TEMPERATURE TOLERANCE

IN

CACTOPHILIC *DROSOPHILA*

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SUMMARY

Temperate zone insect species must be capable of surviving climatic extremes. If genetic variation for survival of extremes exists, this may be expected to be of adaptive significance. A series of tests of high and low temperature tolerance and adaptability were performed on two cactophilic drosophilids, *Drosophila buzzatii* and *Drosophila aldrichi*. In Australia, the two species, accidentally introduced with their host plants since European settlement, share the same cactus rot niche, and have widely overlapping distributions.

D. buzzatii was shown to be able to continue normal development over a wider range of temperatures than most *Drosophila* species. Developmental speeds of various life-cycle stages were shown to vary significantly with temperature.

Adult *D. aldrichi* were found able to survive up to 3 times as long at 41°C as *D. buzzatii* of the same age. Little difference however was found in the survival times of the two species at 0°C. Comparisons between cage populations of *D. buzzatii*, and between synthetic chromosome inversion lines, revealed only limited differences in both heat and cold tolerance.

Electrophoretic analysis was performed on heat and cold shock survivors of both species. In *D. buzzatii*, *Aldox*^{a} and *Est-2*^{d} were associated with heat shock survival, and *Adh-1*^{c} and *Est-1*^{b} with cold shock survival. *Hex*^{c} and *Lap*^{b} were associated with *D. aldrichi* heat shock survival, and *Est-D*^{a} and *Pgm* heterozygotes with cold shock survival of that species.

Chromosome inversion frequency was determined for six pairs of *D. buzzatii* population cages in which one cage of each pair was

kept at 25°C and a daughter/replicate at 18°C. Within-pair differences in karyotype frequencies were significant, but differences between cage pairs were also significant.

The temperature tolerance of cactophilic *Drosophila* was found to be considerably greater than that of more commonly studied cosmopolitan species. This high tolerance of extremes was found to be repeatable across cage populations and other strains. Furthermore, as outlined above, genetic variation in temperature tolerance was observed within both species. That such variation was generally small is not unexpected, as most populations or strains studied were outbred, and any genetic differences would be buffered by homeostatic mechanisms.

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CHAPTER 1

INTRODUCTION

Almost since the founding of genetics as a scientific discipline early this century there has been debate over the quantity and function of genetic variation in natural populations. The division into two camps, the "classical" school (Muller 1939) and the "balance" school (Mather 1943) was not resolved, as initially expected, by the large amount of variation revealed by application of the biochemical technique of electrophoresis to genetics (Lewontin and Hubby 1966; Johnson *et al*. 1966; Harris 1966). The only apparent effect was the introduction of new names; "neutralist" for classical and "selectionist" for balance.

Thus Barker and Mulley (1976) were concerned "not merely to categorise the genetic variation in yet another species of *Drosophila*". They chose a species which, as nearly as possible, fitted the requirements of the ideal organism for population genetic studies (Barker 1977) and would therefore, they hoped, help resolve the dispute between neutralists and selectionists. This species, *Drosophila buzzatii*, is found over a wide area of Eastern Australia, but inhabits only rot pockets of some members of the *Opuntia* genus of cactus (Carson and Wasserman 1965). The importance of the cactus-yeast-*Drosophila* ecological system in population genetics has since been recognized in a conference devoted to the subject (Barker and Starmer 1982).

During their investigation they distinguished a second cactophilic drosophilid, *D. aldrichi* (Mulley and Barker 1977). The

two species are closely related members of the generally cactophilic *mulleri* subgroup from the *repleta* group of *Drosophila* species. Both are specific to the cactus genus *Opuntia* (called "prickly pears" in Australia), in association with which they appear to have originated in South or Central America. Neither species is able to initiate attacks on prickly pear; both depend on other agents to penetrate the tough cactus skin and start the microbial soft rots on which the drosophilids depend for feeding and breeding sites (Barker and Mulley 1976). The most common such agent is *Cactoblastis cactorum*, a moth introduced to Australia from South America as the result of a research program to find a natural predator of prickly pear. This moth was responsible in the 1920's for reducing the status of prickly pear in Australia from plague to botanical curiosity (Murray 1982).

The distributions of *D. buzzatii* and *D. aldrichi* overlap to a considerable extent in Australia, but field collection data suggest a difference in temperature tolerance between the two species. Suitable prickly pear is found from Victoria to North Queensland, but *D. aldrichi* is not found south of the Hunter Valley in N.S.W., and *D. buzzatii* tends to be the less common of the two species in the hotter areas of Queensland.

It is known that the species differ in feeding preferences, (Barker *et al.* 1981b) and specialization would seem possible, as each rot contains a varying mixture of yeast, bacterial and fungal species (Barker 1977; Vacek 1982).

Barker and Mulley (1976) and Mulley (1975) gave more complete accounts of the ecology and introduction to Australia of *D. buzzatii*. The history of the introduction to Australia of *D. aldrichi* is believed to be the same as that of *D. buzzatii*, although the former

was not identified in Australia until field collections in the early 1970's (Barker and Mulley 1976).

The main factors which make *D. buzzatii* and *D. aldrichi* good models for testing population genetic theories are their exclusive dependence on prickly pear and the intermittent distribution of prickly pear. Thus both species have a comparatively simple ecology, and occur as discrete populations with (theoretically) measurable migration rates. In the majority of populations, adults can be collected in genetically "useful" numbers all year round. Both species are amenable to laboratory culture, and the techniques of genetic analysis developed for other *Drosophila* species are generally applicable. Field experiments, such as gene frequency perturbations, are quite practicable (Barker and East 1980).

Barker and Mulley (1976) assayed *D. buzzatii* samples from collection sites covering most of the fly's distribution in Australia. They found 6 of 29 enzyme loci surveyed to be consistently polymorphic. Their data were subjected to a series of multivariate analyses (Mulley *et al*. 1979) in an attempt to associate the genetic variation revealed by electrophoresis to climatic or geographical variables. The factor that showed the most consistent association with genetic variation was temperature.

The following chapters describe a series of experiments which examine the genetic and ecological effects of temperature on *D. buzzatii* and, to a lesser extent, its sibling species *D. aldrichi*.

The first experiment investigated developmental speeds of *D. buzzatii* over a range of temperatures considered representative of wild conditions. Some conclusions on overwintering are deduced from the data.

In the second experiment LT50's (lethal time for 50% of individuals) at 41°C and 0°C were used to make three comparisons; cage populations of *D. buzzatii* versus *D. aldrichi*, inbred homozygous chromosome inversion lines of *D. buzzatii*, and six pairs of *D. buzzatii* population cages, one of each pair adapted to 25°C, the other to 18°C.

In the third experiment, high and low temperature shocks were used to test for association of particular alleles at electrophoretic loci with resistance to extremes of temperature. Survivors of heat shocks were compared to control groups, and survivors of cold shocks were compared to those that died from the cold shocks, for changes in electromorph frequencies.

The final experiment compared chromosome inversion frequencies in the previously mentioned six pairs of *D. buzzatii* population cages.

The last chapter presents a general discussion, and attempts to draw conclusions from the experimental results of the previous chapters.

CHAPTER 2

THE EFFECT OF TEMPERATURE ON DEVELOPMENTAL SPEED

2.1. INTRODUCTION

"It has long been known that poikilothermic animals complete their development more rapidly in warm weather than in cool" (Andrewartha and Birch 1954). This was expressed more rigorously by Davidson (1944), who showed empirically that a logistic curve best predicts the relationship between developmental speed of a single stage of an organism and temperature. However, this curve does not fit measurements of developmental speed made near the limits of the temperature range that permits development (Andrewartha and Birch 1954). At such temperatures developmental speed is generally below that predicted by the logistic curve.

Information of this kind can be of great value to ecologists, for example, in understanding and predicting fluctuations in population numbers in different seasons. A knowledge of the temperature range within which development may occur, and the variation of generation interval within that range, is also of great importance to population geneticists who are concerned with long term genetic trends in natural populations. Potential rates of genetic change with time in natural populations are governed by generation interval and population size.

Of considerable interest to population geneticists is the existence of "temperature races", or populations within a species displaying genetic differences in temperature tolerance. One of the earliest demonstrations of such differentiation was by Timofeeff-Ressovsky (1940) who identified temperature races within the species

Drosophila funebris. In the 1940's Dobzhansky (1950) demonstrated differences in the viability of Drosophila pseudoobscura in cage populations maintained at 25°C, not only between lines with different chromosome inversions, but also between lines with the same chromosome inversion, but collected from different areas. Parsons (1973) notes that 25°C is, for D. pseudoobscura, an extreme temperature. Thus the fitness differences observed by Dobzhansky were, at least in part, differences in temperature tolerance.

Among Drosophila species the permissible temperature range for development generally lies between 12°C and 32°C (see Table 2.1.1). A general conclusion from the information in Table 2.1.1 is that cosmopolitan species have a wider permissible range for development than tropical species. Similarly, from Table 2.1.2 it may be seen that adults of cosmopolitan species survive environmental stresses longer than tropical species. Further, the table shows that adults of D. buzzatii, a temperate species, show higher resistance to desiccation and cold than the cosmopolitan species listed, with the exception of dessication resistance in D. simulans.

It is therefore of interest to know whether D. buzzatii shows an unusually wide developmental temperature range as well as the high resistance to environmental stress shown by adults. The experiments described in the following sections compare developmental speeds of various stages for temperatures representative of the potential range for Drosophila development.

Table 2.1.1. Observed temperature limits to development in *Drosophila* species

Ecological type	Species	Observed range °C	Population origin	Author(s)
Cosmopolitan	*D. melanogaster*	10-31.5	Lebanon & Africa	Tantawy & Mallah (1961)
		12-30	Victoria (Australia)	McKenzie (1978)
		12-32	France	Cohet *et al.* (1980)
		13-32	Africa	"
	D. simulans	12-30	Lebanon & Africa	Tantawy & Mallah (1961)
		12-30	Victoria (Australia)	McKenzie (1978)
		12-31	France	Cohet *et al.* (1980)
		12-32	Africa	"
Tropical	*D. yakuba*	13-31	Africa	Cohet *et al.* (1980)
	D. ananassae	17-32	"	"
	D. iri	17-32	"	"
	D. fraburu	16-28	"	"

Table 2.1.2. Resistance to climatic stress in adult *Drosophila* spp.

Ecological type	Species	Stress (Temperature (°C) or % humidity*)	Time (hrs)	Survival (%)	Population origin	Author(s)
Cosmopolitan	*D. melanogaster*	41°C	0.5	10	USA	Johnson & Powell (1974)
		0°C	24-48	10	"	"
		37°C	13.5	16.7	Vic. (Aust.)	Schenfield & McKecknie (1979)
		-1° to -2°C	7.5	70.6	"	"
		33.5°C	24	19.9	"	Hosgood & Parsons (1968)
		-1°C	48	70.7	"	Parsons (1977)
		-1°C	42	50	Melbourne,	Parsons & McDonald (1978)
		0%	11	50	Vic. (Aust.)	"
		-1°C	48	5.4	Townsville,	Parsons (1977)
					Qld. (Aust.)	"
	D. simulans	-1°C	9	50	Melbourne,	Parsons & McDonald (1978)
		0%	34	50	Vic. (Aust).	"
		-1°C	48	19.7	"	Parsons (1977)
		-1°C	48	16.6	Townsville,	"
					Qld. (Aust).	

Table 2.1.2 - Page 2

Ecological type	Species	Stress (Temperature (°C) or % humidity*)	Time (hrs)	Survival (%)	Population origin	Author(s)
	D. immigrans	-1°C	50	50	Melbourne,	Parsons & McDonald (1978)
		0%	7	50	Vic. (Aust.)	"
Tropical (rainforest)	D. bipectinata	-1°C	5	50	Qld. (Aust.)	"
		0%	6	50	"	"
	D. birchii	-1°C	7	50	"	"
		0%	3	50	"	"
	D. paulistorum	-1°C	6	50	USA	"
		0%	4	50	"	"
Temperate	D. pseudoobscura	-3°C	168	16.2	Colorado (USA)	Crumpacker & Marinkovic (1967)
		-2°C	144	70	"	Jefferson et al. (1974)
	D. buzzatii	-1°C	108	50	Australia	Parsons & McDonald (1978)
		0%	22	50	"	"

*Relative humidity measured at a temperature of 25°C.

2.2. MATERIALS AND METHODS

2.2.1. Introduction

The experiments reported here were conducted during July 1979 on *Drosophila buzzatii* derived from two of six replicate population cages. The establishment of these cages in April 1977 from lines collected at "Yarrawonga", in the N.S.W. Hunter Valley (locality 5, Barker and Mulley (1976)), is described by Barker *et al.* (1981a). These cages are named herein with a code consisting of three letters and two numbers. The first letter denotes the species, the second denotes the collection site from which the cage was established, the third letter the state of origin, the first number is the cage number and the final number (in brackets) is the temperature (°C) at which the cage was maintained. Thus the first cage in this series is denoted BYN1(25).

Experimental material was obtained by egg-sampling of cages. The egg sampling apparatus is described by Barker *et al.* (1981a), by whom it was used for testing the attractiveness of various yeast species to adult *Drosophila*. The populations sampled were maintained in the larger of the cages described by Barker *et al.* (1981a). The recipe for the egg collection medium used in the present experiments is recorded in Appendix A. Sampling was usually for 2 hours, within the range 3 pm to 7 pm, as *D. buzzatii* shows a preference for laying at this time of day. Laying was always at 25°C, as it was not possible to obtain the required number of eggs at lower temperatures. In this series of experiments, cages BYN5(25) and BYN6(25) were used as they were healthy, and reliably gave suitably large egg samples. The medium used for maintenance of cages and samples and for developmental speed testing of all stages was the same as that used for *D. buzzatii* by Barker *et al.* (1981a); autoclaved yeast and sucrose.

With the exception of the experiment on larval emergence, for which eggs were transferred to test temperatures as soon as possible after laying, the eggs were kept at 25°C until they hatched the following evening. The larvae were then collected on the point of a dissecting needle, with the aid of a binocular dissecting microscope, and placed in food vials in cohorts of 50. They were then stored at 25°C until they reached the stage of development required for the particular experiment (for the experiment on larval developmental speed they were transferred immediately to test temperatures).

Temperatures at which developmental speeds were usually recorded were 10°C, 18°C and 25°C, although in a few cases additional information was obtained at 30° and 35°C. The laboratory constant-temperature fly rooms provided 18°C and 25°C with 65-75% relative humidity and a regular light cycle of 12 hours on/12 hours off. A constant temperature cabinet was used for 10°C, and large sterilizing ovens provided 30°C and 35°C, all three being unlit. Humidity was not controlled in the three latter temperatures, but was presumed adequate because of the high water content of the medium.

2.2.2. Larval emergence

To obtain a measure of the time from oviposition to larval emergence at various temperatures, six laying disks were placed in each of cages BYN5(25) and BYN6(25) from 3 p.m. to 4 p.m. After eggs were laid, the disks were cut in half, the halves being assigned to two of three groups. The number of eggs on each half was counted, and the groups were then placed at 10°, 18° or 25°C at 4.30 p.m. Oviposition was at 25°C, and the half hour required for dividing and

counting was at 20°C. The disks were examined daily (at least) until the first larvae were observed, and then as often as necessary to collect all hatching larvae (approximately half-hourly). Larval collection was done at 20°C which may possibly have affected hatch-times recorded, particularly for 10°C, the most different from 20°C, but the time at 20°C was short compared to total time at 10°C, so the bias is unlikely to be great.

2.2.3. Larval development

Time taken from larval emergence to 50% pupation was measured using larvae collected, as they hatched, on the evening of day 1, from eggs sampled from cages BYN5(25) and BYN6(25) in the afternoon of the previous day. Five vials (50 larvae per vial) were placed at each of 10°C, 18°C and 25°C. Cumulative numbers of pupae were recorded twice-daily for 25°C, and daily for 18°C and 10°C.

2.2.4. Pupation

The number of days from the start of pupation to 50% pupation was measured in two slightly different ways, the difference being in the time of distribution to test temperatures, which was also the starting point of recording. The first experiment was begun on the day pupation at 25°C actually began (that is on the first appearance of pupae), the second on the day before the time it was expected that the first pupae would appear at 25°C. For the first method, larvae were collected on day 1 from eggs sampled the previous day from BYN5(25) and BYN6(25) and were divided, approximately equally within cages, among the 3 temperatures at midday on day 8 by which time the first pupae had appeared. Cumulative numbers of pupae were

recorded - initially thrice daily, but tapering off to once daily as the 25° and then 18° samples were completed.

The procedure for measuring speed of pupation when recording started one day earlier was very similar. Third instar larvae were placed at 10°C, 18°C, and 25°C at midday on day 7, having been distributed into vials on day 1, from egg-samples taken the previous afternoon from cages BYN5(25) and BYN6(25). The procedure was otherwise the same as for the previous experiment.

2.2.5. Adult eclosure

Comparison of rates of eclosion was also made in two ways; time from beginning of pupation to 50% eclosure and time from late pupation to 50% eclosure of adults. Not all larvae were collected on the same day for the first test; for 25°C, 30°C, and 35°C, larval collection and placement at test temperatures was one day later than the same operation for 18°C. For measuring time from late pupation to 50% eclosure, larvae were collected on the evening of day 1 from eggs laid the previous afternoon. Groups of vials (containing pupae) were placed at 10°C, 18°C and 25°C on day 11, one day before eclosure was due to begin at 25°C.

2.2.6. Oviposition

The last of this series of observations was a test of "time to commencement of oviposition" at temperatures of 10°C, 18°C, 25°C, 30°C and 35°C for both newly hatched flies and flies aged initially for 9 days at 25°C. For testing newly eclosed flies, the adults were collected on day 12 from larvae allotted to vials on day 1 (from eggs laid the previous afternoon). For mature flies, adults were collected

on day 12 and the experiment was begun on day 21. Matings were set up in standard vials (5 pairs per vial, 5 vials per temperature), and inspected at regular intervals, either until eggs were observed in non-trivial quantities or until it was decided that further observations would yield no additional information.

2.3. RESULTS

2.3.1. Introduction

The tables referred to in this section give mean developmental times with standard errors (s.e.) and the results of *t* tests of the differences between the means. The mean time and s.e. for a particular cage of origin and testing temperature may be read off from the columns in the normal way. To find whether the difference between two developmental times is significant, find one in the columns on the left side of the table and the other in the rows under the right side. The result of the *t* test of the difference can be read off at the point where a line from each temperature would intersect.

2.3.2. Larval emergence

Estimates of the time in hours for 50% of eggs under test to hatch at temperatures of 10°C, 18°C and 25°C are recorded in Table 2.3.1, together with standard errors and the results of *t* tests on the differences between the various times. Times taken at different temperatures were highly significantly different, but there was no difference between cages within temperatures. It can be seen that at 25°C, larval emergence took a little more than one day (on average 1.13 days), but that at 18°C hatching took almost twice as long - approximately 2.17 days. At 10°C almost 9 days were required for 50% of eggs to hatch, and it is possible that the process was marginally accelerated by the necessity for counting emerged larvae at 20°C, since no 10°C room was available. It may be noted that time to hatching at 10°C is still 4 times that at 18°C.

Table 2.3.1. Time in hours, with the results of *t* tests of differences, from egg-laying to 50% of eggs hatched at 10°C, 18°C and 25°C

Cage of Origin	Temperature (°C)	Time (hours)	*t* Test Results					
BYN5(25)	25	27.4±1.0						
	18	52.0±1.0	***					
	10	213.9±1.0	***	***				
BYN6(25)	25	27.3±1.0	ns	***	***			
	18	52.0±1.0	***	ns	***	***		
	10	210.5±1.0	***	***	ns	***	***	
Temperature (°C)			25	18	10	25	18	10
Cage of Origin				BYN5(25)			BYN6(25)	

ns = not significant; *** = P < 0.001

Table 2.3.2. Time in days, with the results of *t* tests of differences, from hatching of larvae to 50% pupation at 10°C, 18°C and 25°C

Cage of Origin	Temperature (°C)	Time (days)	*t* Test Results					
BYN5(25)	25	6.5±1.0						
	18	14.7±1.0	***					
	10	>45	-	-				
BYN6(25)	25	6.8±1.0	ns	***	-			
	18	15.9±1.0	***	ns	-	***		
	10	>45	-	-	-	-	-	
Temperature (°C)			25	18	10	25	18	10
Cage of Origin				BYN5(25)			BYN6(25)	

ns = not significant; * = P < 0.05; *** = P < 0.001; - = not tested

2.3.3. Larval development

No final result was obtained at 10°C. After 1 month a small number of larvae could be observed in each vial, and their size had at least doubled over that of newly hatched larvae. However they had not significantly worked the medium, which had in most cases split vertically and was drying out quite severely. The 10°C investigation was thus terminated in the 2nd month due to the poor state of the medium. After approximately 45 days larvae were still only 2nd instar and may still not have been halfway through development to pupation.

The results for 18°C and 25°C are given in Table 2.3.2, together with standard errors and t tests of differences. Between-temperature differences were highly significant, but there was no real distinction between cage populations. At 18°C half the pupae appeared after a mean of 15.3 days as larvae. At 25°C this time was approximately 6.7 days - less than half the 18°C time.

2.3.4. Pupation

Two experiments were performed to compare pupation rates. The finishing point used, in both cases, was a number of pupae (25) equal to 50% of the initial number of larvae, but two different starting points were used. Table 2.3.3 records the time taken at 10°C, 18°C and 25°C when transfer to test temperatures (and commencement of recording) was done 24 hours before the first pupae were expected at 25°C. As may be seen from Table 2.3.3, pupation at 25°C is very rapid once commenced, since the mean time to 50% pupation was 1.5 days. This process took on average 4 days at 18°C and at least 15 days at 10°C. Results of tests of significance of differences are also shown. Differences between temperatures are all highly

Table 2.3.3. Time in days, with the results of t tests of differences, from 1 day before start of pupation at 25°C, to 50% pupation at 10°C, 18°C and 25°C

Cage of Origin	Temperature (°C)	Time (days)	t Test Results					
BYN5(25)	25	1.4±1.1						
	18	4.2±1.1	***					
	10	15.1±1.1	***	***				
BYN6(25)	25	1.6±1.1	ns	***	***			
	18	3.8±1.1	***	ns	***	***		
	10	17.3±1.1	***	***	ns	***	***	
Temperature (°C)			25	18	10	25	18	10
Cage of Origin				BYN5(25)			BYN6(25)	

ns = not significant; *** = $P < 0.001$

Table 2.3.4. Time in days, with the results of t tests of differences, from initiation of pupation at 25°C, to 50% pupation at 10°C, 18°C and 25°C

Cage of Origin	Temperature (°C)	Time (days)	t Test Results					
BYN5(25)	25	0.41±0.05						
	18	0.96±0.05	*					
	10	2.63±0.05	***	**				
BYN6(25)	25	0.58±0.05	ns	ns	***			
	18	1.46±0.05	**	ns	ns	*		
	10	6.44±0.05	***	***	**	***	***	
Temperature (°C)			25	18	10	25	18	10
Cage of Origin				BYN5(25)			BYN6(25)	

ns = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

significant, but differences between cages within temperatures are not significant.

