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Coexistence of ecologically similar colonising species. II. Population differentiation in *Drosophila aldrichi* and *D. Buzzatii* for competitive effects and responses at different temperatures and allozyme variation in *D. aldrichi*

Robert A. Krebs* and J. S. F. Barker

Department of Animal Science, University of New England, Armidale, NSW 2351, Australia

Key words: Larval competition; viability; size; developmental time; electrophoresis.

Abstract

Drosophila aldrichi and *D. buzzatii* are cactophilic species that colonised Australia about 55–60 years ago. They are sympatric only in Australia. Thus they may be in the process of adapting to new environments and to each other, and diversifying among local, possibly isolated, populations. Larval competitive effects for three populations of each species (Roma, Planet Downs, and Binjour) were measured on semi-natural cactus rots at three temperatures, with preadult viability, developmental time and adult body weight scored for each sex and species. Populations of both species varied in their responses to the other species as competitor, and one *D. buzzatii* population (Roma) reduced larval performance of *D. aldrichi* significantly more than did other *D. buzzatii* populations. Geographic divergence for the three traits was similar in both species, with a relative performance index derived from these traits highest for Roma, second for Binjour, and least for the Planet Downs population of each species. The Roma *D. aldrichi* population was the most different from the other populations for the performance index and in terms of genetic distances derived from allozyme frequencies. Additionally, comparisons of climatic variables among the population localities showed that the Roma environment was most different from the others. Differential natural selection in different areas of the cactus distribution may be a major cause of population divergence in both species. *Drosophila aldrichi* is superior for some fitness components at the highest temperature. Thus temperature

* Present address and address for correspondence: Department of Ecology and Genetics, University of Aarhus, Ny Munkegade, bldg. 540, 8000 Aarhus C, Denmark.

variation throughout the cactus distribution may contribute to the different ranges of these two species, with competitive exclusion of *D. aldrichi* in the southern, cooler region of the cactus distribution, but coexistence in the northern, warmer region.

Introduction

Interspecific competition may control the abundance or geographic distribution of many species of animals (Connell, 1983), but see Underwood (1986) for a critical review of field experiments and their validity in showing the importance or otherwise of interspecific competition as a determinant of community structure. Also, competition may contribute to coevolution of competing species, or to genetic change in one of them (Barker, 1973; Budnik and Brncic, 1974; Christiansen and Loeschcke, 1990).

For two species sharing one or more common resources, each one is part of the biotic environment to which the other may be adapting. At the level of intraspecific competition, Mather and Caligari (1983) recognised two components of competitive interactions, viz. competitive pressure, or the effects exerted by an individual on all other individuals of the population, and the response of the individual to the sum of the competitive effects exerted by all others. These components will be relevant at any level of resource competition, including interspecific competition. Where competing species exist in discrete habitats, and populations are genetically isolated, different effects or responses may evolve in different populations. Populations therefore may be genetically different for competitive ability.

Many studies of interspecific competition have used only one population of each species in a single environment. However, to determine whether there are genetic differences among populations of one species for competitive ability, the populations must be compared in the same environment. If a number of populations of both species are used, and all pair-wise combinations are set in each of two or more environments, then genetic variation and population by environment interactions can be studied in each species. In plants, populations by environment interactions have been studied in the field by transplanting individuals to different habitats (Davies and Snaydon, 1976; Lovett Doust, 1981). However, because of their mobility, animals are difficult to monitor over time in nature, so that laboratory studies using simulated natural environments are necessary.

The well known ecology of the cactophilic *Drosophila* (Barker and Starmer, 1982; Barker et al., 1990) provides an opportunity to study variation among populations on controlled, semi-natural environments within the laboratory. The two cactophilic *Drosophila* present in Australia, *D. aldrichi* and *D. buzzatii*, utilise necrotic cladodes (rots) of several prickly pear cacti: primarily *Opuntia stricta*, *O. tomentosa*, and *O. streptacantha*, and are specific to this cactus niche. The records of the *Opuntia* biological control program indicate that *D. buzzatii* was unintentionally introduced into Australia 55–60 years ago (Barker et al., 1985; Sokal et al., 1987), and subsequent to the deliberate human introduction of the prickly pear control agent, *Cactoblastis cactorum* (Lepidoptera: Pyralidae). *Drosophila aldrichi* almost

certainly was introduced at about the same time from Texas or northern Mexico, again unintentionally in material brought to Australia during the control program. *Drosophila buzzatii* occurs throughout the range of *Opuntia* in Australia, but *D. aldrichi* is present only in the northern part of the cactus distribution.

