temperatures and humidities. J. Stored Prod. Res., 22(3), 135-139.

ROORDA, F. A., SCHULTEN, G. G. & ANDRIESSEN, E. A. M. (1982). Laboratory observations on the development of Tribolium castaneum Herbst. (Col. Tenebrionidae) on millet at different temperatures and relative humidities. S. Angew. Entomol. 93, 446-452.

ROSE, M. R. & CHARLESWORTH, B. (1981a-b). Genetics of life history in Drosophila melanogaster. Genetics, 97, 193-196.

SHAZALI, M. E. H. (1982) The biology and population ecology of four insect pests of stored grain with particular reference to competition and succession. PhD Thesis, University of Reading.

SMITH, R. H. & SIBLY, R. (1985). Behavioural ecology and population dynamics towards and a synthesis. In S. &. Smith (Eds.), Behavioural Ecology (pp. 577-591). Blackwell Sci. Oub, Oxford.

SMITH, R. H. & HOLLOWAY, G. J. (1989). Population genetics and insect introduction. The Entomologist, 108(1&2), 14-27.

SOKOLOFF, A., FRANKLIN, I. R. & LAKHANPAL, R. K. (1966a). Comparative studies with Tribolium (Coleoptera: Tenebrionidae). II. Productivity of T. castaneum(Herbst) and T. confusum duv. on natural, semi-synthetic and synthetic diets. J. Stored Prod. Res., 1, 313-324.

STAUFFER, R. C. (1975). Charles Darwin's Natural Selection. Ed Stauffer, Cambridge University Press. Cambridge, 393 pp.

STEMLEY, P. G. & WILBUR, D. A. (1966). A colour characteristic for sexing live adult lesser grain borers. J. Econ. Entomol., 59, 760-761.

SUZUKI, D. T., GRIFFITHS, A. J. F., MILLER, J. H. & LEWONTIN, R. C. (1989). An introduction to genetic analysis. 4th Ed. W. H. Freeman and Company, New York.

VIA, S. (1991). Variation between strains of the flour beetle Tribolium castaneum in relative performance on 5 flours. Entomologia Experimentalis et Applicata., 60(2), 173-182.

WADE, M. J. & McCAULEY, D. E. (1980). Group selection : the phenotypic differentiation of small populations. Evolution, 34(4), 799-812.

WADE, M. J. & McCAULEY, D. E. (1980). Group selection: the genetic and demographic basis for phenotypic differentiation of small populations of Tribolium castaneum. Evolution, 4(4), 813-821.

WADE, M. J. (1991). Genetic variance of rate of population increase in natural population of flour beetles, Tribolium spp. Evolution, 45(7), 1574-1584.

WOOL, D. & MANHEIM, O. (1980). Genetically-induced susceptibility to malathion in Tribolium castaneum despite selection for resistance. Entomol. Exp. Appl., 28, 183.

WOOL, D. (1982). Family selection for DDT resistance in flour beetle (Tribolium, Tenebrionidae) as a model for group selection of fitness traits. Israel J. of Entomol., 16, 73-85.

WOOL, D., NOIMAN, S., MANHEIM, O. & COHEN, E. (1982). Malathion resistance in Tribolium strains and hybrids : inheritance patterns and possible enzymatic mechanisms (Coleoptera: Tenebrionidae). Biochem. Genet., 20, 621-636.

WOOL, D. & BERGERSON, O. (1986). Random environmental variation and inbreeding: effects on pure-strain and hybrid populations of flour beetles (Tribolium). Can. J. Genet. Cytol., 28, 889-898.

YAMADA, Y. (1973). Fifty percent time to pupation as a measure of developmental time in Tribolium castaneum. Can. J. Genet. Cytol., 15, 223-226.

ZHANG, L. (1989). The estimation for genetic parameters of 30-day old adult weight in Tribolium castaneum. Tribolium Information Bulletin, 29, 111.