The other experiment performed on pupation measured the time from initiation of pupation to 50% pupation. Using this scheme, Table 2.3.4 shows that it took approximately ½ day at 25°C to 50% pupation, just over 1 day on average at 18°C, and a rather variable time at 10°C; 2.6 days for cage BYN5(25) and 6.4 days for cage BYN6(25), for a mean of 4.5 days. Time at 18°C was more than double that at 25°C, and mean time to 50% pupation at 10°C was about 4 times that at 18°C. Table 2.3.4 shows that pupation time at 10°C is always significantly different to the times at other temperatures, and that pupation of larvae from cage BYN6(25) was significantly slower at 10°C than of those from cage BYN5(25). However there were no differences between cages at 25°C and 18°C and in fact differences between 25°C and 18°C both within and between cages are either not significant or significant only at the 5% level.

2.3.5. Adult eclosure

In Table 2.3.5 the mean time in days from initiation of pupation to appearance as adults of 50% of the original number of larvae is recorded for 18°C, 25°C and 30°C with standard errors and the results of t tests of differences. There were no significant differences between cages within temperatures. Time taken at 18°C, approximately 13 days, was highly significantly longer than time at 25°C and 30°C. Developmental time at 25°C and 30°C was very similar, about 6 days, but tended to be slightly quicker at 25°C.

Adult eclosure speed was also compared for 10°C, 18°C and 25°C from late pupation to 50% adult eclosure (Table 2.3.6). Pupae

Table 2.3.5. Time in days, with the results of t tests of differences, from beginning of pupation at 25°C to 50% adult eclosure at 18°C, 25°C and 30°C

Cage of Origin	Temperature (°C)	Time (days)	t Test Results					
BYN5(25)	30	6.7±0.5						
	25	5.7±0.4	ns					
	18	12.8±0.5	***	***				
BYN6(25)	30	6.7±0.7	ns	ns	***			
	25	6.1±0.7	ns	ns	***	ns		
	18	13.9±0.4	***	***	ns	***	***	
Temperature (°C)			30	25	18	30	25	18
Cage of Origin			BYN5(25)			BYN6(25)		

ns = not significant; *** = $P < 0.001$

Table 2.3.6. Time in days, and results of t tests of differences, from late pupation at 25°C to 50% adult eclosure at 10°C, 18°C and 25°C

Cage of Origin	Temperature (°C)	Time (days)	t Test Results					
BYN5(25)	25	1.8±1.1						
	18	5.5±1.1	***					
	10	18.3±1.1	***	***				
BYN6(25)	25	2.1±1.2	ns	***	***			
	18	5.5±1.1	***	ns	***	***		
	10	13.1±1.1	***	***	*	***	***	
Temperature (°C)			25	18	10	25	18	10
Cage of Origin			BYN5(25)			BYN6(25)		

ns = not significant; * = $P < 0.05$; *** = $P < 0.001$

remaining at 25°C completed 50% eclosion in 2 days. At 18°C this stage of development required 5½ days, and at 10°C between 13 and 18 days (depending on cage of origin) was required. _t_ tests show highly significant differences in times between temperatures, but no differences between cages within temperatures except at 10°C ($P < 0.05$).

2.3.6. Oviposition

Two tests of time to commencement of oviposition were performed. In neither case were significant differences between cages, within temperatures, observed. In the first (Table 2.3.7) newly eclosed flies were placed at test temperatures of 10°C, 18°C, 25°C, 30°C and 35°C. No eggs were laid at 10°C or 35°C. All flies died at 35°C, and 10°C appears to be too cold for _D. buzzatii_ to lay eggs. Although no check on mating was made, it should be possible at 10°C for cactophilic _Drosophila_ (Schnebel and Grossfield 1984). Time to commencement of oviposition was at least 7 days at 18°C and 2 days at 25°C and 30°C. _t_ tests showed the time at 18°C to be highly significantly different from the 25°C and 30°C times, while 25°C and 30°C were indistinguishable under the conditions used.

The second test of oviposition involved mature flies aged about 9 days at 25°C (Table 2.3.8). At 10°C there were again no eggs observed, but at 35°C females did lay, and some emerged larvae were observed at this temperature. However all flies, eggs and larvae at 35°C were dead after 6 days. Results at 30°C, 25°C and 18°C were essentially the same during the test period for both oviposition and adult mortality. Thus for this experiment the results were of a "yes" or "no" character, as egg laying was either within the first day or not at all.

Table 2.3.7. Time in days, with the results of _t_ tests of differences, to commencement of egg laying by newly eclosed flies at 18°C, 25°C and 30°C

Cage of Origin	Temperature (°C)	Time (hours)				_t_ Test Results		
BYN5(25)	30	2.4±1.1						
	25	2.4±1.1	ns					
	18	7.8±1.1	***	***				
BYN6(25)	30	2.0±1.1	ns	ns	***			
	25	2.0±1.1	ns	ns	***	ns		
	18	7.0±1.1	***	***	ns	***	***	
Temperature (°C)			30	25	18	30	25	18
Cage of Origin				BYN5(25)			BYN6(25)	

ns = not significant

Table 2.3.8. Time in days, with the results of _t_ tests of differences, to commencement of egg laying by mature flies at 18°C, 25°C and 30°C

Cage of Origin	Temperature (°C)	Time (hours)				_t_ Test Results		
BYN5(25)	30	0.57±1.11						
	25	0.50±1.11	ns					
	18	0.57±1.11	ns	ns				
BYN6(25)	30	0.50±1.11	ns	ns	ns			
	25	0.50±1.11	ns	ns	ns	ns		
	18	1.15±1.11	ns	ns	ns	ns	ns	
Temperature (°C)			30	25	18	30	25	18
Cage of Origin				BYN5(25)			BYN6(25)	

ns = not significant

2.4. DISCUSSION

Parsons and McDonald (1978) divided Drosophila species into three groups - Rainforest, Cosmopolitan and Cactus - and suggested that members of these groups are generally distinguishable from each other by their degree of tolerance to climatic extremes. They based their hypothesis on comparisons of seven Drosophila species for desiccation resistance and cold tolerance. They found that rainforest species showed the least tolerance of desiccation and cold, while the sole cactophilic species tested, D. buzzatii, was easily the most resistant to both stresses.

Drosophila buzzatii was however the only non-Cosmopolitan, temperate zone, species investigated by Parsons and McDonald (1978). Considerably greater cold resistance has been observed in D. pseudo-obscura for example (Table 2.1.2) than in D. buzzatii. Schnebel and Grossfield (1984) distinguished four ecological types of Drosophila; Tropical forest, Cosmopolitan, Desert, and Temperate-montane forest. They were able to demonstrate that each type had a distinctive mating temperature range. The Desert species displayed the highest maximum, but the Temperate-montane group (to which D. pseudoobscura would belong) showed the broadest range of mating temperatures. These four types would thus appear to offer a more accurate categorization of Drosophila species than Parsons and McDonald's 3 types.

Although Parsons and McDonald (1978) only tested adults it might be expected that immature stages would show the same hierarchy of climatic tolerance. Indeed the experimental results just presented, when compared with McKenzie's (1975) results, support this contention. McKenzie (1975) was unable to find immature stages of D. melanogaster in a Victorian winery when the temperature was below

13°C, even when larvae had been released in the winery 2 weeks earlier. By contrast, immature stages of *D. buzzatii* have been shown in the previous section to be able to continue development, albeit very slowly, at 10°C in the laboratory, and all stages may readily be found during winter in the Hunter Valley (source of the cage populations; Section 2.2.1), despite overnight temperatures of 0°C, or even lower, being common. Thus all stages of at least one cactophilic *Drosophila* species show greater resistance to low temperatures than equivalent stages of *D. melanogaster*.

The results reported in the previous section also suggest that *D. buzzatii* has greater high temperature tolerance than usual among *Drosophila* species. Parsons (1978) concluded that the upper limit for resource utilization in *Drosophila* species was 26°C for most species. In contrast, for the stages tested, *D. buzzatii* was able to carry on normal reproductive and developmental processes at 30°C.

Thus while Parsons (1978) observed a permissive temperature range for development among *Drosophila* species of 14°C (12°-26°C), *D. buzzatii* seems certain to be capable of development over a wider range, given the species' proven ability to complete all stages of development at 30°C and to survive at 10°C regardless of developmental stage.

As mentioned in the introduction to this chapter, generation intervals in different seasons and overwintering strategies are of interest to both ecologists and population geneticists. The experimental results presented here may be used to deduce generation intervals, or at least developmental times (egg to adult), for 4 different temperatures. However Vacek (1982) has shown that developmental time in *D. buzzatii* may be strongly diet-related. Thus any

prediction of generation intervals from data presented in this chapter is only relevant to flies reared on laboratory medium.

Developmental time for D. buzzatii on yeast/sucrose/agar medium at 25°C and 30°C is twelve days from laying of egg to eclosure of adult. Add to this two days from eclosure until commencement of egg-laying and the generation interval is calculated as 14 days. At 18°C development from egg to adult takes 28 days. First eggs appear 7 days after adult eclosure. Therefore total generation interval at 18°C is 35 days or 5 weeks. Because of time considerations and because of difficulties with artificial medium over a long storage period the generation interval at 10°C has not been directly measured. However on the assumption of a constant ratio of developmental speed between 10°C and 18°C, an estimate of total developmental time and generation interval at 10°C can be made by extrapolation. This is not a very good assumption (Andrewartha and Birch 1954) as even the results already presented show. For egg-hatching time and pupation, the ratio of time at 10°C to time at 18°C is about 4:1. However for adult eclosure (measuring from late pupation) the ratio has a mean (across cage origins) of about 2.8:1. The other assumption being made is of course that larvae can complete development at 10°C. Given the above, an estimated range of developmental time can be deduced. If a minimum for the ratio between 10° and 18°C is 3:1, and a maximum estimate is 4:1, we have a range of 15-20 weeks from laying of egg to eclosure of adult. Of course a generation interval can't be deduced, since no egg-laying was observed at 10°C. However this type of calculation is of use when discussing overwintering of a species. It is clear from the above that in an area with a winter mean of 10°C or lower, any eggs laid in late autumn would not be expected to complete

development until spring. This proposal of overwintering by immature stages is supported by field studies (Barker *et al.* 1986); rotting cactus cladodes have been found to contain larvae during winter months in the Hunter Valley (locality 5, Barker and Mulley (1976)), and they yielded *D. buzzatii* adults when removed to the laboratory. However it is also true that adults are attracted to baits throughout the winter in the Hunter Valley. These adults almost invariably appear old and the number trapped decreases as winter progresses; that is, they do not appear to be supplemented by newly eclosed adults until spring, usually September (Barker pers. comm.). On present evidence it would appear that no single life-cycle stage is solely responsible for overwintering, but that all stages, virtually in a state of suspended development, may be involved.

CHAPTER 3

THE EFFECT OF TEMPERATURE EXTREMES ON SURVIVAL

3.1. INTRODUCTION

While genetic variation at a particular locus may occur within a species, selection in favour of one or other allele will not occur unless the genetic difference is translated into a phenotypic difference. Such phenotypic differences may not exist if a population is maintained under ideal conditions, or at least may be so small as to make detection and measurement very difficult. Discussing laboratory results for *Drosophila willistoni*, Powell (1973) said "Selection coefficients of the order of 0.01 or less may not be detected in this time (about 2 years), yet this degree of selection would be easily great enough to overcome the influence of genetic drift in most natural populations". A further difficulty with laboratory experiments is that population size is usually small in comparison to wild populations. As Falconer (1981) explained, if the product of effective population size and selection coefficient is approximately ¼ or less, the effect of selection will be overcome by random genetic drift. Thus for a selection coefficient of 0.01, the effective population size would have to be more than 25 before selection would even counterbalance random drift.

Clearly a method of amplifying small selection effects is needed by population geneticists. Wright and Dobzhansky (1946) observed chromosome inversion frequencies in population cages of *Drosophila pseudoobscura* kept at 16.5°C and 25°C. No changes in chromosome inversion frequencies were recorded in cages kept at

16.5°C, but in cages kept at 25°C, inversion frequencies converged to the same equilibrium regardless of initial frequency. Parsons (1973) comments that, for *D. pseudoobscura*, 25°C is a relatively extreme temperature. Therefore the difference in response to 16.5°C and 25°C observed by Wright and Dobzhansky (1946) is most logically explained by magnification at an extreme temperature of an otherwise small or non-existent selection effect. This approach has been used since by a number of authors, although, as may be seen from Table 2.1.2, they have generally used more extreme conditions than Wright and Dobzhansky (1946).

The method of extreme climate shock has not been restricted to examination of chromosome inversions. It has been used to compare lines within species (Hosgood and Parsons 1968; Parsons 1970; Parsons 1977), to test for selection of electrophoretic alleles (Johnson and Powell 1974; Wills *et al.* 1975) and to compare species and groups of species (Parsons and McDonald 1978) (see Table 2.1.2).

Comparison of species in this way seems also to be ecologically meaningful - for example the results of Parsons and McDonald (1978) match well with expectations based on habitat preferences. Information on survival at climatic extremes may thus be a very useful tool for population geneticists, helping understanding of how a population adapts genetically to climate.

In the experiments reported here, the temperatures chosen for heat and cold shocks were intended to produce a severe shock, while still allowing for calculation of the time taken to kill 50% of the sample.

For cold shock this was dictated largely by technical expediency. The temperature chosen, 0°C, is however fairly typical of

a cold winter's night in the New South Wales Hunter Valley (Mulley *et al.* 1979), an area which supports *D. buzzatii* all year round. It is also not very different to the most commonly used cold shock temperature (-1°C) in the results summarized in Table 2.1.2.

From its relatively long survival of desiccation (Table 2.1.2) one would expect *D. buzzatii* to also show resistance to heat stress (Levins 1969). This is supported by collection of *D. buzzatii* in Queensland. Even in the Hunter Valley, daytime summer temperatures may exceed 40°C (Mulley *et al.* 1979). The temperature chosen, 41°C, is the same as that used by Johnson and Powell (1974) for *D. melanogaster*.

Varied doses of these temperatures were used to produce an estimate of the LT50 (Median Lethal Time) for the temperature under test. Comparisons made by this technique were between species (*D. buzzatii* and *D. aldrichi*), between populations, between thermally differently adapted cage populations and between lines differing in gene arrangements.

3.2. MATERIALS AND METHODS

3.2.1. Introduction

A number of findings by earlier workers have been incorporated into these experiments. For example, Hollingsworth and Bowler (1966) reported a decline in heat tolerance with increasing age in *Drosophila subobscura*. Preliminary tests revealed a similar age dependence in *Drosophila buzzatii* - for cold as well as heat tolerance. Accordingly, in the experiments below, the method of egg sampling and larval collection described in Section 2.2 was used to ensure that all flies in a given experiment had eclosed within a 30 hour period and had experienced, as nearly as possible, the same developmental conditions. All egg collection and all rearing of experimental flies was done at 25°C. At the end of the eclosion period, flies were divided into random, single sex, groups of 20, and were then aged for 7 or 8 days at 25°C before being used. For all experiments *D. buzzatii* was reared and aged on the yeast and agar medium described by Barker *et al.* (1981a). The cactus fortified medium used for rearing and ageing *D. aldrichi* is also described by Barker *et al.* (1981a).

Apparatus used for administering temperature shocks was quite simple. For heat shocks, a water bath was used, the temperature being maintained at 41°C by a laboratory immersion stirrer equipped with heating coil and thermostat. The variation in water bath temperature with this equipment was usually less than ±0.5°C. Within-vial temperature variations and warm-up time were not checked, but Schnebel and Grossfield (1984) found the fluctuations to be never more than 1.5°C for temperatures between 3°C and 38°C. They also found that time to reach temperature equilibrium (when vials first

entered the apparatus) never exceeded 3.5 minutes. Since their vials were placed in holes in an aluminium block, while these experiments used a water bath for heat shocks, which should provide better heat transfer, it may be assumed that time to reach equilibrium for heat shocks never exceeded 3.5 minutes. For cold shocks, two different methods of maintaining 0°C were used, but within any experiment only one method was used. Thus results from the two sets of apparatus are never directly compared. The first was an ice-water bath, kept in a commercial refrigerator, and the second was a constant temperature cabinet set to 0°C. Although vials in the latter would not have reached equilibrium as quickly as those in a water bath, cooling-down time would still have been only about 1-2% of total cold shock duration.

With regard to storage of flies during experiments, Maynard Smith (1958) showed that D. subobscura did not survive as long at 33°C when in empty vials as they did when given access to food. This was true regardless of whether flies in empty vials were supplied with dry air, or air saturated with H_2O. In preliminary experiments, flies were exposed to both heat and cold shocks in vials containing their normal food medium with the intention of approximating natural conditions. However the chance of flies sticking to the medium was found to be so great that the use of medium was abandoned in favour of the method used by Johnson and Powell (1974); a wad of Kleenex tissue was pressed to the bottom of each treatment vial and moistened with about .5 ml of 2 gm/100 ml sucrose solution.

Humidity in the vials was not measured, but was presumed to be high, as condensation quickly formed on the inside walls of vials removed from 41°C, and some condensation was observed inside vials kept at 0°C. It was decided to use moist air rather than dry air in

experimental vials to avoid confounding the effects of desiccation with temperature shock, despite the finding of Lamb and McDonald (1973) that determining the point of death from heat shock was more difficult for D. melanogaster shocked in moist air.

Regarding determination of the point of death, Lamb and McDonald (1973) and Ushakov (1977), who both used Drosophila melanogaster, and Maynard Smith (1957), using D. subobscura, all chose the point at which flies ceased responding to stimuli (such as tapping their container) as the point of death. Ushakov (1977) noted, however, that 25% of D. melanogaster would recover after reaching this stage if removed to an intermediate temperature. Initial tests of D. buzzatii showed that all flies appeared dead after less than 30 minutes at 41°C (this included warm-up time), but that complete recovery within 24 hours of virtually all flies was possible for exposure times up to about 45 minutes. This general pattern of recovery from coma was also observed at 0°C. Thus temperature shocks were performed by exposing a series of vials for different, predetermined, lengths of time, and then scoring the number dead after a fixed recovery period had elapsed.

For both heat and cold shocks there was not a readily recognizable distinction between survivors and dead flies, but rather a continuum of increasing paralysis. The point of "death" was therefore arbitrarily defined to be that point at which a fly could not stand up. For cold shocks the scoring of survivors was done 2-3 hours after removal from 0°C. Since recovery from heat narcosis took longer than recovery from cold shock, counting of survivors was not done till 24 hours after removal from 41°C. However, since most flies died over a relatively short time span (the results show a sigmoid

dose-response curve), varying the criterion of death would not be expected to have much effect on LT50's.

All data were analysed by the method of logistic regression using the computer package GLIM3 (Baker and Nelder 1978). This produced a linear regression against time for the logit of proportion dead. A logit of a proportion p is ln[p/(1-p)]. The mean lethal time, or LT50, has a logit of zero.

In a preliminary analysis of each data set, a separate regression was determined for each sex within each parental origin group, and then the residual was calculated for each observation from its own regression. A very large residual was taken as a sign that the data point was inconsistent with its replicates and the remainder of that group and sex, and that observation was excluded from the succeeding analysis. This typically amounted to less than 5 exclusions among 120 observations.

The data were then reanalysed. Initially all regressions within an experiment were calculated using a single slope, with separate y (vertical axis - logit of proportion dead) intercepts for each group and sex combination. Next, separate slopes were fitted for each sex, then for each group. If the residual deviance (analogous to the residual sum of squares in an ordinary regression) was significantly reduced by the fitting of separate slopes for sex or group, then separate slopes were fitted for each sex and group combination (subgroup). On all occasions where the residual deviance was significantly reduced by the fitting of separate slopes for each subgroup, it was found that one subgroup (that is, one sex within one group) had a markedly different slope to all other subgroups. Reanalysis, excluding the abberant subgroup, always showed no

remaining significant difference in slope between the groups. The LT50s, and their standard errors, recorded in Section 3.3, were calculated using a separate y intercept for each subgroup, and the smallest number of slopes consistent with maximum significant reduction in residual deviance. Where a subgroup was excluded, its LT50 was calculated from its independent slope and y intercept. Such cases, where the regression lines may intersect in a biologically meaningful area, are presented graphically in Section 3.3. The standard errors presented with the LT50s were found by taking the square root of the corrected variance. The variance was calculated from the slope, the y intercept, their standard errors and the correlation between them, while the correction factor was the mean deviance (residual deviance ÷ degrees of freedom). The correction to the variance was necessary because the data were more variable than expected for purely binomial sampling. The inflation of the error reflects heterogeneity in the experimental flies, which Finney (1978) blamed on inevitable incomplete randomization. Thus the observed excess variability is to be expected in such experiments, rather than being a feature of this particular series of experiments.

3.2.2. Comparison of *D. buzzatii* and *D. aldrichi*

The sources of *Drosophila buzzatii* for this comparison were population cages BYN5(25) and BYN6(25) (as described in Section 2.2). *Drosophila aldrichi* were also drawn from 2 cages for each experiment, one from "Yarrawonga" in the New South Wales Hunter Valley (cage AYN1(25) for all experiments) and one of Queensland origin. Cage AYN1(25) is one of the two *D. aldrichi* population cages whose establishment is described by Barker *et al.* (1981a). One heat shock

and two cold shocks were performed. For the heat shock and the first cold shock (both carried out in October 1979) cage ACQ1(25) was used as the source for Queensland flies (see Table 3.2.1). This cage was established in October 1978 from six iso-female lines, the founder females having been collected at three localities in central Queensland (Barker, pers. comm.). A repeat cold shock was performed the following month. Due to a population crash in cage ACQ1(25) (caused apparently by mite infestation), cage AHQ1(25) was used as the source of Queensland D. aldrichi. This cage was established in January 1977 from mass cultures derived from emergences from 37 cactus rots collected at Hemmant, near Brisbane (locality 31 in Barker and Mulley (1976)), in November 1978 (Barker, pers. comm.).

Table 3.2.1 lists the number of days that flies were aged before temperature shock, the most common number of replicates within each treatment group, the total number of replicates used and the treatment times for the three experiments outlined above.

3.2.3. Comparison of D. buzzatii from two different areas

A heat shock was used to compare genetic adaptation to climate of flies of the same species but from widely separated populations. Survival time at 41°C for D. buzzatii from "Yarrawonga" (cages BYN3(25) and BYN4(25)) was compared with survival time of Hemmant origin D. buzzatii (cages BHQ1(25) and BHQ2(25)). The Hemmant cages were established at the same time and in the same manner as the D. aldrichi Hemmant cages described in Section 3.2.2. Experimental flies were derived from egg samples (as described in Section 2.2). The egg sampling and heat shock were carried out in March 1979. Details of ageing, replicates and treatment times are recorded in Table 3.2.2.

Table 3.2.1. Experimental design parameters of heat and cold shock comparisons of <u>Drosophila buzzatii</u> and <u>Drosophila aldrichi</u>

	Experiment											
	Heat shock				First cold shock				Second cold shock			
Species	D. buzzatii		D. aldrichi		D. buzzatii		D. aldrichi		D. buzzatii		D. aldrichi	
Cage origin 1	"Yarrawonga"		"Yarrawonga"		"Yarrawonga"		"Yarrawonga"		"Yarrawonga"		"Yarrawonga"	
2	"Yarrawonga"		Central Qld.		"Yarrawonga"		Central Qld.		"Yarrawonga"		Southern Qld.	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Age in days when treated	8		8		7		7		7		7	
Treatment lengths*	60	70	90	90	3	3	3	3	3.5	3.5	3	3+
	70	80	100	100	4	4	4	4	4	4	3.5	3.5++
	80	90	110	110	4.5	4.5	5	5	4.5	4.5	4	4
	90	100	120	120	5	5	5.5	5.5	5	5	4.5	4.5
			130	130	5.5	5.5	6	6	5.5	5.5	5	5
			140	140			6.5	6.5	6	6	5.5	5.5
			150	150					6.5	6.5	6	6
Replicates/ treatment/cage†	3	3	3	3	2	2	2	2	3	3	3	3
Mean replicates/cage	11	11	20	20	8	8	10	10	19	19	16	16

* Minutes for heat shock, days for cold shock

† Mode

\+ Southern Qld. only

++ Yarrawonga only

Table 3.2.2. Experimental design parameters for the heat shock comparison of *D. buzzatii* from "Yarrawonga" (N.S.W.) and Hemmant (Qld.)

	Cage							
	BYN3(25)		BYN4(25)		BHQ1(25)		BHQ2(25)	
Origin	"Yarrawonga"		"Yarrawonga"		Hemmant		Hemmant	
Sex	M	F	M	F	M	F	M	F
Age in days when treated	7	7	7	7	7	7	7	7
Treatment lengths (min)	50	60	50	60	60	60	60	60
	60	70	60	70	75	75	70	70
	70	80	70	80	80	90	75	75
	80		80	90	90		80	80
			90	100			85	85
							90	90
Replicates/treatment*	3	2	3	3	2	2	3	3
Total replicates	12	6	15	14	8	7	16	14

* Mode

3.2.4. Comparison of *D. buzzatii* from differently adapted population cages

In August 1978 a replicate of each of the six *D. buzzatii* population cages described in Section 2.2 was established. The replicates, derived by egg sampling, were then maintained at 18°C while the original cages remained at 25°C. Thus the replicate cages are designated BYN1(18) to BYN6(18). Generation interval at 25°C is about 2 weeks, so the 25°C cages would have completed approximately 32 generations at the time of the establishment of the 18°C cages.

The experiments described below investigated short term genetic adaption to temperature in the 25°C and 18°C population cages. The first experiment was a heat shock comparison of each 25°C cage with its 18°C counterpart. This was in March 1979, by which time the 25°C cages would have undergone a further 14 generations. The 18°C cages, for which the generation interval is estimated to be at least 5 weeks (see Section 2.4), would be expected to have completed at most 5 generations. Tabulation of experimental parameters for this experiment can be found in Table 3.2.3.

Completing all heat shocks took a week, testing 2 cages per day. Some difficulty was encountered in obtaining sufficiently large numbers of flies from a single egg-sample, particularly from the 18°C cages. The order of the heat shocks was thus dependent on the order in which adequate larval samples were obtained. The implication is that samples from a cage pair, that is a 25°C cage and its 18°C replicate, were not necessarily tested on the same day.

A repeat experiment was performed, designed to overcome the possibility that the results observed in the first experiment were due more to maternal cytoplasmic preconditioning than to genetic changes.

Table 3.2.3. Experimental design parameters for heat shock comparison of *D. buzzatii* 25°C and 18°C population cages of "Yarrawonga" (N.S.W.) origin

	Cage											
	BYN1		BYN2		BYN3		BYN4		BYN5		BYN6	
	(25)	(18)	(25)	(18)	(25)	(18)	(25)	(18)	(25)	(18)	(25)	(18)
Age in days when treated	7	7	7	7	7	7	7	7	7	7	7	7
Treatment lengths (min.)	60	60	60	60	50[1]	50	50[1]	50	45	40[1]	60	40[1]
	75	70[1]	75	70	60	60	60	60[1]	60	50	75	50
	90	80	90	80	70	70	70	70	75	60	90	60
	105	90	105	90[1]	80	80	80	80	90	70	105	70
	120	100[2]	120			90[2]	90		105	80	120	80
							100[2]		120	90		90[2]
Replicates/treatment*												
M	3	3	3	3	3	3	3	2	3	3	3	3
F	2	3	2	2	2	2	3	3	3	3	2	3
Mean replicates/cage	22	23	24	20	19	20	26	16	34	31	27	28

* Mode

1 Males only

2 Females only

Such an effect is known to occur in the diapause pattern of the moth genus Bombyx; while the moth displays genetic variation for diapause, this can be overruled by maternal effects (Andrewartha and Birch 1954). More recently, Fleuriel (1976) demonstrated gene-cytoplasm interactions, affecting male viability and resistance to CO_2, in Drosophila melanogaster. Therefore a reversed environment intermediate generation was used to produce experimental flies. This intermediate generation was derived by egg sampling from cages BYN5(25), BYN5(18), BYN6(25) and BYN6(18). Each sample was divided in two, one half being raised at 25°C, the other at 18°C. In this way, a control and a reversed environment parental group was available for each original cage. Two egg-samples were taken from this intermediate parental group; one was used for a heat shock (41°C) and the other for a cold (0°C) shock.

Since the initial egg samples for this repeat experiment were made in August 1979, a further 10 generations among the 25°C cages and 4 generations among the 18°C cages would have been completed since the egg-samples for the initial experiment were taken. The total number of generations from the establishment of the 18°C cages till the beginning of the repeat experiment is thus estimated to be 24 generations among the 25°C cages and 9 generations for the 18°C cages.

Details of numbers of flies and their treatment are recorded in Table 3.2.4 for both the heat and cold shock. Within a temperature shock and a particular cage group (e.g. BYN5(25) and BYN5(18)), all trials were run concurrently.

3.2.5. Comparison of chromosome arrangements in D. buzzatii

In Australian populations, D. buzzatii has only one common chromosome inversion (Carson and Wasserman 1965), the "2j" inversion.

Table 3.2.4. Experimental design parameters for heat and cold shock comparisons of differently adapted *Drosophila buzzatii* population cages of "Yarrawonga" (N.S.W.) origin

	Experiment															
	Heat Shock								Cold Shock							
Cage	BYN5				BYN6				BYN5				BYN6			
Maintenance Temp. °C	(25)		(18)		(25)		(18)		(25)		(18)		(25)		(18)	
Age in days when treated	7		7		7		7		7		7		7		7	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Treatment lengths in minutes (heat shock) or days (cold shock)	50	60	50	60	50	55	50	55	3	5.5	5.5	5.5	3	3	3	3
	60	70	60	70	55	60	55	60	3.5	6	6	6	3.5	3.5	3.5	3.5
	70	80	70	80	60	65	60	65	5.5	6.5	6.5	6.5	4	4	4	4
	80	90	80	90	65	70	65	70	6	7	7	7	4.5	4.5	4.5	4.5
						75	70	75	6.5				5.5	5.5	5.5	5.5
									7				6	6	6	6
													6.5	6.5	6.5	6.5
													7	7	7	7
Replicates/treatment/parental group*	3	3	3	3	3	3	3	3	3	2	2	2	2	2	2	2
Total replicates Parental group 25°C	12	12	12	12	12	15	15	15	17	12	6	6	14	14	15	14
18°C	11	12	12	12	12	15	12	14	6	6	6	7	12	12	13	13

*Mode

This inversion is, as its name implies, on the second chromosome. It has been found to be polymorphic in all populations adequately sampled (Wasserman 1962; Carson 1965; Fontdevila *et al*. 1981; Fontdevila *et al*. 1982; Barker *et al*. 1985; Barker and Watt, unpublished data; see also Table 3.4.2), and its frequency in some areas is higher than that of the standard arrangement (Carson and Wasserman 1965; Table 3.4.2).

For two collection sites, "Yarrawonga" (N.S.W.) and Hemmant (Qld.), two lines homozygous for the standard chromosome arrangement and two lines homozygous for the 2j inversion were established by inbreeding. Chromosome examination was performed using the method of Wasserman (1954). Since it was desired to test the heterozygous chromosome arrangement as well as the homozygotes, it was decided to test all lines as F_1 hybrids, with the intention of cancelling out any heterotic advantage accruing to the heterozygote due to heterozygosity in the genetic background. Accordingly each line was bred up in vials until sufficient matings were established for all lines to produce 300 pairs of adults over about 1 week. These adults were collected as virgins and placed, with a group of opposite sex from another line, in small population cages. The second chromosome inversion is designated "j", and the standard arrangement "st". The above mating scheme produced F_1 flies with second chromosome complement (chromosome of male parent origin given first) st/st, st/j, j/j and j/st. Each of the four lines was thus used twice as a parent, once as male parent and once as female.

The F_1 flies were produced by egg-sampling from the mating cages, followed by the established procedures of heat and cold shocks. Number of days aged before treatment, replicates used per treatment and treatment times for each sex, line and treatment are recorded in Table 3.2.5.

Table 3.2.5. Experimental design parameters for heat and cold shock comparisons of F_1 chromosome inversion lines of *Drosophila buzzatii* from "Yarrawonga" and Hemmant

	Experiment							
	Heat Shock				Cold Shock			
Origin of Lines	"Yarrawonga"		Hemmant		"Yarrawonga"		Hemmant	
Age in days when treated	7		7		7		7	
Sex	M	F	M	F	M	F	M	F
Treatment lengths in minutes (heat shock) or days (cold shock)	60	60	60	65	3	3	3.5	4
	65	65	65	70	4	4	4	4.5
	70	70	70	75	4.5	4.5	4.5	5
	75	75	75	80	5	5	5	5.5
	80	80	80	85	5.5	5.5	5.5	6
					6	6		
Replicates/line/treatment*	3	3	3	3	3	3	3	3
Total replicates/line	15	15	14	15	18	18	15	15

*Mode

3.3. RESULTS

3.3.1. Comparison of D. buzzatii and D. aldrichi

Three experiments were performed to compare the temperature tolerance of *D. buzzatii* and *D. aldrichi*. In the first, a heat shock (Table 3.3.1), the mean survival time of *D. buzzatii* was 63 minutes, while the mean survival time of *D. aldrichi* was 108 minutes for "Yarrawonga" (N.S.W.) origin flies and 151 minutes for central Queensland origin flies. Within each sex, the two *D. buzzatii* groups were not significantly different from each other, but the differences between *D. aldrichi* groups and between *D. buzzatii* and *D. aldrichi* were all highly significant. Regarding between-sex comparisons within a cage of origin, *D. aldrichi* males and females were not significantly different. By contrast, between *D. buzzatii* males and females, a pattern was established that is repeated, with very few exceptions, through all the experiments involving calculations of LT50's; *D. buzzatii* females lived significantly longer than *D. buzzatii* males of the same cage origin.

A final point of interest for this heat shock comparison is that the regression of logit of proportion dead against time for *D. aldrichi* was smaller than that for *D. buzzatii*. The regression lines used to calculate the LT50's are presented in Figure 3.3.1.

In summary, Table 3.3.1 shows clearly that *D. aldrichi* is more heat tolerant than *D. buzzatii*.

The two further experiments performed to compare the two species were both cold shocks. The second cold shock was performed because of the low replication level in the first (see Table 3.2.1). In the first cold shock (Table 3.3.2) the mean survival time was 3.6 days for *D. buzzatii* and 3.9 days for *D. aldrichi*, and in the second

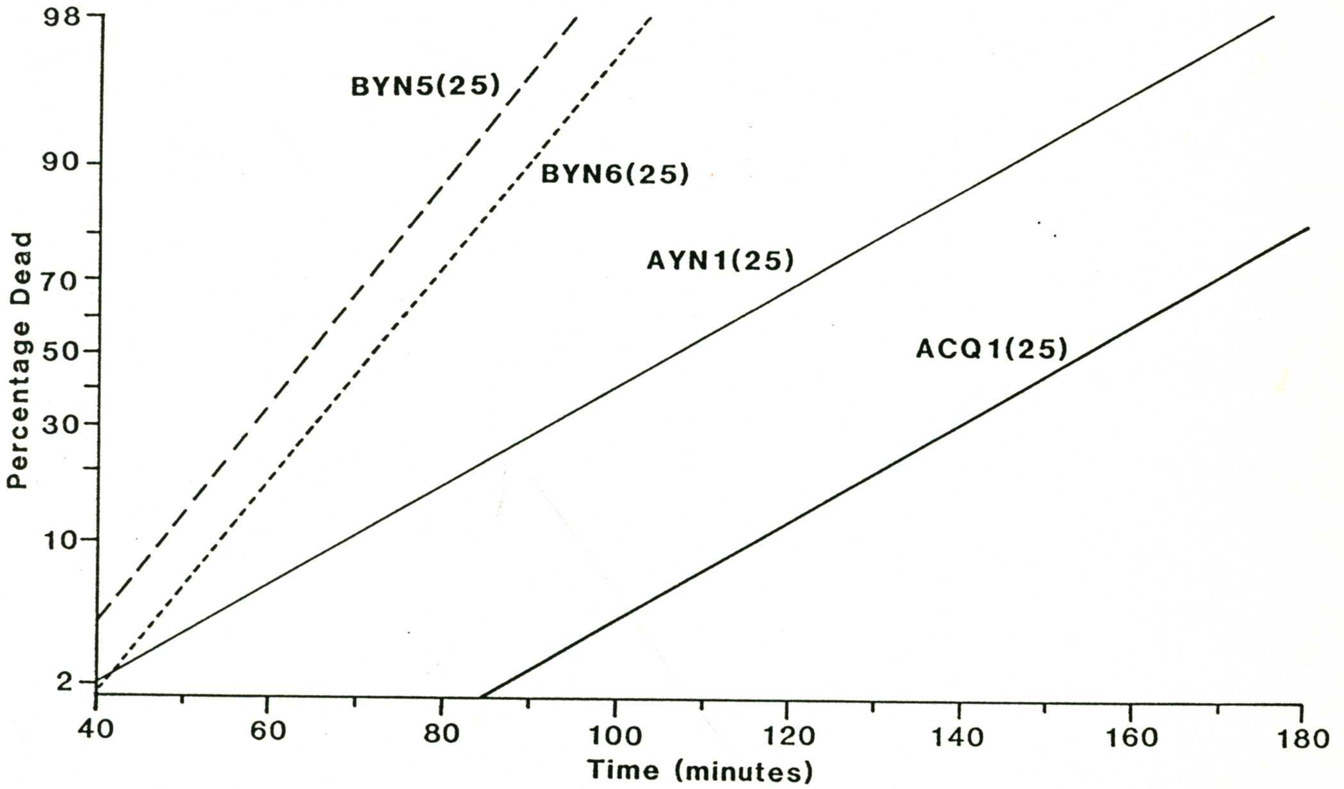

Figure 3.3.1. Elapsed time at 41°C versus percent dead for female <u>Drosophila buzzatii</u> from cages BYN5(25) (– – – –) and BYN6(25) (-------), and <u>Drosophila aldrichi</u> from cages AYN1(25) (———) and ACQ1(25) (———).

Table 3.3.1. Time in minutes to 50% death (LT50) at 41°C with standard errors, with the results of *t* tests of differences, for *Drosophila aldrichi* and *Drosphila buzzatii*

Species	Origin	Cage No.	Sex	LT50±se	*t* Test Results							
D. aldrichi	Qld.	ACQ1(25)	M	148.3±3.2								
			F	153.2±3.5	ns							
D. aldrichi	N.S.W.	AYN1(25)	M	109.5±2.8	***	***						
			F	106.3±2.9	***	***	ns					
D. buzzatii	N.S.W.	BYN5(25)	M	57.6±2.2	***	***	***	***				
			F	64.7±2.7	***	***	***	***	*			
D. buzzatii	N.S.W.	BYN6(25)	M	58.5±2.1	***	***	***	***	ns	*		
			F	71.4±2.0	***	***	***	***	***	***	***	
		Sex			M	F	M	F	M	F	M	F
		Cage No.			ACQ1(25)		AYN1(25)		BYN5(25)		BYN6(25)	

ns = not significant; * P < 0.05; *** P < 0.001

Table 3.3.2. Time in days to 50% death (LT50) at 0°C, with the results of _t_ tests of differences, for _Drosophila aldrichi_ and _Drosophila buzzatii_; first comparison

Species	Origin	Cage No.	Sex	LT50 ±se	_t_ Test Results							
Drosophila aldrichi	Qld.	ACQ1(25)	M	3.61±0.15								
			F	4.14±0.16	*							
	N.S.W.	AYN1(25)	M	3.94±0.15	ns	ns						
			F	3.74±0.17	ns	ns	ns					
Drosophila buzzatii	N.S.W.	BYN5(25)	M	3.37±0.22	ns	**	*	ns				
			F	3.70±0.17	ns	ns	ns	ns	ns			
	N.S.W.	BYN6(25)	M	3.24±0.19	ns	***	**	*	ns	ns		
			F	3.98±0.16	ns	ns	ns	ns	*	ns	**	
		Sex			M	F	M	F	M	F	M	F
		Cage No.			ACQ1(25)		AYN1(25)		BYN5(25)		BYN6(25)	

ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

shock (Table 3.3.3) the corresponding means were 5.2 days (D. buzzatii) and 5.5 (D. aldrichi). While no standard errors are attached to these means, (some of the LT50's pooled to calculate the means are themselves significantly different), they seem consistent in pointing to D. aldrichi having superior cold resistance to D. buzzatii.

In examining the individual comparisons it may be observed that while LT50's for D. aldrichi males and females do not differ consistently, females of D. buzzatii survive longer than males from the same cage population. As previously noted, this was found to be the norm for D. buzzatii. In the first cold shock (Table 3.3.2) D. aldrichi males of N.S.W. origin lived significantly longer than males from either D. buzzatii cage. This apparently clear result was however contradicted by the D. aldrichi females from N.S.W., whose LT50 fell between those of the two D. buzzatii female samples. It was further confused by the results of the second cold shock (Table 3.3.3), in which N.S.W. origin D. aldrichi (males and females) had (non significantly) smaller LT50's than either D. buzzatii cage. However the Hemmant (Qld.) origin D. aldrichi sample showed much greater cold shock resistance than all other flies in the experiment for all but one comparison ($P < .05$). Thus while the general trend suggests superior cold resistance in D. aldrichi, the inconsistencies in the detailed results throw some doubt on this conclusion.

3.3.2. Comparison of D. buzzatii from two different areas

In section 3.3.1, besides D. aldrichi being found to be superior in heat resistance to D. buzzatii, it was shown that D. aldrichi from a population cage derived from central Queensland was more heat tolerant than D. aldrichi from "Yarrawonga", N.S.W. (Table

Table 3.3.3. Time in days to 50% death (LT50) at 0°C, with the results of *t* tests of differences, for *Drosophila aldrichi* and *Drosophila buzzatii*; second comparison

Species	Origin	Cage No.	Sex	LT50±se	*t* Test Results							
Drosophila aldrichi	Qld.	AHQ1(25)	M	6.15±0.13								
			F	5.88±0.11	ns							
	N.S.W.	AYN1(25)	M	4.93±0.10	***	***						
			F	5.09±0.09	***	***	ns					
Drosophila buzzatii	N.S.W.	BYN5(25)	M	5.11±0.10	***	***	ns	ns				
			F	5.53±0.09	***	*	***	***	**			
	N.S.W.	BYN6(25)	M	5.12±0.09	***	***	ns	ns	ns	**		
			F	5.15±0.10	***	***	ns	ns	ns	**	ns	
		Sex			M	F	M	F	M	F	M	F
		Cage No.			AHQ1(25)		AYN1(25)		BYN5(25)		BYN6(25)	

ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

3.3.1). By contrast, D. buzzatii from Hemmant near Brisbane, Queensland do not show clear evidence of greater heat tolerance than D. buzzatii from "Yarrawonga" (Table 3.3.4). The mean LT50 was 71 minutes for "Yarrawonga" flies and 75 minutes for Hemmant flies, but the only significant difference between the populations is the "Yarrawonga" cage B3 males are significantly less tolerant of heat than flies from either Hemmant cage. All other significant differences are between sexes, within and between cages of origin.

3.3.3. Comparison of D. buzzatii from differently adapted population cages

The first experiment was a simple heat shock (41°C) comparison of direct samples from each of the six Drosophila buzzatii cages stored at 25°C with their 18°C counterparts.

The mean LT50 determined for cage BYN1(25) was 84 minutes, while that for BYN1(18) was 70 minutes (Table 3.3.5). Within-sex cage differences were highly significant. For cages BYN2(25) and BYN2(18) the respective mean LT50's were 82 and 75 minutes, but due to different regression coefficients between the two cages (Fig. 3.3.2), only within-cage comparisons are valid (Table 3.3.6). The mean LT50's for cages BYN3(25) and BYN3(18) were 84 and 70 minutes respectively (Table 3.3.7) and for cages BYN4(25) and BYN4(18) they were 82 and 74 minutes respectively (Table 3.3.8); neither pair of cages showed any significant same-sex differences,however. Males of the BYN4 cage pair had a larger regression coefficient than their female counterparts (Fig. 3.3.3). Both males and females of cage BYN5(25) (mean survival time 82 minutes) survived significantly longer than their same-sex counterparts from cage BYN5(18) (mean survival time 68 minutes) (Table

Table 3.3.4. Time in minutes to 50% death (LT50) at 41°C, with the results of *t* tests of differences, for *Drosophila buzzatii* from "Yarrawonga" (N.S.W.) and Hemmant (Qld.)

Origin	Cage No.	Sex	LT50 ±se	*t* Test Results							
"Yarrawonga"	BYN3(25)	M	59.8±2.5								
		F	77.3±3.3	***							
"Yarrawonga"	BYN4(25)	M	63.9±2.3	ns	**						
		F	81.5±3.3	***	ns	***					
Hemmant	BHQ1(25)	M	70.4±3.4	*	ns	ns	**				
		F	77.1±3.3	***	ns	**	ns	ns			
Hemmant	BHQ2(25)	M	69.8±2.2	**	ns	ns	**	ns			
		F	83.2±2.4	***	ns	***	ns	**	ns	***	
		Sex		M	F	M	F	M	F	M	F
		Cage No.		BYN3(25)		BYN4(25)		BHQ1(25)		BHQ2(25)	

ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

Table 3.3.5. Time in minutes to 50% death (LT50) at 41°C, with the results of t tests of differences, for Drosophila buzzatii from cages BYN1(25) and BYN1(18)

Cage No.	Sex	LT_{50} ±se	t Test Results			
BYN1(25)	M	73.3±2.2				
	F	94.0±2.4	***			
BYN1(18)	M	59.3±2.2	***	***		
	F	80.1±2.0	*	***	***	
		Sex	M	F	M	F
		Cage No.	BYN1(25)		BYN1(18)	

* = P < 0.05; *** = P < 0.001

Table 3.3.6. Time in minutes to 50% death (LT50) at 41°C, with the results of t tests of differences, for Drosophila buzzatii from cages BYN2(25) and BYN2(18)

Cage No.	Sex	LT50 ±se	t Test Results			
BYN2(25)	M	76.0±2.5				
	F	87.4±3.2	**			
BYN2(18)	M	59.1±7.6	ns	**		
	F	87.4±8.5	ns	ns	*	
	Sex		M	F	M	F
	Cage No.		BYN2(25)		BYN2(18)	

ns = not significant; * = P < 0.05; ** = P < 0.001

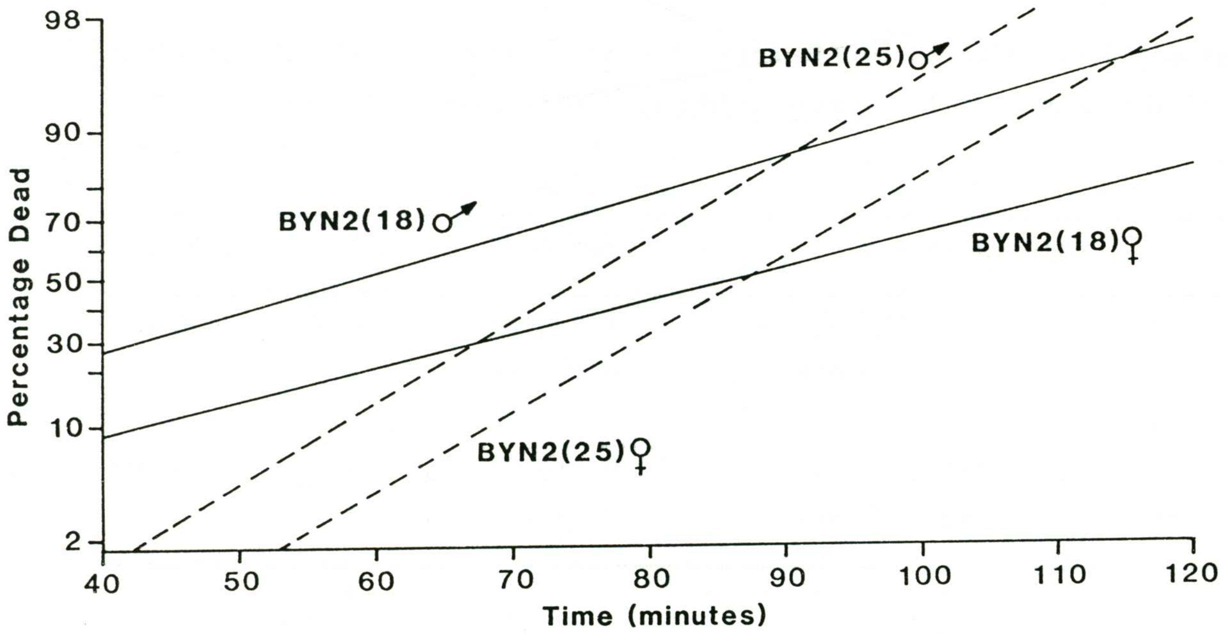

Figure 3.3.2. Elapsed time at 41°C versus percent dead for male and female <u>Drosophila</u> <u>buzzatii</u> from cages BYN2(25) (---) and BYN2(18) (———).

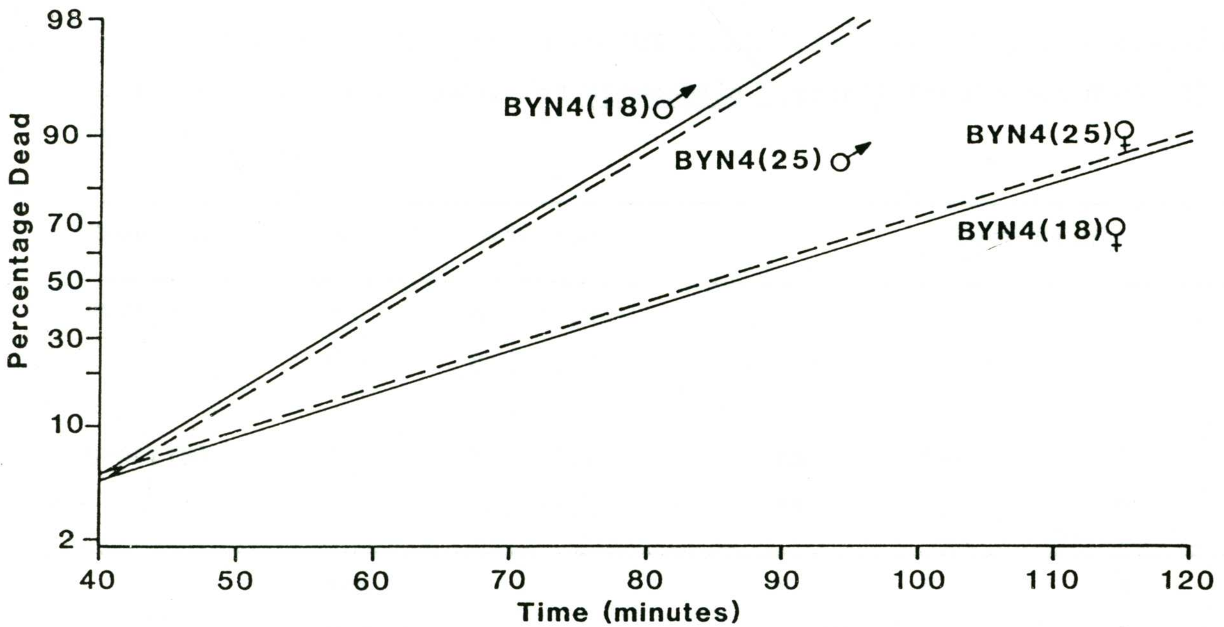

Figure 3.3.3. Elapsed time at 41°C versus percent dead for male and female <u>Drosophila</u> <u>buzzatii</u> from cages BYN4(25) (----) and BYN4(18) (———).

Table 3.3.7. Time in minutes to 50% death (LT50) at 41°C, with the results of t tests of differences, for Drosophila buzzatii from cages BYN3(25) and BYN3(18)

Cage No.	Sex	LT50 ±se	t Test Results			
BYN3(25)	M	61.3±2.7				
	F	78.0±3.9	***			
BYN3(18)	M	57.6±3.0	ns	***		
	F	79.8±3.5	***	ns	***	
	Sex		M	F	M	F
	Cage No.		BYN3(25)		BYN3(18)	

ns = not significant; *** = P < 0.001

Table 3.3.8. Time in minutes to 50% death (LT50) at 41°C, with the results of t tests of differences, for Drosophila buzzatii from cages BYN4(25) and BYN4(18)

Cage No.	Sex	LT50 ±se	t Test Results			
BYN4(25)	M	64.3±2.0				
	F	85.1±3.2	***			
BYN4(18)	M	63.5±2.7	ns	***		
	F	86.4±6.2	**	ns	**	
	Sex		M	F	M	F
	Cage No.		BYN4(25)		BYN4(18)	

ns = not significant; ** = P < 0.01; *** = P < 0.001

3.3.9). Mean survival times for cages BYN6(25) and BYN6(18) were 83 minutes and 74 minutes respectively. The males of the two cages had significantly different LT50's, BYN6(25) males surviving the longer (Table 3.3.10).

In summary, flies sampled from 25°C populations survived longer than those of the same sex drawn from 18°C in 10 out of 12 comparisons. In 5 of these 10 comparisons the differences were significant. Neither of the remaining 2 comparisons was significant.

In the repeat of this experiment only cages BYN5(25), BYN5(18), BYN6(25) and BYN6(18) were used. However, from each cage two groups were taken to be parents of the actual experimental flies - one group from each cage was reared at 25°C, the other at 18°C. The mean LT50 for the 25°C groups from both BYN5 cages was 67 minutes (Table 3.3.11). For the 18°C parental groups the LT50s were 66 and 72 minutes for BYN5(25) and BYN5(18) cage origins respectively. The only significant within-sex difference observed within parental rearing temperatures was between females whose parents were reared at 18°C. Those descended from BYN5(18) actually had a longer LT50 than those from BYN5(25).

The four BYN6 groups, BYN6(25), 25°C and 18°C parents and BYN6(18), 25°C and 18°C parents, produced mean survival times of 63, 65, 65 and 64 minutes respectively. The males of cage BYN6(25), whose parents were reared at 25°C, survived for a shorter time than males of the other three groups, between which survival time at 41°C did not differ (Table 3.3.12). Among females, only those from cage BYN6(25) with parents reared at 25°C lived significantly longer than the shortest lived group, those from BYN6(18) whose parents were reared at 18°C.

Table 3.3.9. Time in minutes to 50% death (LT50) at 41°C, with the results of *t* tests of differences, for *Drosophila buzzatii* from cages BYN5(25) and BYN5(18)

Cage No.	Sex	LT50 ±se	*t* Test Results			
BYN5(25)	M	72.4±2.1				
	F	91.8±2.1	***			
BYN5(18)	M	58.6±1.8	***	***		
	F	78.1±1.9	*	***	***	
	Sex		M	F	M	F
	Cage No.		BYN5(25)		BYN5(18)	

* = P < 0.05; *** = P < 0.001

Table 3.3.10. Time in minutes to 50% death (LT50) at 41°C, with the results of *t* tests of differences, for *Drosophila buzzatii* from cages BYN6(25) and BYN6(18)

Cage No.	Sex	LT50 ±se	*t* Test Results			
BYN6(25)	M	81.0±2.5				
	F	84.7±2.9	ns			
BYN6(18)	M	66.9±2.2	***	***		
	F	82.0±2.5	ns	ns	***	
	Sex		M	F	M	F
	Cage No.		BYN6(25)		BYN6(18)	

ns = not significant; *** = P < 0.001

Table 3.3.11. Time in minutes to 50% death (LT50) at 41°C, with the results of *t* tests of differences, for controls and exchanged temperature groups of *Drosophila buzzatii* from cages BYN5(25) and BYN5(18)

Cage No.	Parental Rearing Temp.	Sex	LT50 ±se	*t* Test Results							
BYN5(25)	25°C	M	61.4±1.9								
		F	72.8±1.8	***							
BYN5(25)	18°C	M	67.3±2.0	*	*						
		F	65.2±2.0	ns	**	ns					
BYN5(18)	25°C	M	64.9±1.9	ns	**	ns	ns				
		F	69.7±1.9	**	ns	ns	ns	ns			
BYN5(18)	18°C	M	67.5±2.1	*	ns	ns	ns	ns	ns		
		F	77.1±2.1	***	ns	**	***	***	*	*	
	Sex			M	F	M	F	M	F	M	F
	Cage No.			BYN5(25)		BYN5(25)		BYN5(18)		BYN5(18)	
	Parental Rearing Temp.			25°		18°		25°		18°	

ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

Table 3.3.12. Time in minutes to 50% death (LT50) at 41°C, with the results of *t* tests of differences, for controls and exchanged temperature groups of *Drosophila buzzatii* from cages BYN6(25) and BYN6(18)

Cage No.	Parental Rearing Temp.	Sex	LT50 ±se	*t* Test Results							
BYN6(25)	25°C	M	54.4±1.2								
		F	71.2±1.2	***							
BYN6(25)	18°C	M	61.4±1.2	***	***						
		F	69.4±1.1	***	ns	***					
BYN6(18)	25°C	M	61.9±1.2	***	***	ns	***				
		F	69.6±1.1	***	ns	***	ns	***			
BYN6(18)	18°C	M	60.3±1.2	***	***	ns	***	ns	***		
		F	67.8±1.1	***	*	***	ns	***	ns	***	
	Sex			M	F	M	F	M	F	M	F
	Cage No.			BYN6(25)		BYN6(25)		BYN6(18)		BYN6(18)	
	Parental Rearing Temp.			25°		18°		25°		18°	

ns = not significant; * = P < 0.05; *** = P < 0.001

Thus neither of the above 4-way heat shock comparisons supports the result of the 6 original pairwise comparisons. For experimental flies whose parents were reared at the same temperature there was no case where the flies originating from a 25°C cage survived heat shock significantly longer than flies from the equivalent 18°C cage.

The reversed environment parental groups were used to produce a second batch of flies which were subjected to a cold shock (0°C). For cage BYN5(25), (parents reared at 25°C and 18°C) and cage BYN5(18), (parents raised at 25°C and 18°C), the mean LT50s were 5.2, 5.2, 5.4 and 6.0 days respectively. Cage BYN5(18) origin flies whose parents were reared at 18°C lived significantly longer than their same sex counterparts from all other groups (Table 3.3.13). Between-sex comparisons are meaningless as the males have a smaller regression coefficient than the females and the regression lines cross (Fig. 3.3.4).

Initial analysis of the BYN6 groups revealed significantly different regression coefficients for all groups and sexes. Exclusion of the most aberrant of these (males derived from BYN6(18) whose parents were reared at 25°C) removed the between-groups difference but not the between-sexes difference in regression coefficients. Samples of the regression lines used to calculate the LT50's are illustrated in Fig. 3.3.5. The LT50 for males from cage BYN6(18), whose parents were reared at 25°C, was calculated from the separate male regression coefficient (Fig. 3.3.5). The mean LT50's for flies derived from cage BYN6(25), 25°C and 18°C parents, and from cage BYN6(18), 25°C and 18°C parents, are 4.7, 4.8, 4.8, and 4.6 respectively (Table 3.3.14). Cage BYN6(25) origin males whose parents were reared at 18°C lived

Table 3.3.13. Time in days to 50% death (LT50) at 0°C, with the results of *t* tests of differences, for controls and exchanged temperature groups of *Drosophila buzzatii* from cages BYN5(25) and BYN5(18)

Cage No.	Parental Rearing Temp.	Sex	LT50 ± se	*t* Test Results							
BYN5(25)	25°C	M	5.10±0.21								
		F	5.36±0.11	ns							
BYN5(25)	18°C	M	5.09±0.24	ns	ns						
		F	5.27±0.14	ns	ns	ns					
BYN5(18)	25°C	M	5.19±0.22	ns	ns	ns	ns				
		F	5.59±0.11	*	ns	ns	ns	ns			
BYN5(18)	18°C	M	5.81±0.16	**	*	**	*	*	ns		
		F	6.11±0.08	***	***	***	***	***	***	ns	
	Sex			M	F	M	F	M	F	M	F
	Cage No.			BYN5(25)		BYN5(25)		BYN5(18)		BYN5(18)	
	Parental Rearing Temp.			25°		18°		25°		18°	

ns = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

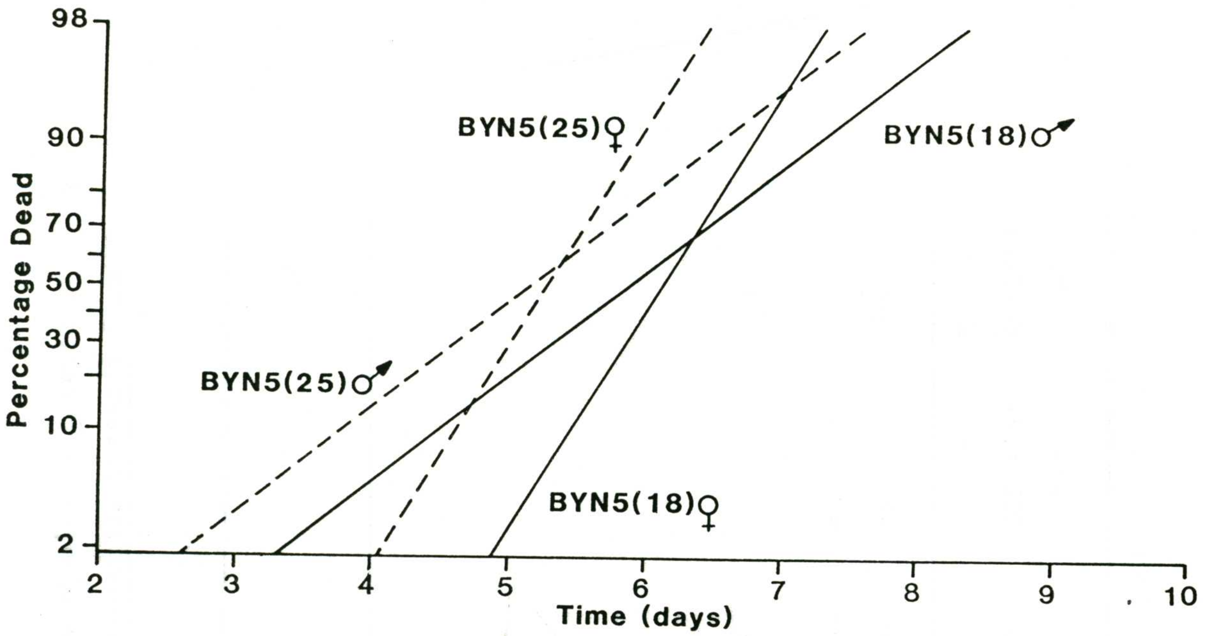

Figure 3.3.4. Elapsed time at 0°C versus percent dead for male and female Drosophila buzzatii from cages BYN5(25) (---) and BYN5(18) (——), reared for the preceding generation at 18°C.

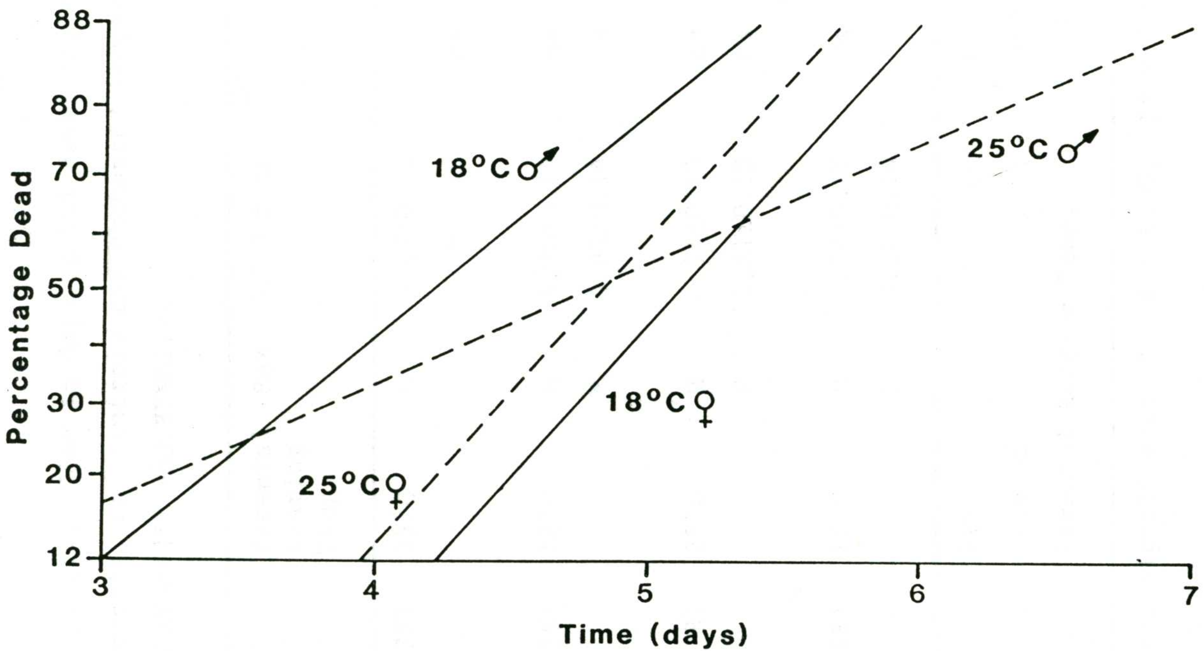

Figure 3.3.5. Elapsed time at 0°C versus percent dead for male and female Drosophila buzzatii from cage BYN6(18), preceding generation reared at 25°C (---) and 18°C (——).

Table 3.3.14. Time in days to 50% death (LT50) at 0°C, with the results of _t_ tests of differences, for controls and exchanged temperature groups of _Drosophila buzzatii_ from cages BYN6(25) and BYN6(18)

Cage No.	Parental Rearing Temp.	Sex	LT50 ± se	_t_ Test Results							
BYN6(25)	25°C	M	4.62±0.18								
		F	4.86±0.13	ns							
BYN6(25)	18°C	M	4.70±0.17	ns	ns						
		F	4.92±0.14	ns	ns	ns					
BYN6(18)	25°C	M	4.74±0.19	ns	ns	ns	ns				
		F	4.81±0.13	ns	ns	ns	ns				
BYN6(18)	18°C	M	4.18±0.15	ns	**	*	***	*	**		
		F	5.10±0.14	*	ns	ns	ns	ns	ns	***	
	Sex			M	F	M	F	M	F	M	F
	Cage No.			BYN6(25)		BYN6(25)		BYN6(18)		BYN6(18)	
	Parental Rearing Temp.			25°		18°		25°		18°	

ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

significantly longer than cage BYN6(18) males whose parents were reared at 18°C, but there were no differences between females of the four groups.

To sum up, the initial series of heat shocks produced strong evidence in favour of differential temperature adaptation between the 25°C and 18°C cages. This however is not supported by the repeat series of heat shocks or the cold shocks. Indeed the high cold resistance of cage BYN5(18) origin flies, whose parents were raised at 18°C, suggests that cytoplasmic preadaptation may be more important in the short time scale than any genetic adaptation.

3.3.4. Comparison of chromosome arrangements in D. buzzatii

In the comparison of survival of chromosome lines from the Hunter Valley after heat (41°C) shock, the most prominent feature is the large difference in survival time between males and females of the st/st line (Table 3.3.15). In fact this difference is so large that, for all four lines, and regardless of sex, the st/st males have the smallest LT50 ($P < 0.01$) and the st/st females the largest ($P < 0.01$). However the means of the two sexes differ little between lines - for the st/j line the mean LT50 was 71 minutes and for all three other lines it was 70 minutes.

Among Hemmant origin chromosome lines, the st/st line shows the highest heat shock resistance (Table 3.3.16). The male LT50 was however not significantly longer than that for j/j males, and st/st females did not survive significantly longer than st/j females.
The means of male and female LT50s for each of the four groups are as follows: 73 minutes for the st/st line, 70 minutes for the j/j line, 67 minutes for the st/j line and, for the j/st line, 68 minutes.

Table 3.3.15. Time in minutes to 50% death (LT50) at 41°C, with the results of *t* tests of differences, for chromosome inversion lines of *Drosophila buzzatii* from "Yarrawonga" (N.S.W.)

Chromosome arrangement M parent/F parent	Sex	LT50 ± se	*t* Test Results							
st/st	M	59.3±1.7								
	F	80.8±1.7	***							
j/j	M	65.6±1.4	**	***						
	F	73.5±1.4	***	**	***					
st/j	M	70.7±1.3	***	***	**	ns				
	F	71.7±1.4	***	***	**	ns	ns			
j/st	M	66.4±1.4	**	***	ns	***	*	**		
	F	74.3±1.4	***	**	***	ns	ns	ns	***	
Sex			M	F	M	F	M	F	M	F
Chromosome arrangement (M parent/F parent)			st/st		j/j		st/j		j/st	

ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

Table 3.3.16. Time in minutes to 50% death (LT50) at 41°C, with the results of _t_ tests of differences, for chromosome inversion lines of _Drosophila buzzatii_ from Hemmant (Qld.)

Chromosome arrangement M parent/F parent	Sex	LT50 ± se	_t_ Test Results							
st/st	M	66.3±1.1								
	F	80.4±1.2	***							
j/j	M	63.6±1.2	ns	***						
	F	76.2±1.1	***	*	***					
st/j	M	56.5±1.6	***	***	***	***				
	F	77.5±1.2	***	ns	***	ns				
j/st	M	62.9±1.2	*	***	ns	***	***	***		
	F	73.6±1.1	***	***	***	ns	***	*	***	
Sex			M	F	M	F	M	F	M	F
Chromosome arrangement (M parent/F parent)			st/st		j/j		st/j		j/st	

ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

Replicate samples from the same sets of lines were subjected to cold (0°C) shocks. Initial analysis of "Yarrawonga" results showed that there were significant differences between regression coefficients of lines and sexes. Exclusion of the data for males of the st/st line and reanalysis removed the within-sex differences in regression coefficients between chromosome lines (Fig. 3.3.6). For st/st males the LT50 was derived from the original analysis, but for all other groups the reanalysis provided the estimates of LT50s (Fig. 3.3.6). Females of the st/st line were superior to all other lines (Table 3.3.17, $P < 0.001$) in their cold resistance, while st/st males, although not significantly different to j/j males, produced the smallest LT50 of any line. However the mean of male and female LT50's was 5 days for all lines.

The LT50's at 0°C for the Hemmant lines (Table 3.3.18) reveal that, for both males and females, the j/st line showed the greatest cold resistance (the difference between j/st and st/st females was not significant). The mean LT50's of the four groups were 4.0 for the st/st line, 3.8 for j/j, 3.6 for st/j and 4.2 for the j/st line.

In conclusion, unlike Wright and Dobzhansky (1946), who showed a clear relationship between temperature and chromosome inversions, it is not possible, from the above results, to deduce a simple link between extreme temperature survival and chromosome inversion. Relationships between the st (standard) arrangement and heat tolerance and between j/st and cold tolerance do however receive limited support from both sets of lines.

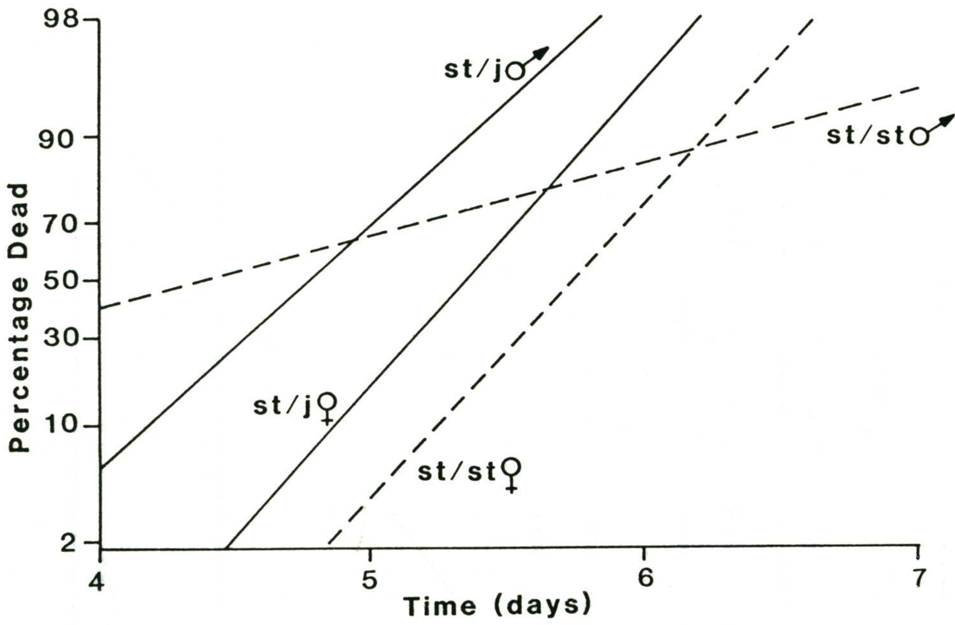

Figure 3.3.6. Elapsed time at 0°C versus percent dead for males and females of Drosophila buzzatii st/st (----) and st/j (———) Fl chromosome inversion lines.

Table 3.3.17. Time in days to 50% death (LT50) at 0°C, with the results of _t_ tests of differences, for _Drosophila buzzatii_ chromosome inversion lines of "Yarrawonga" (N.S.W.) origin

Chromosome arrangement M parent/F parent	Sex	LT50 ± se	_t_ Test Results							
st/st	M	4.40±0.14								
	F	5.76±0.05	***							
j/j	M	4.56±0.07	ns	***						
	F	5.24±0.06	***	***	***					
st/j	M	4.79±0.07	*	***	*	***				
	F	5.36±0.05	***	***	***	ns	***			
j/st	M	4.82±0.07	*	***	**	***	ns	***		
	F	5.51±0.05	***	***	***	***	***	*	***	
Sex			M	F	M	F	M	F	M	F
Chromosome arrangement (M parent/F parent)			st/st		j/j		st/j		j/st	

ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

Table 3.3.18. Time in days to 50% death (LT50) at 0°C, with the results of *t* tests of differences, for *Drosophila buzzatii* chromosome inversion lines of Hemmant (Qld.) origin

Chromosome arrangement M parent/F parent	Sex	LT50 ± se	*t* Test Results							
st/st	M	3.16±0.10								
	F	4.76±0.12	***							
j/j	M	3.05±0.12	ns	***						
	F	4.62±0.07	***	ns	***					
st/j	M	3.25±0.16	ns	***	ns	***				
	F	3.94±0.12	***	***	***	***				
j/st	M	3.61±0.08	***	***	***	***	*	**		
	F	4.84±0.07	***	ns	***	*	***	***	***	
Sex			M	F	M	F	M	F	M	F
Chromosome arrangement (M parent/F parent)			st/st		j/j		st/j		j/st	

ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

3.4. DISCUSSION

Some workers have shown evidence of a threshold temperature above which normal development or ageing processes are overlaid by the effect of heat stress. Maynard Smith (1958) put this threshold at 31°C for *Drosophila subobscura*, although for the same species Hollingsworth (1969) found it to lie between 28 and 29°C. Miquel *et al.* (1976) suspected that the maximum "normal" temperature for *D. melanogaster* may be as low as 27°C. This threshold is most easily observed as a sudden change in the slope of the plot of survival time *vs.* temperature (Hollingsworth 1969). While this threshold was not determined for either of the two species under study here, the temperature chosen for heat shock study (41°C), plus the high humidity in treatment vials (see section 3.2.1), ensured that all flies suffered a severe heat shock.

Thirty minutes at 41°C was found by Johnson and Powell (1974) to be sufficient to kill 90% of *D. melanogaster*. By comparison, the two cactophilic species studied here (*D. aldrichi* and *D. buzzatii*) showed much greater resistance to high temperatures (Section 3.3.1). As suggested by the prevalence of *D. aldrichi* in the more northern parts of the *Opuntia* spp. distribution (Mulley and Barker 1977), the heat tolerance of *D. aldrichi* is much greater than that of *D. buzzatii*. Further, a clear distinction between *D. aldrichi* populations is revealed in their comparative heat tolerances, with central Queensland *D. aldrichi* surviving, on average, 43 minutes (40%) longer than the Hunter Valley population.

That such a distinction between populations was not observed for "Yarrawonga" and Hemmant populations of *D. buzzatii*, despite the Hunter Valley being almost 1,000 km south of the Brisbane area, may be partly explained by reference to Table 3.4.1. Despite the overall

mean temperature being higher for Hemmant, the highest expected mean daily maximum (in January) is 3°C higher for the Hunter Valley than for Brisbane. In contrast, the annual mean for central Queensland is 3°C higher than that for the Hunter Valley, and the mean daily expected maxima for all 4 representative months are also at least 3°C higher than for the Hunter Valley.

Table 3.4.1. Representative seasonal maximum and minimum temperatures in °C for Victoria and S.E. South Australia, the Hunter Valley (N.S.W.), Hemmant (Brisbane, Qld.) and central Queensland. Source: Climatic Atlas of Australia (1975)

Month	Area			
	Vic. & S.A.	Hunter Valley	Brisbane	Central Qld.
January				
max.	27-33	27-33	27-30	33-36
min.	12-18	12-18	18-21	18-21
April				
max.	18-24	21-27	24-27	24-30
min.	6-12	6-12	15-18	9-15
July				
max.	9-15	12-18	18-21	18-21
min.	0-6	0-6	6-12	0-6
October				
max.	18-24	24-27	24-27	27-33
min.	6-9	6-12	15-18	12-15
Annual mean	15	16	20	20

From the July expected daily temperature minima (Table 3.4.1), it can be inferred that there is no greater selection for cold resistance in the Hunter Valley than in central Queensland. The fact that *D. aldrichi* from Hemmant survived longer than *D. aldrichi* from "Yarrawonga" (Section 3.3.1) was unexpected, given the mild winters experienced in the Brisbane area (Table 3.4.1). Considering that *D. buzzatii* are found in Victoria (Carson and Wasserman 1965), while *D. aldrichi* are not known south of the Hunter Valley (Mulley and Barker 1977), it was also unexpected that the cold shock survival of *D. aldrichi* should be as good or better than that of *D. buzzatii*. Perhaps the difference in the laboratory diet (Section 3.2.1) gave *D. aldrichi* an advantage, regardless of temperature.

The initial comparison of pairs of cages, one of each pair adapted to 25°C, the other to 18°C, appeared to support the hypothesis that loss of heat resistance would occur in a cooler climate or season. Flies of 25°C origin survived longer than their 18°C origin counterparts in 10 of 12 comparisons, and 5 of these 10 differences were significant. In contrast, in no case did 18°C origin flies survive significantly longer than flies of the same sex drawn from 25°C cages (Section 3.3.3). However, Dr H. Soliman (pers. comm.) pointed out that this observed difference was not necessarily genetic. Thus the repeat experiment designed to break down short term physiological (as distinct from genetic) adaptation, was performed. The results of this repeat experiment do not support those of the former, suggesting that indeed there was physiological (non-genetic) preadaptation, or else that the significant differences observed were an experimental artifact.

Scoring of chromosome inversion frequencies in populations of *D. buzzatii* covering most of its Australian distribution suggests a North-South cline in 2j frequency (Table 3.4.2). While there is little difference in the 2j inversion frequencies of the Hunter Valley and of Victoria and South Australia, they are consistently higher than the mean for Queensland populations. This is not inconsistent with the maximum and minimum temperatures listed in Table 3.4.1, which show that the Hunter Valley temperatures are more similar to Victorian and South Australian temperatures than they are to central Queensland temperatures.

Table 3.4.2. Second chromosome inversion frequencies, with binomial standard errors, of *D. buzzatii* populations in eastern Australia

Area	j Inversion frequency	No. Chromosomes examined	Date	Source
Qld.	0.47±0.02	438	August 1974	Watt (1975)
"Yarrawonga" (N.S.W.)	0.65±0.05	99	July-Sept. 1975	Watt (1975)
Vic. & S.A.	0.69±0.09	26	August 1975	Watt (1975)
Vic.	0.69±0.06	80	July 1961	Carson & Wasserman (1965)

The above association of the standard chromosome arrangement with higher temperatures and of the 2j inversion with cooler temperatures is given some support by the results of laboratory heat and cold shock experiments (Section 3.3.4). There is evidence for superior survival of heat shock by homozygous st flies, especially those of Hemmant origin. While homozygous st females also showed high cold shock resistance, the most consistently cold resistant flies were the j/st heterozygotes, although there is no indication as to why this heterozygote should be superior to the reverse cross.

CHAPTER 4

THE EFFECT OF TEMPERATURE EXTREMES ON ELECTROPHORETIC ALLELE FREQUENCIES

4.1. INTRODUCTION

A great deal of work by population geneticists has centred on the technique of electrophoresis since its first application to population genetics about 20 years ago (Lewontin and Hubby 1966; Harris 1966). One advantage of this technique is that it can give a number of quantifiable phenotypes, each usually the product of a single locus, from the one individual. Further, heterozygotes can usually be distinguished from homozygotes. In contrast, because morphological traits are generally the end product of a series of enzymes, each coded for by a different locus, a single trait may result from any of a number of similar mutants which actually originate at different loci. While it is possible to determine which locus produces a particular mutant by genetic mapping, this is a much more laborious process than electrophoresis. Further, because of the phenomenon of dominance, it is usually only possible to distinguish the homozygote of a dominant allele from its heterozygote by examining the progeny of test matings. Thus a great deal of low-level variation is not observed in morphological features.

Attempts to test both the selectionist and neutralist positions, using electrophoretic methods, have been a feature of recent experiments in population genetics. For example, Johnson and Powell (1974) explained a natural North-South cline in *Alcohol dehydrogenase* (*Adh*) frequency of *Drosophila melanogaster* by showing

that one allele increased in frequency among the survivors of heat shock, and that the other allele was at a higher frequency among cold shock survivors. Powell (1973) found no changes at one locus of *D. melanogaster* in population cages kept at different temperatures, but strong directional changes in two other loci. Admittedly not all such studies have been so successful; Schenfield and McKechnie (1979) found no differential selection between *α-glycerophosphate dehydrogenase* alleles at extremes of temperature, and Gionfriddo and Vigue (1978) found no correlation between maxima, minima or weekly mean temperature and *Adh* allele frequencies.

Barker and Mulley (1976) chose to study *Drosophila buzzatii* "because ... of its potential for the study of selective forces acting in natural populations". They reported the variation found at about 30 loci of that species over most of its distribution in Australia. Of these 30 loci, six were found to show consistent variation. Using canonical correlation and multiple regression methods, Mulley *et al.* (1979) attempted to relate variation between Australian populations at these six loci to climatic and geographical measurements.

They found significant variation between collection sites for all loci, much of which was shown to be related to geographical location alone, but some of which was found to be independent of simple geographical parameters. Variation of gene frequencies with geographical location is ambiguous, for while some aspects of climate which are potential selective agents change progressively with geographical location, regular geographic clines in gene frequencies may also be caused by genetic drift and migration. Since temperature is an important component of climate which varies considerably with geographical location, laboratory experiments involving temperature

were seen as a logical progression from the field experiments. The finding of significant correlation between facets of temperature variation and both the "c" allele of Esterase-2 ($Est\text{-}2^{c}$) and "b" allele of Phosphoglucomutase (Pgm^{b}) by Mulley et al. (1979) further emphasizes the potential use of laboratory studies.

It was therefore decided to test both D. buzzatii and D. aldrichi for changes in frequencies of alleles, at all commonly polymorphic enzyme loci, among the survivors of both heat (41°C) and cold (0°C) shocks. The selection of these shock temperatures is discussed in Section 3.1.

4.2. MATERIALS AND METHODS

4.2.1. Introduction

While egg-sampling, as described in Section 2.2, was used to produce D. aldrichi for heat and cold shock, a different method of generating experimental flies was used for the heat and cold shock performed on D. buzzatii, as a reliable method of egg sampling D. buzzatii had not been developed at the time these experiments were performed. The basic method consisted of taking a random sample of adults from a cage (by emptying the cage and anaesthetising the flies) and setting up the mature adults from this sample (easily distinguished by abdominal colour) in food vials as double pair matings. Males were included in each vial to remove uncertainty as to whether or not the females had been previously mated. Since almost all females began laying within 24 hours of being placed in the vials, stages of development remained quite uniform across vials. Adults for an experiment were collected within 24 hours of eclosure and assigned to treatment vials. Each treatment vial received only one fly from each mating vial, thus eliminating sibling effects within treatments.

For the first experiment performed (a heat shock; described in Section 4.2.2), two such collections were made. One group was aged for one week before treatment, the other was treated immediately after collection. In this and in preliminary trials, it was noted that the survival of both high and low temperature shocks by newly eclosed flies was much higher than by mature flies, which emphasized the importance of careful control over the age of flies in such experiments. To simplify subsequent experiments it was decided to age all flies for one week before exposure to temperature shocks. At this age the adults are close to peak reproductive performance; any

advantage in tolerance of extremes by particular genotypes of this age would be expected to lead to an increase in their genetic contribution to the next generation.

Vials used for treatments contained a pad of tissue moistened with sucrose, as described in Section 3.2.1. Treatment times were calculated to produce approximately 50% mortality, thus ensuring that the treatment was a severe shock, while retaining sufficient survivors for estimating gene frequencies from electrophoresis.

On the completion of the predetermined treatment time, cold-shocked flies were allowed 2 hours to recover (as for the cold shocks described in Chapter 3) before separation of the dead from the survivors. Immediately this separation was completed, both groups were frozen until electrophoresis could be performed.

Only survivors of heat-shocks, and an untreated control group, were frozen for electrophoresis, as the heat would be expected to have caused at least partial denaturation of the enzymes of non-survivors. The recovery time allowed before separation of living and dead flies, 24 hours, was the same as for heat shocks described in Chapter 3.

The criterion of death for both heat and cold shocks was as described in Section 3.2.1.

Electrophoretic procedures used for *D. buzzatii* were described by Barker *et al.* (1986) and Barker and Mulley (1976). For *D. aldrichi* a description of the electrophoretic procedures may be found in Appendix B. Enzyme loci screened for *D. buzzatii* were *Esterase-1* (*Est-1*), *Esterase-2* (*Est-2*), *β-n-acetyl-hexosaminidase* (*Hex*; previously called *Pyranosidase* (Barker 1981), *Phosphoglucomutase*

(Pgm), Aldehyde oxidase (Aldox) and Alcohol dehydrogenase-1 (Adh-1). For *D. aldrichi* the loci examined were Esterase-C (Est-C) (Queensland populations only), Esterase-D (Est-D), Esterase-F (Est-F) (male specific; New South Wales populations only), β-n-acetyl-hexosaminidase (Hex), Phosphoglucomutase (Pgm), Malate dehydrogenase (Mdh) and Leucine aminopeptidase (Lap).

For the above loci, gene frequencies, genotype frequencies, and homozygote *vs.* heterozygote frequencies of survivors *vs.* controls (or non-survivors) were compared using contingency table analysis (Lancaster 1969). This analysis works through all alleles (or genotypes) for all treatment groups using, in effect, pairwise comparisons, finally producing a single χ^2 value. If significant this indicates that one (or more) treatment group(s) is different to the other(s). Where such a difference was revealed, the gene frequencies are tabulated to permit identification of the allele showing shock tolerance, and whether the response was consistent. Besides testing total frequencies, all possible interactions between cage, sex etc. were tested.

4.2.2. Heat shock of D. buzzatii

Two "Yarrawonga" 25°C cages, BYN1(25) and BYN3(25) were sampled twice according to the method described in Section 4.2.1. The second sample succeeded the first by one week. Flies from the first sample were aged one week before treatment, and flies from the second sample were treated within 24 hours of being collected from mating vials.

To ensure that sufficient flies were killed, two treatment times were used for each age group - 60 and 90 minutes for those aged

one week, and 90 and 120 minutes for those not aged. An untreated control group was also kept for each age group. Since sexes were tested separately, 6 treatment vials (3 of males and 3 of females) were used for each age-group from each cage. Each treatment vial initially contained 30 flies, although due to losses during ageing and from electrophoresis failures the number available for statistical analysis was generally less than 30.

4.2.3. Cold shock of D. buzzatii

As for the heat-shock, two "Yarrawonga" cages, BYN5(25) and BYN6(25), were sampled to produce double-pair matings. The experiment was replicated by collecting new eclosures on two consecutive days; all experimental flies were then aged for seven days before use. Shock treatment for these flies was then started on two consecutive days. Treatment length was 5 days. For each treatment group, 1 vial of each sex was used from each cage of origin, each vial containing 40 flies initially.

4.2.4. Heat shock of D. aldrichi

As mentioned in Section 4.2.1, D. aldrichi for experimental use were generated by egg sampling according to the method of Section 2.2.1. Three population cages were used, two of "Yarrawonga" origin, AYN1(25) and AYN2(25) and one of Queensland origin, AHQ2(25) (see Section 3.2.1). Heat shock treatment lengths employed are listed in Table 4.2.1. The differing shock lengths reflect attempts, based on preliminary experiments, to bracket the LT50 for each population. Those surviving after 24 hours recovery time, plus an untreated control group, were then frozen till electrophoretic assays could be

performed. Three replicates for each sex were used for each treatment time - giving a total of 18 treatment vials per cage. Each treatment vial contained 20 flies. Unlike D. buzzatii, not all assays could be performed on each fly; to assay all known variable enzymes required three gels, and, with the starch gel system, the supernatant of a single fly is sufficient for only 2 gels. Thus each fly could be assayed for only two-thirds of known variable enzymes. They were assigned such that the frequency of each enzyme was based on two-thirds of the survivors and two-thirds of the controls.

Table 4.2.1. Treatment lengths for heat and cold shocks of Drosophila aldrichi

	Heat shock length (minutes)					
	AYN1(25)		AYN2(25)		AHQ2(25)	
M	170	180	130	150	150	160
F	180	190	130	150	150	160
	Cold shock length (days)					
	ANY1(25)		AYN2(25)		AHQ1(25)	
M	6	-	6	7	5.5	6.5
F	6	7	-	7	5.5	6.5

4.2.5. Cold shock of D. aldrichi

Egg samples of 3 population cages, two of "Yarrawonga" origin, AYN1(25) and AYN2(25) and one from Queensland, AHQ1(25), were used in this experiment. Treatment lengths are recorded in Table 4.2.1. In cases where more than one treatment length was used, all flies were withdrawn from the cold after the shorter period and allowed 2 hours to recover before those already dead were separated. Survivors up to that point were then returned to the shock environment for a further 24 hours, after which the final separation of dead and

alive flies was made. Three replicates for each sex and each cage were used, each replicate containing 20 flies. As for *D. aldrichi* heat shock, only two-thirds of the enzymes being assayed could be scored on an individual fly, and the final estimate of each gene frequency is based on two-thirds of the sample.

4.3. RESULTS

4.3.1. Introduction

For each electrophoresis experiment a table of χ^2 values, obtained from contingency table analysis, is presented. These tables show main treatment effects (χ^2 values for frequency differences)for alleles, for genotypes, and for comparisons of homozygotes vs. heterozygotes. They also show all interactions (e.g. for sex and/or cage) with treatment effect. For any significant main effect the frequencies from all treatments are tabulated separately. Shock treatment lengths used are recorded in Section 4.2. At the 5% level of significance used, 1 in 20 comparisons may be expected to give a significant result due solely to chance. Examination of the actual frequencies for consistency of response to shocks of differing length helps to distinguish real gene frequency changes from chance artifacts.

The initial contingency table analyses of Drosophila aldrichi gene frequencies after cold and heat shocks included Queensland origin flies with N.S.W. origin flies. The number of significant cage by treatment interactions was so great (9 out of 12 enzyme analyses) that the data was reanalysed, treating Qld. and N.S.W. origin flies separately. This reduced cage by treatment interactions involving the N.S.W. cages to two (there was only one Qld. cage).

4.3.2. Heat shock of D. buzzatii

Esterase-2 (Est-2) was the only locus of those examined that produced a significant response to heat-shock among recently eclosed flies (Table 4.3.1). However its allele frequencies (Table 4.3.2) showed no consistent trend beyond the association of the "d" allele with survival in males.

Table 4.3.1. χ^2 values from contingency table analysis of electromorph frequencies among heat shock survivors and controls of Drosophila buzzatii of "Yarrawonga" (N.S.W.) origin; flies not aged before exposure to heat shock.

	Enzyme locus					
	Est-1	Est-2	Hex	Pgm	Aldox	Adh-1
Shock response						
Alleles	3.9	16.1*	0.6	0.1	0.6	1.9
Genotypes	7.9	19.8	4.2	-	-	3.1
Het. vs Hom.	1.0	0.6	3.8	0.1	2.5	1.9
Sex x Shock response						
Alleles	7.8	14.3*	8.6*	1.7	0.5	0.2
Genotypes	11.1	20.6	8.7	-	-	1.5
Het. vs Hom.	0.5	2.3	3.1	1.8	3.4	1.1
Cage x Shock response						
Alleles	1.1	5.3	8.2*	1.6	0.8	1.6
Genotypes	2.8	15.5	8.9	-	-	5.3
Het. vs Hom.	0.5	0.0	6.0*	1.6	4.1	4.3
Cage x Sex x Shock response						
Alleles	9.4	0.5	3.3	1.0	1.5	1.0
Genotypes	10.1	10.7	4.6	-	-	1.9
Het. vs Hom.	1.2	6.4*	1.2	1.1	7.2*	0.6
Degrees of freedom						
Alleles	4	6	2	2	2	2
Genotypes	8	16	4	-	-	4
Het. vs Hom.	2	2	2	2	2	2

* $P < 0.05$

- Rare allele not present in homozygous form

Table 4.3.2. Frequencies of Esterase-2 alleles, with binomial standard errors, for heat shock survivors and controls of "Yarrawonga" (N.S.W.) origin *Drosophila buzzatii* not aged before exposure to heat shock

Sex		Shock lengths (minutes)		
		0	90	120
Males				
	Alleles			
	a	.46±.06	.44±.05	.26±.05
	b	.38±.06	.28±.04	.38±.05
	c	0	.06±.02	0
	d	.16±.04	.22±.04	.36±.05
No. flies assayed		37	52	39
Females				
	Alleles			
	a	.36±.05	.30±.05	.35±.06
	b	.34±.05	.48±.05	.35±.06
	c	.04±.02	.04±.02	0
	d	.27±.04	.18±.04	.29±.06
No. flies assayed		52	47	31

In comparison Aldehyde oxidase (Aldox), which was the only enzyme to show a significant response in flies aged for one week before heat treatment (Table 4.3.3), showed a consistent trend in allele frequencies (Table 4.3.4). The observed significant difference between the two cage populations (Table 4.3.3) can be seen to be largely a product of the difference in their gene frequencies (Table 4.3.4). Although the sample sizes are small, the binomial standard

Table 4.3.3. χ^2 values from contingency table analysis of electromorph frequencies among heat shock survivors and controls of Drosophila buzzatii of "Yarrawonga" (N.S.W.) origin. Flies aged one week before exposure to heat shock

	Enzyme locus					
	Est-1	Est-2	Hex	Pgm	Aldox	Adh-1
Shock response						
Alleles	3.4	6.2	0.6	1.7	7.7*	0.2
Genotypes	3.6	19.7	2.8	-	-	1.6
Het. vs Hom.	2.2	5.1	2.4	0.8	1.6	1.1
Sex x Shock response						
Alleles	5.1	4.5	0.9	0.1	2.4	1.6
Genotypes	9.7*	14.4	3.4	-	-	1.7
Het. vs Hom.	2.7	0.8	0.9	0.1	0.6	0.4
Cage x Shock response						
Alleles	4.4	5.1	1.6	Δ	7.5*	1.7
Genotypes	2.2	14.0	4.1	-	-	4.9
Het. vs Hom.	0.2	0.9	3.6	Δ	0.7	2.7
Cage x Sex x Shock response						
Alleles	1.2	2.5	6.3	Δ	1.1	0.6
Genotypes	3.5	9.1	7.2	-	-	0.6
Het. vs Hom.	1.7	0.5	2.8	Δ	0.1	0.1
Degrees of freedom						
Alleles	4	6	2	2	2	2
Genotypes	4	18	4	-	-	4
Het. vs Hom.	2	2	2	2	2	2

* $P < 0.05$

- Rare allele not present in homozygous form

Δ Rare allele only observed in one cage

Table 4.3.4. Frequencies of Aldehyde oxidase alleles, with binomial standard errors, for heat shock survivors and controls of "Yarrawonga" (N.S.W.) origin Drosophila buzzatii aged one week before exposure to heat shock

Cage		Shock lengths (minutes)		
		0	60	90
BYN1(25)				
	Alleles			
	a	.83±.04	.96±.03	.92±.08
	b	.17±.04	.04±.03	.08±.08
No. flies assayed		42	23	6*
BYN3(25)				
	Alleles			
	a	.99±.01	1.0	1.0
	b	.01±.01	0	0
No. flies assayed		59	51	15

*No male survivors

errors provided show that the difference between control and treatment groups is significant for cage BYN1(25). Furthermore, the 60 and 90 minute treatment survivors are not different from one another, both indicating that Aldox^a is the more heat tolerant allele.

4.3.3. Cold shock of D. buzzatii

Both Esterase-1 (Est-1) and Alcohol dehydrogenase-1 (Adh-1) showed significant responses to cold shock (Table 4.3.5). Since there were no significant interactions for alleles or genotypes in either of

Table 4.3.5. χ^2 values from contingency table analysis of electromorph frequencies among cold shock survivors and non-survivors of Drosophila buzzatii of "Yarrawonga" (N.S.W.) origin

	Enzyme locus					
	Est-1	Est-2	Hex	Pgm	Aldox	Adh-1
Shock response						
Alleles	8.2**	6.1	0.7	2.2	1.2	5.4*
Genotypes	8.2*	12.7	1.7	-	-	5.6
Het. vs Hom.	3.5	1.1	1.6	2.2	0.0	0.0
Sex x Shock response						
Alleles	0.9	6.6	2.8	0.0	3.3	2.9
Genotypes	3.1	11.6	5.6	-	-	5.4
Het. vs Hom.	2.8	3.5	0.0	0.0	6.1*	5.8*
Cage x Shock response						
Alleles	0.0	6.7	0.0	4.1*	0.6	0.1
Genotypes	0.7	3.0	1.9	-	-	0.4
Hom. vs Het.	0.0	1.3	0.2	4.2*	0.0	0.6
Emergence Day x Shock response						
Alleles	0.0	1.7	0.8	2.3	1.0	0.0
Genotypes	0.1	10.0	0.7	-	-	0.1
Hom. vs Het.	0.4	0.0	0.0	2.3	2.0	0.0
Cage x Sex x Shock response						
Alleles	0.0	6.4	0.4	2.6	0.1	3.1
Genotypes	0.1	6.2	2.1	-	-	1.2
Hom. vs Het.	0.4	0.3	0.1	2.7	0.9	1.0
Emergence Day x Sex x Shock response						
Alleles	0.2	1.7	0.5	0.0	2.4	0.0
Genotypes	0.9	7.2	0.5	-	-	3.0
Hom. vs Het.	0.0	0.5	1.8	0.0	1.4	3.4

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	Enzyme locus					
	Est-1	Est-2	Hex	Pgm	Aldox	Adh-1
Emergence Day x Cage x Shock response						
Alleles	1.3	3.3	2.5	1.4	0.0	0.2
Genotypes	1.6	6.2	4.1	-	-	0.4
Hom. vs Het.	1.0	1.0	4.0*	1.4	0.1	1.1
Emergence Day x Cage x Sex x Shock response						
Alleles	0.0	2.3	0.1	1.7	0.0	3.3
Genotypes	0.3	5.7	0.7	-	-	3.5
Hom. vs Het.	0.3	2.0	0.0	1.7	0.0	1.6
Degrees of freedom						
Alleles	1	3	1	1	1	1
Genotypes	2	8	2	-	-	2
Hom. vs Het.	1	1	1	1	1	1

* $P < 0.05$

** $P < 0.01$

\- Rare allele not present in homozygous form

these enzymes they appear to be more than chance effects. The significant result for genotypes of Est-1 is a reflection of the change in allele frequencies, as the cold shock survivors and non-survivors (Table 4.3.6) are in Hardy-Weinberg equilibrium. Table 4.3.6 shows that $Est\text{-}1^b$ is associated with cold shock survival. Similarly $Adh\text{-}1^c$ appears to be associated with cold survival (Table 4.3.7).

4.3.4. Heat shock of D. aldrichi

Among "Yarrawonga" origin flies, 4 enzyme loci showed significant differences in electromorph frequencies between heat shock survivors and untreated controls (Table 4.3.8). Of these, Esterase-D (Est-D) and Esterase-F (Est-F) showed no consistent response to treatment (Table 4.3.9 and Table 4.3.10), suggesting that the observed significance may have been due mainly to chance. In contrast, the consistent increase in the frequency of the β-n-acetyl-hexosaminidase (Hex) "c" allele (Table 4.3.11) with increased duration of heat shock indicates a link between Hex and high temperature tolerance. The standard errors indicate that, while the treated groups differ significantly from the control, they are not significantly different from one another. The fourth locus, Leucine aminopeptidase (Lap), showed a significant interaction, for allele frequencies, of sex with treatment (Table 4.3.8). It may be seen in Table 4.3.12 that this interaction was mainly due to a low Lap^b frequency in female controls. The observed differences between males and females are therefore likely to have been caused by sampling error. The strength of the shock response (Table 4.3.8) is however such that there is probably a real effect of heat shock even after correcting for the sampling error.

Table 4.3.6. Frequencies of Esterase-I alleles and genotypes, with binomial standard errors, for cold shock survivors and non-survivors of "Yarrawonga" (N.S.W.) origin *Drosophila buzzatii*; 5 day shock

Result Category	Dead	Alive
Alleles		
a	.38±.03	.27±.03
b	.62±.03	.73±.03
Genotypes		
aa	.15±.03	.09±.02
ab	.46±.04	.35±.04
bb	.39±.04	.56±.04
No. flies assayed	150	149

Table 4.3.7. Frequencies of Alcohol dehydrogenase-1 alleles, with binomial standard errors, for cold shock survivors and non-survivors of "Yarrawonga" (N.S.W.) origin *Drosophila buzzatii*; 5 day shock

Result Category	Dead	Alive
Alleles		
b	.54±.03	.44±.03
c	.46±.03	.56±.03
No. flies assayed	143	145

Table 4.3.8. χ^2 values from contingency table analysis of electromorph frequencies among heat shock survivors and controls of "Yarrawonga" (N.S.W.) origin *Drosophila aldrichi*

	Enzyme locus					
	Est-D	Est-F	Hex	Pgm	Mdh	Lap
Shock response						
Alleles	10.5**	19.9*	9.5*	0.8	2.6	16.0***
Genotypes	11.0*	34.6	17.3	3.7	-	15.3**
Het. *vs* Hom.	6.1*	4.4	1.8	2.1	2.5	5.1
Sex x Shock response						
Alleles	0.7	Δ	7.4	0.5	0.9	7.1*
Genotypes	2.9	Δ	15.2	1.4	-	7.2
Het. *vs* Hom.	1.9	Δ	0.7	0.5	1.7	4.8
Cage x Shock response						
Alleles	14.5***	8.3	1.8	4.0	1.4	1.4
Genotypes	15.4**	25.6	10.3	5.7	-	1.9
Het. *vs* Hom.	8.8*	0.7	0.6	1.4	0.9	0.5
Cage x Sex x Shock response						
Alleles	2.0	Δ	2.1	0.9	0.2	0.1
Genotypes	10.7*	Δ	5.7	6.2	-	2.2
Het. *vs* Hom.	10.5**	Δ	1.1	3.6	0.7	1.8
Degrees of freedom						
Alleles	2	8	4	2	2	2
Genotypes	4	28	10	4	-	4
Het. *vs* Hom.	2	2	2	2	2	2

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

\- Rare allele present only in heterozygous form

Δ Not present in females

Table 4.3.9. Frequencies of Esterase-D alleles, genotypes and homozygotes vs heterozygotes, with binomial standard errors, for heat shock survivors and controls of "Yarrawonga" (N.S.W.) origin *Drosophila aldrichi*

Result Category	Cage AYN1(25)			Cage AYN2(25)		
	Control	Shock* length 1	Shock* length 2	Control	Shock* length 1	Shock* length 2
Alleles						
a	.79±.03	.80±.03	.73±.05	.55±.03	.76±.04	.75±.04
b	.21±.03	.20±.03	.27±.05	.45±.03	.24±.04	.25±.04
Genotypes						
aa	.60±.05	.61±.05	.49±.07	.29±.05	.62±.06	.59±.07
ab	.39±.05	.38±.05	.49±.07	.53±.05	.28±.05	.32±.06
bb	.01±.01	.01±.01	.02±.02	.18±.04	.10±.04	.09±.04
Heterozygotes	.39±.05	.38±.05	.49±.07	.53±.05	.28±.05	.32±.06
Homozygotes	.61±.05	.62±.05	.51±.07	.47±.05	.72±.05	.68±.06
No. flies assayed	104	94	45	101	68	56

*Heat shock lengths in Table 4.2.1

Table 4.3.10. Frequencies of Esterase-F alleles, with binomial standard errors, for heat shock survivors and controls of "Yarrawonga" (N.S.W.) origin *Drosophila aldrichi*

	Control	Shock* length 1	Shock* length 2
Alleles			
a	.07±.02	.17±.03	.04±.02
b	.29±.03	.21±.03	.33±.05
c	.26±.03	.25±.04	.25±.05
d	.15±.03	.09±.02	.11±.04
e	.23±.03	.29±.04	.28±.05
No flies assayed	89	75	38

*Heat shock lengths in Table 4.2.1

Table 4.3.11. Frequencies of β-n-acetyl-hexosaminidase alleles, with binomial standard errors, for heat shock survivors and controls of "Yarrawonga" (N.S.W.) origin *Drosophila aldrichi*

	Control	Shock* length 1	Shock* length 2
Alleles			
a	.36±.02	.30±.03	.26±.03
b	.15±.02	.17±.02	.15±.02
c	.49±.02	.54±.03	.59±.03
No. flies assayed	225	154	106

*Heat shock lengths in Table 4.2.1

Table 4.3.12. Percentages of Leucine aminopeptidase alleles and genotypes, with binomial standard errors, for heat shock survivors and controls of "Yarrawonga" (N.S.W.) origin Drosophila aldrichi

Sex	Control	Shock* length 1	Shock* length 2
Males			
Alleles			
b	.77±.03	.81±.03	.78±.05
c	.23±.03	.19±.03	.22±.05
No. flies assayed	115	64	37
Females			
Alleles			
b	.69±.03	.88±.03	.87±.04
c	.31±.03	.12±.03	.13±.04
No. flies assayed	109	80	45
Males and Females			
Genotypes			
bb	.54±.03	.72±.04	.68±.05
bc	.37±.03	.26±.04	.29±.05
cc	.09±.02	.03±.01	.02±.02
No. flies assayed	224	144	82

*Heat shock lengths in Table 4.2.1

The significant genotype frequency variations (Table 4.3.8) are almost certainly a product of gene frequency changes, as the treated groups (Table 4.3.12) are in Hardy-Weinberg equilibrium. Lap^b favoured survival, and the two treatment groups were not different.

For Hemmant origin Drosophila aldrichi (Table 4.3.13), two enzymes showed significant differences between treated and control groups. Hex showed a significant increase in homozygotes among survivors of the longer treatment (Table 4.3.14). Although Malate dehydrogenase (Mdh) allele frequencies were not significantly different (Table 4.3.13), they are presented with the genotype frequencies in Table 4.3.15. A large difference in Mdh^b frequency after the shorter treatment (Table 4.3.15) was also reflected in the "bb" genotype frequency, although there was no deviation from Hardy-Weinberg equilibrium. The lack of consistency across treatments for both these enzymes suggests that chance effects are the most probable cause of the observed significant results.

Thus among Drosophila aldrichi, the clearest evidence of a link between particular electromorphs and heat shock tolerance came from Hex, with some evidence for a similar effect at the Lap locus, in flies of "Yarrawonga" origin.

4.3.5. Cold shock of D. aldrichi

Only the male-specific Est-F showed significant electromorph frequency differences between dead and surviving flies of "Yarrawonga" origin (Table 4.3.16). Allele frequencies are presented in Table 4.3.17, but, as implied by the presence of a significant cage by treatment interaction (Table 4.3.16), there is no consistent response across cages. Therefore this result is best treated as a chance

Table 4.3.13. χ^2 values from contingency table analysis of electromorph frequencies among heat shock survivors and controls of Hemmant (Qld.) origin *Drosophila aldrichi*

	Enzyme locus					
	Est-C	Est-D	Hex	Pgm	Mdh	Lap
Shock response						
Alleles	1.5	5.6	6.9	3.1	4.5	1.9
Genotypes	18.5	6.1	14.5	-	14.2**	2.1
Het. *vs* Hom.	1.6	2.0	7.7*	5.3	0.6	1.3
Sex x Shock response						
Alleles	3.2	1.0	5.8	1.2	0.3	1.2
Genotypes	11.8	4.1	10.8	-	0.9	3.0
Het. *vs* Hom.	0.2	1.9	2.0	0.7	0.9	2.5
Degrees of freedom						
Alleles	6	2	4	2	2	2
Genotypes	16	4	10	-	4	4
Het. *vs* Hom.	2	2	2	2	2	2

* P < 0.05

** P < 0.01

\- Rare allele not present in homozygous form

Table 4.3.14. Frequencies of β-n-acetyl-hexosaminidase heterozygotes and homozygotes, with binomial standard errors, for heat shock survivors and controls of Hemmant (Qld.) origin *Drosophila aldrichi*

	Shock lengths (minutes)		
	0	150	160
Heterozygotes	.54±.05	.58±.08	.30±.08
Homozygotes	.46±.05	.42±.08	.70±.08
No. flies assayed	102	38	37

Table 4.3.15. Frequencies of *Malate dehydrogenase* genotypes, with binomial standard errors, for heat shock survivors and controls of Hemmant (Qld.) origin *Drosophila aldrichi*

	Shock lengths (minutes)		
	0	150	160
Alleles			
a	.85±.02	.75±.05	.84±.04
b	.15±.02	.25±.05	.16±.04
Genotypes			
aa	.72±.04	.63±.08	.67±.07
ab	.27±.04	.25±.07	.33±.07
bb	.01±.01	.12±.05	0
No. flies assayed	103	40	40

Table 4.3.16. χ^2 values from contingency table analysis of electromorph frequencies among cold shock survivors and non-survivors of "Yarrawonga" (N.S.W.) origin Drosophila aldrichi

	Enzyme locus					
	Est-D	Est-F	Hex	Pgm	Mdh	Lap
Shock response						
Alleles	3.9	16.2*	6.0	4.3	0.5	0.1
Genotypes	6.7	36.6*	13.8	4.7	-	2.0
Het. vs Hom.	1.2	4.2	1.0	3.0	0.5	0.8
Sex x Shock response						
Alleles	1.1	Δ	9.1	1.2	1.3	3.0
Genotypes	5.3	Δ	15.1	4.3	-	4.3
Het. vs Hom.	1.9	Δ	2.5	3.1	1.3	2.6
Cage x Shock response						
Alleles	4.9	19.2*	2.9	2.6	0.9	4.3
Genotypes	7.9	28.6	21.6*	4.0	-	6.9
Het. vs Hom.	1.1	1.0	4.2	1.7	1.0	5.3
Cage x Sex x Shock response						
Alleles	0.7	Δ	3.4	0.8	1.6	1.5
Genotypes	2.3	Δ	11.4	6.1	-	6.4
Het. vs Hom.	1.9	Δ	3.7	0.2	1.7	6.1*
Degrees of freedom						
Alleles	2	8	4	2	2	2
Genotypes	4	22	10	4	-	4
Het. vs Hom.	2	2	2	2	2	2

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

\- Rare allele not present in homozygous form

Δ Not present in females.

Table 4.3.17. Frequencies of Esterase-F alleles, with binomial standard errors, for cold shock survivors and non-survivors of "Yarrawonga" (N.S.W.) origin Drosophila aldrichi

Cage		Dead 6†	Dead 7†	Alive*
AYN1(25)				
	Alleles			
	a	.08±.03	-	.07±.03
	b	.36±.05	-	.24±.06
	c	.35±.05	-	.28±.06
	d	0	-	.02±.02
	e	.21±.05	-	.40±.06
No. flies assayed		40	-	29
AYN2(25)				
	Alleles			
	a	.08±.04	.02±.03	.05±.02
	b	.45±.08	.36±.10	.40±.05
	c	.21±.07	.39±.10	.32±.04
	d	0	0	.07±.02
	e	.26±.07	.22±.09	.16±.03
No. flies assayed		38	22	59

*Shock treatment terminated after 6 days for AYN1(25), 7 days for AYN2(25)

†Cold shock duration, days

occurrence. The frequencies of the 15 genotypes at this locus are not tabulated, since the low numbers of each genotype, and the small differences in frequencies between survivors and non-survivors, combine to make interpretation difficult. The low numbers also make tests for Hardy-Weinberg equilibrium meaningless.

Among Hemmant (Qld.) origin flies, cold shock survivors and non-survivors displayed highly significant differences for both allele and genotype frequencies of Est-D, and a significant response to treatment for the ratio of homozygotes to heterozygotes of Phosphoglucomutase (Pgm) (Table 4.3.18). Although there were interactions with sex recorded for Est-D, examination of Table 4.3.19 shows that the difference in the response of the sexes was in magnitude, not direction. The only exception was the "ab" heterozygotes, but the standard errors suggest that this was not significant. Both allele and genotype results point to Est-D^{a} showing cold tolerance relative to Est-D^{b}. All samples were in Hardy-Weinberg equilibrium, implying that the observed selection was at the allele level. While Table 4.3.20 suggests heterozygote superiority in Pgm, with no homozygotes present for the low frequency "c" allele it is impossible to test whether this is true heterozygote advantage, or selection for Pgm^{c}. It should be noted that Pgm allele frequencies of alive and dead flies were significantly different at the 10% level (Table 4.3.18).

In summary, although enzymes from "Yarrawonga" Drosophila aldrichi did not show any response to cold shock, for Hemmant flies both Est-D and Pgm showed evidence of selection by cold shock.

Table 4.3.18. χ^2 values from contingency table analysis of electromorph frequencies among cold shock survivors and non-survivors of Hemmant (Qld.) origin *Drosophila aldrichi*

	Enzyme locus					
	Est-C	Est-D	Hex	Pgm	Mdh	Lap
Shock response						
Alleles	6.1	18.9***	1.4	5.8	1.1	2.3
Genotypes	21.7	17.5**	3.5	-	1.6	-
Het. *vs* Hom.	3.2	0.1	0.4	6.3*	1.2	0.0
Sex x Shock response						
Alleles	18.2**	9.6**	4.5	0.9	0.8	2.9
Genotypes	25.7	10.6*	14.1	-	1.6	-
Het. *vs* Hom.	0.1	1.9	2.2	1.0	0.8	4.8
Degrees of freedom						
Alleles	6	2	4	2	2	2
Genotypes	18	4	10	-	4	-
Het. *vs* Hom.	2	2	2	2	2	2

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

\- Rare allele not present in homozygous form

Table 4.3.19. Frequencies of Esterase-D alleles and genotypes, with binomial standard errors, for cold shock survivors and non-survivors of Hemmant (Qld.) origin *Drosophila aldrichi*

	Male			Female		
Category	Dead 5.5*	Dead 6.5*	Alive 6.5*	Dead 5.5*	Dead 6.5*	Alive 6.5*
Alleles						
a	.45±.06	.45±.04	.60±.04	.33±.05	.55±.05	.61±.06
b	.55±.06	.55±.04	.40±.04	.67±.05	.45±.05	.39±.06
Genotypes						
aa	.20±.07	.23±.04	.41±.06	.11±.04	.28±.07	.34±.08
ab	.50±.09	.42±.05	.38±.06	.43±.07	.53±.08	.53±.08
bb	.30±.08	.35±.05	.21±.05	.45±.07	.19±.06	.13±.05
No. flies assayed	30	90	61	53	43	38

*Cold shock duration, days

Table 4.3.20. Frequencies of Phosphoglucomutase heterozygotes and homozygotes, with standard errors, for cold shock survivors and non-survivors of Hemmant (Qld.) origin *Drosophila aldrichi*

	Dead 5.5*	Dead 6.5*	Alive 6.5*
Heterozygotes	.07±.03	.16±.03	.20±.03
Homozygotes	.93±.03	.84±.03	.80±.03
No. flies assayed	84	167	138

*Cold shock duration, days

4.4. DISCUSSION

The results presented for *Drosophila buzzatii* in this chapter, while supporting the hypothesis of selection for particular electromorphs by temperature, do not entirely match the conclusions drawn from the analyses of Mulley *et al.* (1979). For example the *Est-2*^{c} allele, which shows the strongest associations with environmental variables of any allele at any locus in the results of Mulley *et al.*, was at too low a frequency in the four cage populations sampled for the experiments of this chapter to show any significant changes. In fact in a number of samples its frequency was zero (e.g. Table 4.3.2).

On the positive side, *Est-2*^{d}, found by Mulley *et al.* (1979) to be associated with high summer maximum temperature, was shown in Section 4.3.2 to be related to survival of heat shock, at least in males. *Est-1*^{b} was found in Section 4.3.3 to be associated with cold shock survival, while Mulley *et al.* found a positive association of this allele with latitude; that is, its frequency increased in the southern, or colder, part of *D. buzzatii's* distribution. Less directly, *Adh-1*^{b} was found to be negatively associated with latitude (increased to the North) by Mulley *et al.*, while the "c" allele was shown in Section 4.3.3 to favour cold shock survival. The only other significant result from this chapter involving *D. buzzatii* was the association of *Aldox*^{a} with heat shock survival. Mulley *et al.* (1979) found this allele to be positively associated with latitude, which would be expected to imply association with cold rather than heat.

For *D. aldrichi*, esterases (*Est-D* and *Est-F*) showed a number of significant associations, but only one consistent response; *Est-D*^{a} was elevated in frequency among survivors of cold shock in the

Queensland cage samples. The only other significant association with cold survival was for heterozygotes of Pgm, also from the Queensland population. Under heat stress, two enzyme loci showed significant responses; in the New South Wales populations, Hex^c and Lap^b were associated with survival, and in the Queensland population Hex homozygotes increased in frequency among survivors.

The use (in most cases) of two treatment lengths, was an attempt to find the shock treatment producing the largest differences, if any, between the survivors and controls (for heat shock) or non-survivors (for cold shock). For example, if after heat shock too small a proportion was killed, any change in gene frequency among the survivors may not have been observable, due to inevitable variations in the initial frequencies of all samples. If too many were killed, the standard errors of the survivor's gene frequencies may have been too large to permit the observation of significant differences. An added factor is that, as shown in Chapter 3, the sexes may have differing LT50's.

In the D. buzzatii results there were no obvious examples of the above scenarios; however for D. aldrichi two cases of interest may be noted. For "Yarrawonga" origin flies, the differences in Hex frequencies between the control and the shorter heat shock are of borderline significance (Table 4.3.11), while the survivors of the longer heat shock are quite clearly significantly different to the controls. After cold shock of Hemmant origin flies, the two groups of dead males do not differ for Est-D frequencies (Table 4.3.19), although they do differ significantly from the survivors, while females that died during the shorter period differed significantly from both those that died during the longer shock and from survivors.

Note that the percentage dead after each time interval implies that male D. aldrichi from Queensland are more cold tolerant than females, a trend supported by results presented in Table 3.3.3.

Thus it seems clear that the use of (at least) two treatment times can increase the chance of identifying repeatable gene frequency changes among temperature stress survivors.

Despite these observations of associations between extreme temperature survival and gene frequency changes, for many enzymes it may well be fruitless to speculate as to their possible roles in temperature tolerance. Wills et al. (1975) comment pointedly that it is "improbable that the marker alleles which these workers just happen to have the capability of detecting are the ones responsible for the observed selective effect". Further, Mulley et al. (1979) recorded that, in D. buzzatii, Est-1, Est-2 and Aldox (all of which showed significant associations with temperature shock) are on the second chromosome, which carries the only inversions known in Australian D. buzzatii populations. This increases the probability of linkage of these loci to others directly involved in temperature tolerance. Thus, until proven otherwise, an electromorph should really be viewed in rather the same light as a chromosome inversion - as a way of observing selection (or its absence) on a closely linked block of loci.

An indication that a particular enzyme locus or class of loci is more than a marker for other loci is the significant involvement of that enzyme locus or class in thermal tolerance across more than one species. In Section 4.3 esterase loci showed significant responses to temperature shock in both D. buzzatii and D. aldrichi. Perhaps even more interestingly, Alcohol dehydrogenase,

found in Section 4.3.3 to be involved in the survival by D. buzzatii of cold shock, was shown by Johnson and Powell (1974) to be associated with both heat and cold shock survival in D. melanogaster. These findings support the case for biochemical studies into the involvement of alcohol dehydrogenase and the esterases in extreme temperature survival.

CHAPTER 5

THE EFFECT OF REARING TEMPERATURE ON CHROMOSOME INVERSION FREQUENCIES

5.1. INTRODUCTION

In a series of papers, Theodosius Dobzhansky showed that the frequency of chromosome inversions varied between populations, and that this variation could be correlated with climatic differences (e.g. Dobzhansky 1943). He then showed, using population cages (e.g. Wright and Dobzhansky 1946), that the frequencies of chromosome inversions were influenced by temperature in *Drosophila pseudoobscura*.

The technique of using population cages to study selective effects has been used since in many different ways. For instance Powell (1973) compared changes in allele frequencies of three *Drosophila willistoni* enzyme loci in population cages kept at 25°C or 19°C. He was unable to determine whether selection observed was really at loci scored, or due to selection for chromosome inversions with which selected loci were associated.

While establishing the (biochemical) reasons for changes due to selection in enzyme allele frequencies is potentially simpler, the selection coefficients for blocks of loci associated with inversions are potentially much larger. Thus the task of establishing that selection does occur, in response to a particular stress, may be much simplified by first examining chromosome inversions. This chapter compares the chromosome inversion frequencies of *Drosophila buzzatii* drawn from population cages maintained at 25°C, against those drawn from sister cage populations maintained at 18°C.

5.2. MATERIALS AND METHODS

The chromosome inversions considered are both on the second chromosome of D. buzzatii, and are the only inversions known in Australia for this species. The more common is the "2j" inversion, mentioned in Section 3.2.5. In some populations, the 2j inversion has a higher frequency than the standard arrangement. The other chromosome inversion, designated "$2z^3$" by Carson and Wasserman (1965), was first identified in an Australian population by the present author in 1975. At present it has been detected only in the "Yarrawonga" (Hunter Valley, New South Wales) population (locality 5, Barker and Mulley (1976)).

The source material for this experiment was the twelve "Yarrawonga" population cages (6 maintained at 25°C, and 6 daughter/replicates maintained at 18°C) referred to in Sections 2.2 and 3.2.4. All cages were transferred to the University of New England (Armidale) late in 1979. Egg-samples for this experiment were made by Mr. P.D. East in April 1980, according to the method of Section 2.2, and mailed to Sydney for cytological examination. Salivary glands of third instar larvae were dissected out and squashed in orcein stain according to the method of Wasserman (1954). To prevent all larvae simultaneously reaching the required stage for dissection, some vials were temporarily stored at 18°C to retard development. Storage temperature otherwise was 25°C. Fifty larvae from each cage were scored, providing a sample of 100 second chromosomes per cage. All karyotypes (3 homokaryotypes and 3 heterokaryotypes) were distinguishable and were scored separately. Contingency table analysis (described in Section 4.2.1) was used to test for differences between the cages in inversion frequencies. As established in Section 3.2.5, the designation "st" is used for standard, and "j" for the 2j inversion. In addition, the designation "z" will be used for the $2z^3$ inversion.

5.3. RESULTS

Contingency table analysis revealed significant differences in chromosome arrangement frequency between parent/daughter pairs of population cages (Table 5.3.1). However, there was a highly significant interaction of cage of origin with maintenance temperature. The actual frequencies of the chromosome arrangements are presented in Table 5.3.2. From this table it may be seen that cages BYN1(25), BYN2(25) and BYN6(25) generally showed an increase in j frequency, and a decrease in st frequency, over their 18°C counterparts. In the other three cage pairs, the differences for both the j and st frequencies were generally not as large as were their standard errors. In three cage pairs the z inversion was at a higher frequency in 25°C cages than in 18°C cages. However it was not found at all in BYN4(25), BYN5(25) and BYN6(25). Thus the only pattern to emerge is for higher j frequencies and lower st frequencies in the 25°C cages.

Table 5.3.1. χ^2 values from contingency table analysis of chromosome arrangement frequencies in _Drosophila buzzatii_; samples from 12 population cages, 6 maintained at 25°C and the other 6 at 18°C

	χ^2	df
Cage maintenance temperature	10.6^{**}	2
Cage maintenance temperature vs. Cage of origin	47.3^{**}	10

$^{**}P < 0.01$

Table 5.3.2. Chromosome arrangement frequencies in *Drosophila buzzatii* 25°C and 18°C population cages, with binomial standard errors

Cage of origin	Gene Arrangement Frequencies st	2j	$2z^3$	Sample Size
BYN1(25)	.16±.04	.70±.05	.14±.03	100
BYN1(18)	.30±.05	.66±.05	.04±.02	100
BYN2(25)	.18±.04	.75±.04	.07±.03	100
BYN2(18)	.36±.05	.62±.05	.02±.01	100
BYN3(25)	.37±.05	.54±.05	.09±.03	100
BYN3(18)	.39±.05	.58±.05	.03±.02	100
BYN4(25)	.21±.04	.79±.04	0	100
BYN4(18)	.13±.03	.81±.04	.06±.02	100
BYN5(25)	.14±.03	.86±.03	0	100
BYN5(18)	.10±.03	.86±.03	.04±.02	100
BYN6(25)	.05±.02	.95±.02	0	100
BYN6(18)	.29±.05	.69±.05	.02±.01	100

5.4. DISCUSSION

In the results presented in Section 5.3, the observed interaction with cage of origin for gene arrangement frequency (Table 5.3.1) could, at least in part, be due to genetic drift. For the 25°C cage populations, the absence of the z inversion from three of six samples (Table 5.3.2) appears to be a classic illustration of the effects of drift, as the z frequency in the remaining three 25°C cages was higher (with a mean of 10%) than in any 18°C population. The observation of this gene arrangement in all six 18°C cages proves that it was initially present in all 25°C cages, as the 18°C cages were derived from the 25°C cages.

On the other hand, when one looks at the results for the j and st arrangements (Table 5.3.2), there is some evidence for selection. Virtually all large differences between cage pairs involve high j (and low st) in the 25°C cage. In cases where j is at a higher frequency in the 18°C cage, the difference is never greater than the standard errors of the frequencies being compared.

It may be seen from Table 3.4.2 and Table 5.3.2 that the j inversion frequencies observed in the "Yarrawonga" origin cage populations are relatively high in comparison to those found in wild populations. It was argued in Section 3.4 that Table 3.4.2 gave evidence of an increase from north to south in j frequency among wild populations, and that a possible explanation for the observed cline was a decrease in mean temperature from north to south. However if temperature was the only factor affecting chromosome inversion frequencies in laboratory populations, then the 18°C cages would be expected to have higher j frequencies than the corresponding 25°C cages. Thus it seems likely that factors other than temperature are affecting inversion frequencies in the cage populations.

Given that D. buzzatii continue development over a relatively wide range of temperatures (Chapter 2), and that adults and larvae may be collected year-round in the N.S.W. Hunter Valley (Barker et al. 1986), despite temperatures ranging from below 0°C in winter to above 40°C in summer, it seems unlikely that either 18°C or 25°C would be severe stress temperatures for this species. It may thus be expected that direct effects of temperature on the cage populations would be difficult to observe, particularly over the short term.

However, there is qualitative evidence that differences exist between the 25°C and 18°C laboratory environments. For example, larvae in a vial at 18°C did not work the medium as effectively as an equal number of larvae reared at 25°C. The medium was also more subject to drying out at 18°C during the longer larval development time. Together, these factors produced a quite different physical appearance in the medium, and presumably some nutritional variation. Other such differences were observed in the progression of both bacterial and mould infections of the medium, and of mite infestations in the cages. It even became necessary to periodically transfer 18°C vial stocks to the 25°C room to avoid extinction of the line. Thus, differences in chromosome arrangement frequencies between 25°C and 18°C cages may be indirectly related to temperature through temperature-dependant environmental differences.

In a classic paper, Wright and Dobzhansky (1946) proposed heterozygote advantage to explain the changes in gene arrangement frequencies in populations of Drosophila pseudoobscura maintained at 25°C. It is now believed that, for D. pseudoobscura, 25°C actually constitutes a stress (Parsons, 1973). Assuming for the moment that the 18°C laboratory cage environment does apply stress to D. buzzatii,

it could be hypothesized that there will be heterozygote advantage under those conditions. The design of this experiment did not, however, permit testing of this hypothesis.

The problem that remains is that, even though a single set of observations may be able to demonstrate that changes in frequency have occurred, it is not possible to distinguish between oscillation of frequencies about new equilibria and approach to fixation of one arrangement. Using the minimum generation intervals deduced in Chapter 2, the maximum number of generations that may have been completed since establishment of the 18°C cages is 43 for 25°C populations and 17 for the 18°C populations. It is therefore possible that the j arrangement is approaching fixation in all populations, and that it is higher in a majority of 25°C populations only because of the larger number of generations completed at that temperature.

CHAPTER 6

GENERAL DISCUSSION

6.1. RECAPITULATION OF RESULTS

The preceding four chapters represent a diversity of experimental approaches to both population genetics and ecology, within two species of cactophilic *Drosophila*; *D. buzzatii* and *D. aldrichi*. The common thread through all four experiments is the investigation of the response to different temperatures by these poikilothermic insects.

In the first experiment (Chapter 2) the emphasis was perhaps more on ecology than genetics. The aim was to quantify developmental rates of *D. buzzatii*. Egg samples were taken from two *D. buzzatii* population cages, and were used to compare the developmental speeds of all major life cycle stages at a range of temperatures. Since both population cages were established from the same source material only two years prior to the experiment, genetic differences were not expected to be large. However there was a tendency for later pupation among larvae from one cage (in one case this was significant at 10°C), and earlier eclosure (only apparent at 10°C) of pupae from the same cage.

Ecological information obtained from the experiments recorded in Chapter 2 suggests that *D. buzzatii* continues normal development over a broader range of temperatures than either tropical or cosmopolitan species. This result was not surprising, given the wide distribution of this species documented by Carson and Wasserman (1965), Barker (1982), Fontdevila *et al.* (1981), Fontdevila *et al.* (1982), and Barker *et al.* (1985).

The second group of experiments (Chapter 3) explored the relative temperature tolerance, at extreme high and low temperatures, of genetically differing groups of adult cactophilic Drosophila. Comparisons were made both within and between species. The most dramatic differences observed were between the two species, D. buzzatii and D. aldrichi, and between populations of D. aldrichi, for high temperature shock. The two species were not distinguishable by cold shock. Comparisons between cage populations, and between chromosome inversion lines, of D. buzzatii did not reveal strong, repeatable differences in survival of extreme temperatures, although there was evidence that the line homozygous for the standard chromosome arrangement may have survived longer at high temperatures. Also the heterozygote between the 2j chromosome inversion and the standard arrangement did show some tendency to higher cold tolerance than the other lines.

The method of heat and cold shocks was also used in Chapter 4, as a way of testing for differential survival of enzyme electromorphs. In both species, some allozymes showed significant associations with extreme temperature shock. For D. buzzatii, frequency changes at esterase loci were associated with both heat and cold shocks. Associations of Aldehyde oxidase with heat shock, and Alcohol dehydrogenase-1 with cold shock were also noted. In D. aldrichi, four loci showed significant associations with temperature shock; Esterase-D and Phosphoglucomutase with cold shock, and β-n-acetyl-hexosaminidase and Leucine aminopeptidase with heat shock.

The final experiment, Chapter 5, compared the chromosome makeup of 6 pairs of population cages. One member of each cage pair had been maintained at 25°C, the other at 18°C. The results were highly heterogeneous, due largely to the loss of the '$2z^3$' inversion

from 3 cages. There was evidence that the frequency of the j arrangement was higher in 25°C cages than in their 18°C daughter-replicates, and that selection was a factor in the divergence between cage pairs. However it was really not possible to tell from the single sample presented whether the cages were oscillating about eventual equilibrium points, or whether the j arrangement was approaching fixation in all cages, the faster generation rate at 25°C accounting for the usually higher j frequency in 25°C cages than in 18°C cages.

6.2. THE KNOWN ECOLOGY OF D. BUZZATII AND D. ALDRICHI

The two members of the mulleri subgroup studied in the preceding chapters, *Drosophila buzzatii* and *D. aldrichi*, apparently co-exist in the cactus rot habitat. Adults of both species have only been trapped from the vicinity of cactus plants, and have been observed eclosing from the same rots (Mulley and Barker 1977). In the N.S.W. Hunter Valley (locality 5, Barker and Mulley (1976)), both species reach a population peak in autumn, following the early autumn breeding season of the moth *Cactoblastis cactorum*, which initiates the cactus rots (Barker *et al*. 1986).

However the ecology of the two species is not identical. *D. buzzatii* displays a spring population peak in the Hunter Valley not shared by *D. aldrichi* (Barker *et al*. 1986), *D. buzzatii* has a wider distribution than *D. aldrichi* (Barker 1982) and, as shown in Chapter 3, *D. buzzatii* has lower heat tolerance and less strict nutritional requirements than *D. aldrichi*.

While Taylor and Powell (1977) found significant genetic heterogeneity in collections of *D. persimilis* from different habitats within a small area, in *D. buzzatii* such variation between adjacent habitats was observed at only two loci of six examined by Barker *et al*. (1986). Taylor and Powell (1977) attributed the observed genetic variation to different habitat preferences of the different genotypes. Barker *et al*. (1986) hypothesised that *D. buzzatii* may also exercise habitat selection, but at the individual rot level. They considered that this may be important in the maintenance of polymorphism.

At first glance the cactus niche does not appear to provide much habitat variation, although it is true that there are differences in climate and in prickly pear species across the Australian distribution of *D. buzzatii*. However, Barker *et al*. (1984) tabled a

list of 42 yeast species collected from Australian *Opuntia* rots. They found that, on average, there were less than three yeast species per rot, although the number of species increased with the estimated age of the rot. Cactus rots also contain bacteria. No attempt has yet been made to identify the bacterial species found in rots, but Vacek (1982) showed that a bacterial community derived from a number of rots was, for rearing larvae, nutritionally at least the equal of any of eight yeasts collected from cactus rots. In fact the bacterial communities in individual rots may well be as variable in species composition and nutritional qualities as the yeasts have been shown to be. Alcohol and ion concentrations have also been found to vary between rots, and to have associations with allozyme variation (Barker 1982).

The observations by Barker *et al.* (1981a), Barker *et al.* (1981b), Vacek (1982), and Vacek *et al.* (1986) of differential yeast attractivity, and by Barker *et al.* (1981b) of genetic differences (at one locus) between flies attracted to different yeasts are strong evidence that habitat selection takes place in both *D. buzzatii* and *D. aldrichi*. At present, no experiments have been performed to determine the preferences of either species for other components of environment such as light, humidity, temperature or pH. Certainly differences in temperature preference would seem likely, particularly between the species, but probably within species as well, given the observed variations in heat tolerance (Chapter 3). In the natural environment, however, variations in temperature tolerance among adults would be more likely to be expressed as variations in the number of hours per day suitable for seeking out new rots, feeding and oviposition. Thus *D. aldrichi* might be expected to have greater opportunity in hot

conditions to exercise habitat selection than *D. buzzatii*. If the observed difference in heat tolerance between adults of the two species is reflected in their larvae, then a degree of niche separation may be imposed during summer, as *D. aldrichi* larvae would be able to utilize rots too hot for *D. buzzatii*.

Another facet of the *Drosophila* life cycle affected by temperature is the generation interval (Section 2.4). For *D. buzzatii*, and presumably *D. aldrichi*, this is also affected by diet. Vacek (1982) found that development time from egg to adult of *D. buzzatii* varied from 15 days if bacteria was included in the diet, to 28 days for one of eight yeasts tested.

The temperature at which the above elapsed times were measured was not mentioned by Vacek, but, if it was a constant 25°C (as in experiments reported by Vacek *et al.* (1986)), then the laboratory medium used in Chapter 2 actually permitted faster development (by a minimum of 3 days) than the 'natural' media tested by Vacek (1982).

In Chapter 2 it was shown that the period from adult eclosure to commencement of oviposition was 2 days at 25°C and 7 days at 18°C. Let us assume that this maturation time would remain constant, regardless of diet. The generation interval under natural conditions might therefore be expected to lie between 17 and 30 days, given a mean temperature of 25°C. If the ratio between developmental speed at 25°C and at 18°C remains constant across diets, then, using the 18°C generation interval derived on laboratory medium in Section 2.4, the equivalent periods for the media used by Vacek would range from 42 to 70 days.

This increase in generation intervals as compared to the results of Chapter 2 gives added weight to the hypothesis put forward in Section 2.4 that, in the cooler sections of the Australian range of D. buzzatii (e.g. the Hunter Valley), eggs laid in late autumn may not finally emerge as adults until the following spring.

While some progress has been made in understanding the ecology of these two cactophilic species, it is clear that there is much still to be learned. For instance, it is still not known what restricts the southern distribution of D. aldrichi, or whether Queensland populations of this species have more annual population peaks than the single, autumn, peak observed in the N.S.W. Hunter Valley. It is not known whether D. buzzatii and D. aldrichi compete for exactly the same resources, or whether there is micro-niche separation, e.g. in selective foraging for preferred yeast or bacterial species. It is not known for certain how D. buzzatii adults survive the heat of summer days (although it is assumed that they avoid injurious temperatures by sheltering in leaf-litter), or just what temperatures larvae can survive in cactus rots. Neither is anything known about the ability of either species to acclimatize to heat or cold.

As more is learnt about the ecology of the two species, so it should become possible to design better experiments to uncover the genetic nature of their adaptive strategies. For instance, if it were found that larvae of the two species preferentially browse different yeast colonies, then a study of nutritional requirements, and especially of their genetic control, may increase understanding of how the two species coexist.

6.3. GENETIC ASPECTS OF TEMPERATURE TOLERANCE

There are two obvious facets to temperature tolerance. Firstly there is the ability to continue normal life (feeding, reproducing, etc.) at temperatures outside the optimum for that species. This was examined experimentally, for *Drosophila buzzatii*, in Chapter 2, and has been further discussed in the preceding section. Secondly there is the ability to survive extreme temperatures, at least for short periods. Some aspects of this were investigated in Chapters 3 and 4 for both *D. buzzatii* and *Drosophila aldrichi*.

That strain differences exist for extreme temperature tolerance in *Drosophila* species has been known for many years (Parsons 1973). The results of Chapter 3 show that such inter-strain differences also exist for *D. buzzatii* and *D. aldrichi*. Such differences have been shown to be genetic (Parsons 1973), and one possible cause is differences in performance, at extremes, by alleles at enzyme loci such as those found to be variable by electrophoresis. A strain difference would then be simply a difference in the frequencies of a few critical allozymes. This theory was tested in Chapter 4, using the known electrophoretic variation for *D. buzzatii* and *D. aldrichi*.

Although some statistically significant associations were observed, this is really insufficient proof of an enzyme's direct involvement in temperature tolerance. However, if biochemical evidence of allozyme differences at temperature extremes in vitro could be related to survival rates of flies carrying the different allozymes, then this might be sufficient evidence that the locus that codes for these allozymes does play a part in extreme temperature tolerance. One *D. buzzatii* enzyme, Esterase-2 (EST-2) has been the subject of a study of thermostability and enzyme kinetics (East 1982).

This enzyme has also been associated with high temperature survival in Chapter 4 of this study, with variability in temperature by Barker *et al.* (1986), and with temperature effects by Mulley *et al.* (1979). In his study, East (1982) found stability at 50°C to be highest for EST-2^{b}, and lowest for EST-2^{c}, with EST-2^{d} and EST-2^{a} intermediate. In measurements of enzyme kinetics, EST-2^{c} also had the lowest Vmax/Km for virtually all conditions tested. This may explain why *Est-2*^{c} was found to be at low frequencies in population cages (Table 4.3.2), and in natural populations (Barker *et al.* 1986; Mulley *et al.* 1979), but sheds no light on why it seemed to have a selective advantage in some areas (Mulley *et al.* 1979). The allozyme associated with heat-shock survival in Chapter 4 was EST-2^{d} (Table 4.3.2). This was intermediate in heat stability at 50°C, and showed a strikingly high Vmax/Km at pH 7.5 and 40°C. However EST-2^{b} and EST-2^{a} had equal or higher thermostability, and high Vmax/Km's over a much wider range of conditions than EST-2^{d}. Thus in-vitro biochemical tests do not appear to offer accurate prediction of an allozyme's in-vivo performance. While East's thermal stability results showed a steady, predictable, decay in enzyme activity with time, the table of rate constants reveals very complex relationships with temperature and pH. This serves to emphasize the necessity of knowing conditions such as pH and substrate for an enzyme at various temperatures in-vivo before any attempt at prediction from in-vitro results can be made.

Of course it always remains possible that a positive association with survival of a locus for which one can visualize phenotypes may be due to effects at some unknown, closely linked, locus. This becomes statistically more probable in species where crossing-over is largely suppressed by the presence of many chromosome

inversions. Although D. buzzatii has only two polymorphic inversions in Australia, both on the second chromosome, Est-2 (for example) is on the second chromosome, so linkage disequilibrium remains a possible cause of the positive association of this locus with heat shock survival.

A further problem with electrophoresis is that a single technique does not necessarily reveal all alleles at a locus. Barker et al. (1985) report an allele of D. buzzatii's Est-1 locus that was discovered as a result of buffer changes. In the results of Chapter 4 of this volume, this new allele was not distinguished from Est-1[a]. Using a variety of electrophoretic techniques, Aquadro et al. (1982) found 23 allozymes at the Est-1 locus in deer mice (Peromyscus maniculatus), where only 8 were known previously. Thus a significant change in an allozyme's frequency among temperature shock survivors could be masked by another allozyme of equal mobility.

A further problem in discerning phenotypic differences (in survival) that can be linked to major gene effects such as allozyme differences is what has often been called "genetic background", or genetic architecture. In the 1950's, Lerner (1954) and others demonstrated less phenotypic variation in response to environmental fluctuations by outbred populations than by inbred lines. Gibson and Oakeshott (1982) discussed this effect in relation to Adh studies. This phenomenon may be expected to have affected the results of both Chapters 3 and 4, as outbred cage populations were generally used as source material.

Part of this background effect is caused by "modifiers" or regulatory genes. Tepper et al. (1982) demonstrated that modifiers of EST-6 activity in D. melanogaster lay on a different chromosome to the Est-6 locus, and also that differences in EST-6 activity between D.

melanogaster and D. simulans, which share the same alleles at this locus, were caused by regulatory genes. Thus differences between allozymes observed in-vitro or in inbred lines, may be largely masked by the action of regulatory genes in natural populations.

A more promising area of research into high temperature survival may be heat shock polypeptides. While most protein synthesis stops in Drosophila at temperatures of 35°C or above, certain loci are activated by heat stress and produce organic molecules known as heat-shock polypeptides (hsp's). It has also been shown that anything that causes oxygen shortage will stimulate them (Ashburner and Bonner 1979). While the purpose of these polypeptides is not fully understood, it seems probable that they play a role in the enduring of, or recovery from, metabolic insults.

Production of hsp's was not taken into account in the design of the heat-shock experiments (Chapters 3 and 4). Survival time of D. buzzatii at 41°C was certainly long enough to permit some transcription, as hsp production starts within 10 minutes of initial exposure to high temperature, but the shock temperature may have been above the optimum for hsp production, which is 36-38°C in D. melanogaster (Ashburner and Bonner 1979). It would be of interest to know if relatively heat-resistant species such as D. buzzatii, and especially D. aldrichi, differ from the pattern of hsp production observed in D. melanogaster and D. hydei, for example in temperature optimum for their production.

Thus it may be seen that, while a little is now known of the genetics of temperature tolerance in cactophilic Drosophila, we are still a long way from a full understanding of what it is that gives D. buzzatii and D. aldrichi greater temperature tolerance than many other Drosophila species.

REFERENCES

Andrewartha, H.G., and Birch, L.C. (1954). 'The Distribution and Abundance of Animals'. (University of Chicago Press : Chicago and London.)

Aquadro, C.F., and Avise, J.C. (1982). An assessment of 'hidden' heterogeneity within electromorphs at three enzyme loci in deer mice. Genetics 102, 269-284.

Ashburner, M., and Bonner, J.J. (1979). The induction of gene activity in Drosophila by heat shock. Cell 17, 241-254.

Baker, R.J., and Nelder, J.A. (1978). 'The GLIM System Release 3, Generalized Linear Interactive Modelling'. (NAG : Oxford.)

Barker, J.S.F. (1977). Cactus-breeding Drosophila - a system for the measurement of natural selection. In 'Lecture Notes in Biomathematics'. Vol. 19. (Eds F.B. Christiansen and T. Fenchel.) pp. 403-430. (Springer-Verlag : Berlin.)

Barker, J.S.F. (1981). Selection at allozyme loci in cactophilic Drosophila. In 'Genetic Studies of Drosophila Populations'. (Eds J.B. Gibson and J.G. Oakeshott.) pp. 161-184. (The Australian National University : Canberra.)

Barker, J.S.F. (1982). Population genetics of Opuntia breeding Drosophila in Australia. In 'Ecological Genetics and Evolution. The Cactus-Yeast-Drosophila Model System'. (Eds J.S.F. Barker and W.T. Starmer.) pp. 209-224. (Academic Press Australia : Sydney.)

Barker, J.S.F., and East, P.D. (1980). Evidence for selection following perturbation of allozyme frequencies in a natural population of Drosophila. Nature 284, 166-168.

Barker, J.S.F., East, P.D., Phaff, H.J., and Miranda, M. (1984). The ecology of the yeast flora in necrotic Opuntia cacti and of associated Drosophila in Australia. Microb. Ecol. 10, 379-399.

Barker, J.S.F., East, P.D., and Weir, B.S. (1986). Temporal and microgeographic variation in allozyme frequencies in a natural population of Drosophila buzzatii. Genetics 112, 577-611.

Barker, J.S.F., and Mulley, J.C. (1976). Isozyme variation in natural populations of Drosophila buzzatii. Evolution 30, 213-233.

Barker, J.S.F., Parker, G.J., Toll, G.L., and Widders, P.R. (1981a). Attraction of Drosophila buzzatii and D. aldrichi to species of yeasts isolated from their natural environment. I. Laboratory experiments. Aust. J. Biol. Sci. 34, 593-612.

Barker, J.S.F., Sene, F. de M., East, P.D., and Pereira, M.A.Q.R. (1985). Allozyme and chromosomal polymorphism of Drosophila buzzatii in Brazil and Argentina. Genetica 67, 161-170.

Barker, J.S.F., and Starmer, W.T. (1982). 'Ecological Genetics and Evolution. The Cactus-Yeast-Drosophila Model System'. (Academic Press Australia : Sydney.)

Barker, J.S.F., Toll, G.L., East, P.D., and Widders, P.R. (1981b). Attraction of Drosophila buzzatii and D. aldrichi to species of yeasts isolated from their natural environment. II. Field experiments. Aust. J. Biol. Sci. 34, 613-624.

Carson, H.L. (1965). Chromosomal morphism in geographically widespread species of Drosophila. In 'The Genetics of Colonizing Species'. (Eds H.G. Baker and G.L. Stebbins.) pp. 503-531. (Academic Press : New York.)

Carson, H.L., and Wasserman, M. (1965). A widespread chromosomal polymorphism in a widespread species, Drosophila buzzatii. Am. Nat. 99, 111-115.

Climatic Atlas of Australia; Map Set 1. Temperature. (1975). Department of Science, Bureau of Meteorology. (Australian Government Publishing Service : Canberra.)

Cohet, Y., Vouidibio, J., and David, J.R. (1980). Thermal tolerance and geographical distribution : a comparison of cosmopolitan and tropical endemic _Drosophila_ species. _J. Therm. Biol._ 5, 69-74.

Crumpacker, D.W., and Marinkovic, D. (1967). Preliminary evidence of cold temperature resistance in _Drosophila pseudoobsura_. _Am. Nat._ 101, 505-514.

Davidson, J. (1944). On the relationship between temperature and rate of development of insects at constant temperatures. _J. Anim. Ecol._ 13, 26-38.

Dobzhansky, Th. (1943). Genetics of natural populations. IX. Temporal changes in the composition of populations of _Drosophila pseudoobscura_. _Genetics_ 28, 162-186.

Dobzhansky, Th. (1950). Genetics of natural populations. XIX. Origin of heterosis through natural selection in populations of _Drosophila pseudoobscura_. _Genetics_ 35, 288-302.

Dobzhansky, Th. (1955). A review of some fundamental concepts and problems of population genetics. _Cold Spring Harbor Symp. Quant. Biol._ 20, 1-5.

East, P.D. (1982). Non-specific esterases of _Drosophila buzzatii_. In 'Ecological Genetics and Evolution. The Cactus-Yeast-_Drosophila_ Model System'. (Eds J.S.F. Barker and W.T. Starmer.) Pp. 323-338. (Academic Press Australia : Sydney.)

Falconer, D.S. (1981). Introduction to Quantitative Genetics. Second Edition. (Longman : London.)

Finney, D.J. (1978). 'Statistical Method in Biological Assay'. Third edition. (Charlie Griffin and Company Limited : London.)

Fleuriel, A. (1976). Presence of the heriditary rhabdovirus sigma and polymorphism for a gene for resistance to this virus in natural populations of Drosophila melanogaster. Evolution 30, 735-739.

Fontdevila, A. (1982). Recent developments on the evolutionary history of the Drosophila mulleri complex in South America. In 'Ecological Genetics and Evolution. The Cactus-Yeast-Drosophila Model System'. (Eds J.S.F. Barker, and W.T. Starmer.) pp. 81-95. (Academic Press Australia : Sydney.)

Fontdevila, A., Ruiz, A., Alonso, G., and Ocana, J. (1981). Evolutionary history of Drosophila buzzatii. I. Natural chromosomal polymorphism in colonized populations of the old world. Evolution 35, 148-157.

Gibson, J.B. and Oakshott, J.G. (1982.) Tests of significance of the alcohol dehydrogenase polymorphism in Drosophila melanogaster: paths, pitfalls and prospects. In 'Ecological Genetics and Evolution, The Cactus-Yeast-Drosophila model system.' (Eds JSF Barker and WT Starmer) pp 291-306. (Academic Press, New York)

Gionfriddo, M.A., and Vigue, C.L. (1978). Drosophila alcohol dehydrogenase frequencies and temperature. Genet. Res. 31, 97-101.

Harris, H. (1966). Enzyme polymorphisms in man. Proc. Roy. Soc. B. 164, 298-310.

Hollingsworth, M.J. (1969). Temperature and length of life in Drosophila. Exp. Geront. 4, 49-55.

Hollingsworth, M.J., and Bowler, K. (1966). The decline in ability to withstand high temperature with increase in age in Drosophila subobscura. Exp. Geront. 1, 251-257.

Hosgood, S.M.W., and Parsons, P.A. (1968). Polymorphism in natural populations of Drosophila for the ability to withstand temperature shocks. Experientia 24, 727-728.

Jefferson, M.C., Crumpacker, D.W., and Williams, J.S. (1974). Cold temperature resistance, chromosomal polymorphism and interpopulation heterosis in Drosophila pseudoobscura. Genetics 76, 807-822.

Johnson, F.M., Kanapi, C.G., Richardson, R.H., Wheeler, M.R., and Stone, W.S. (1966). An analysis of polymorphisms among isozyme loci in dark and light *Drosophila ananassae* strains from America and Western Samoa. *Proc. Nat. Acad. Sci. USA* 56, 119-125.

Johnson, F.M., and Powell, A. (1974). The alcohol dehydrogenases of *Drosophila melanogaster* : frequency changes associated with heat and cold shock. *Proc. Nat. Acad. Sci. USA* 71, 1783-1784.

Lamb, M.J., and McDonald, R.P. (1973). Heat tolerance changes with age in normal and irradiated *Drosophila melanogaster*. *Exp. Geront.* 8, 207-217.

Lancaster, H.O. (1969). 'The Chi-squared Distribution'. (John Wiley & Sons : New York.)

Lerner, I.M. (1954). 'Genetic Homeostasis'. (Oliver and Boyd : Edinburgh.)

Levins, R. (1969). Thermal acclimation and heat resistance in *Drosophila* species. *Am. Nat.* 103, 483-499.

Lewontin, R.C., and Hubby, J.L. (1966). A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. *Genetics* 54, 595-609.

Marinkovic, D., Crumpacker, D.W., and Salceda, V.M. (1969). Genetic loads and cold temperature resistance in *Drosophila pseudoobscura*. *Am. Nat.*, 103, 235-246.

Mather, K. (1943). Polygenic inheritance and natural selection. *Biol. Rev.* 18, 32-64.

Maynard Smith, J. (1957). Temperature tolerance and acclimatization in *Drosophila subobscura*. *J. Exp. Biol.* 34, 85-96.

Maynard Smith, J. (1958). The effects of temperature and of egg laying on the longevity of *Drosophila subobscura*. *J. Exp. Biol.* 35, 832-842.

McKenzie, J.A. (1975). The influence of low temperature on survival and reproduction in populations of *Drosophila melanogaster*. *Aust. J. Zool.* 23, 237-247.

McKenzie, J.A. (1978). The effect of developmental temperature on population flexibility in *Drosophila melanogaster* and *D. simulans*. *Aust. J. Zool.* 26, 105-112.

Miquel, J., Lundgren, P.R., Bensch, K.G., and Atlan, H. (1976). Effects of temperature on the life span, vitality and fine structure of *Drosophila melanogaster*. *Mech. Ageing Develop.* 5, 347-370.

Muller, H.J. (1939). Reversibility in evolution considered from the standpoint of genetics. *Biol. Rev.* (*Camb.*) 14, 261-280.

Mulley, J.C. (1975). Isozyme Variation in *Drosophila buzzatii*. Unpublished Master's Thesis. (University of Sydney.)

Mulley, J.C., and Barker, J.S.F. (1977). The occurrence and distribution of *Drosophila aldrichi* in Australia. *Drosophila Inform. Serv.* 52, 151-152.

Mulley, J.C., James, J.W., and Barker, J.S.F. (1979). Allozyme genotype-environment relationships in natural populations of *Drosophila buzzatii*. *Biochem. Genet.* 17, 105-126.

Murray, N.D. (1982). Ecology and evolution of the *Opuntia-Cactoblastis* ecosystem in Australia. In 'Ecological Genetics and Evolution. The Cactus-Yeast-*Drosophila* Model System'. (Eds J.S.F. Barker and W.T. Starmer.) pp. 17-30. (Academic Press Australia : Sydney.)

Parsons, P.A. (1970). Genetic heterogeneity in natural populations of *Drosophila melanogaster* for ability to withstand dessication. *Theor. Appl. Genet.* 40, 261-266.

Parsons, P.A. (1973). Genetics of resistance to environmental stresses in *Drosophila* populations. *Ann. Rev. Genet.* 7, 239-265.

Parsons, P.A. (1977). Resistance to cold temperature stress in populations of *Drosophila melanogaster* and *D. simulans*. *Aust. J. Zool.* 25, 693-698.

Parsons, P.A. (1978). Boundary conditions for *Drosophila* resource utilization in temperate regions, especially at low temperatures. *Am. Nat.* 112, 1063-1074.

Parsons, P.A., and McDonald, J. (1978). What distinguishes cosmopolitan and endemic *Drosophila* species? *Experientia* 34, 1445-1446.

Powell, J.R. (1973). Apparent selection of enzyme alleles in laboratory populations of *Drosophila*. *Genetics* 75, 557-570.

Schenfield, E., and McKechnie, S.W. (1979). The α-glycerophosphate dehydrogenase (α-gpdh) polymorphism in *Drosophila melanogaster*: adult survival under temperature stress. *Theor. Appl. Genet.* 54, 235-237.

Schnebel, E.M., and Grossfield, J. (1984). Mating-temperature range in *Drosophila*. *Evolution* 38, 1296-1307.

Tantawy, A.O., and Mallah, G.S. (1961). Studies on natural populations of *Drosophila*. I. Heat resistance and geographical variation in *Drosophila melanogaster* and *D. simulans*. *Evolution* 15, 1-14.

Taylor, C.E., and Powell, J.R. (1977). Micro-geographic differentiation of chromosomal and enzyme polymorphisms in *Drosophila persimilis*. *Genetics* 85, 681-695.

Tepper, C.S., Richmond, R.C., Terry, A.L., and Senior, A. (1982). Studies of esterase 6 in *Drosophila melanogaster*. XI. Modification of esterase 6 activity by unlinked genes. *Genet. Res., Camb.* 40, 109-125.

Timofeeff-Ressovsky, N.W. (1940). Mutations and geographical variation. In 'The New Systematics'. (Ed. J. Huxley.) pp. 73-136. (Oxford University Press : London.)

Ushakov, B.P. (1977). The environmental temperature and physiological polymorphism of populations. IV. The effect of heat acclimation on the intensity and the genetic effectiveness of selection caused by heating. J. Therm. Biol. 2, 177-182.

Vacek, D.C. (1982). Interactions between microorganisms and cactophilic Drosophila in Australia. In 'Ecological Genetics and Evolution. The Cactus-Yeast-Drosophila Model System'. (Eds J.S.F. Barker and W.T. Starmer.) pp. 175-190. (Academic Press Australia : Sydney.)

Vacek, D.C., East, P.D., Barker, J.S.F., and Soliman, M.H. (1986). Feeding and oviposition preferences of Drosophila buzzatii for microbial species. Biol. J. Linn. Soc. 24, 175-187.

Wasserman, M. (1954). Cytological studies of the repleta group. Univ. Texas Publ. 5422, 130-152.

Wasserman, M. (1962). Cytological studies of the repleta group of the genus Drosophila: V. The mulleri subgroup. Univ. Texas. Publ. 6205, 85-117.

Watt, A.W. (1975). A temporal study of chromosome inversion frequencies in Drosophila buzzatii. Unpublished undergraduate thesis, Uni of Sydney.

Wills, C., Phelps, J., and Ferguson, R. (1975). Further evidence for selective differences between isoalleles in Drosophila. Genetics 79, 127-141.

Wright, S., and Dobzhansky, Th. (1946). Genetics of natural populations. XII. Experimental reproduction of some of the changes caused by natural selection in certain populations of Drosophila pseudoobscura. Genetics 31, 125-156.

APPENDIX A

PREPARATION OF EGG COLLECTION MEDIUM

The apparatus was essentially that used by Barker _et al._ (1981a) for yeast attractivity experiments. It consisted of agar discs of about 33 mm diameter, mounted in depressions machined into the smaller ends of tapered corks. (These corks fitted holes in the bases of plastic population cages). However, unlike Barker _et al._ (1981a), no yeast was smeared on the discs. The recipe for the agar discs was also slightly different.

To make the discs, 1.25 gm of agar was added to a solution of 25 ml (approx.) of sieved cactus slurry (see Barker _et al._ 1981a), plus 1 ml acetic acid and 2 ml ethanol, made up to 100 ml with water. This solution was boiled, then poured into a glass-based gel mould, to a depth of 3-4 mm. When set, the gel was covered with plastic film, and refrigerated until needed. Discs cut from the gel were loaded onto corks upside down, that is, exposing the surface that had been against the base of the mould. This was found to be critical, as _D. buzzatii_ and _D. aldrichi_ females, which inject their eggs into the medium, would rarely lay on the disc surface exposed during initial cooling of the gel.

APPENDIX B

ELECTROPHORESIS PROTOCOLS FOR *D. ALDRICHI*

All electrophoresis was performed on starch gels, but three different buffer systems were used. These were continuous Tris-borate-EDTA, continuous Tris-citric acid, and discontinuous Tris-citric acid (Barker *et al.* 1986; Barker and Mulley 1976). Malate dehydrogenase (MDH), Phosphoglucomutase (PGM), and Aldehyde oxidase (ALDOX) were run on Tris-borate-EDTA, β-n-acetyl-hexosaminidase (HEX) and Leucine aminopeptidase (LAP) were run on continuous Tris-citric acid, and for the esterases the discontinuous buffer system was used.

All enzymes assayed had been previously studied in *D. buzzati*, and the staining methods described by Barker *et al.* (1986) and Barker and Mulley (1976) were used.