Where sympatric, these two species have been reared from the same field collected rots (Barker et al., 1984), so that larvae may compete for food in nature. A study of competitive interactions in the laboratory (Krebs and Barker, 1991) showed that *D. buzzatii* had a competitive advantage over *D. aldrichi* in population cages, in larval competition on an *o. stricta* substrate and for several life history attributes, but only a single population of each species was used, and all experiments were done at 25° C. In the region where *D. aldrichi* and *D. buzzatii* are sympatric, different populations are exposed to environments that differ in climate, in cactus species present and in the microorganisms (bacteria and yeasts) present in rots (Mulley et al., 1979; Barker et al., 1984). Here we examine: (i) whether populations from different parts of the region of sympatry differ in competitive ability, and (ii) effects of temperature on competitive ability. Only competition at the larval stage is considered, and the traits studied were larva to adult viability, developmental time and adult body weight.

Genetic differences among populations also may be estimated from allozyme frequencies. Divergence among populations for allozyme frequencies has been documented in *D. buzzatii* (Barker and Mulley, 1976; Sokal et al., 1987), but no studies have been made for *D. aldrichi*. Thus allozyme frequencies at 20 loci of *D. aldrichi* were estimated to allow comparisons with population differences in the quantitative traits.

Materials and methods

Strains of flies

Drosophila aldrichi and *D. buzzatii* were collected at three sites in Queensland in October 1989: Roma, an open habitat with scattered low shrubs and trees, Planet Downs, a dense scrub of Brigalow trees (*Acacia harpophylla* F. Muell.), and Binjour, open fields on a hillside adjacent to a wooded area (Fig. 1). *Opuntia tomentosa*, the velvety tree pear, was the only cactus present at Roma and Binjour, and was the most common cactus at Planet Downs, where a few *O. stricta* also occurred.

Competition experiments

For each species, ten isofemale lines from each population were maintained (except for Roma *D. aldrichi* with only seven lines) on autoclaved sucrose-agar-cactus medium (Starmer and Barker, 1986) for four generations before use in the experiments. Each line was maintained at 20 pairs of parents per generation (4 vials × 5 pairs). To minimise inbreeding, five pairs of progeny were taken from each

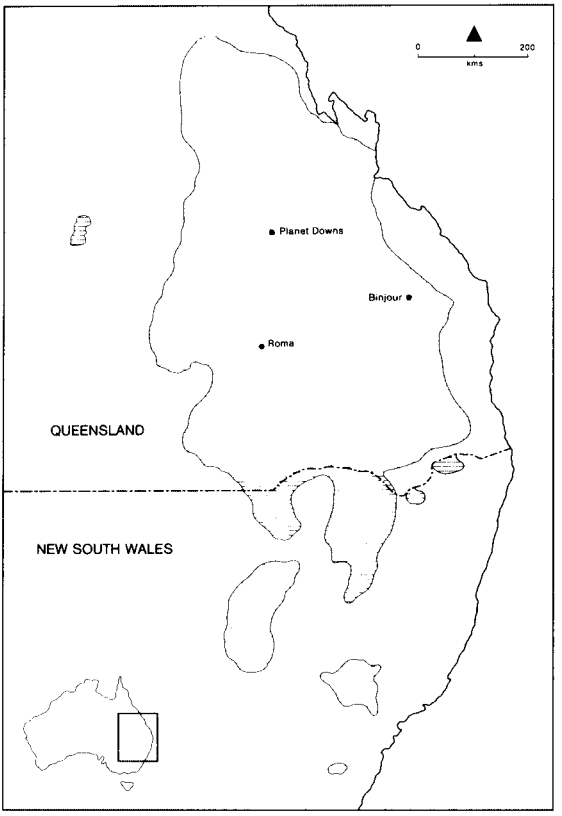

Fig. 1. Localities from which *D. aldrichi* and *D. buzzatii* were collected. The hatched areas show the distribution of the main *Opuntia* infestations in 1920.

vial, mixed and distributed to four new vials each generation. At the fourth generation, 10 pairs of mature flies from each isofemale line (within each species and locality) were pooled, held for two days to permit intermating among lines (multiple mating is common in both of these species, unpubl.), and then serially transferred for three days to produce a mass population in ten bottles. Virgin progeny from these bottles were mixed and set up in 40 bottles (50 pairs per bottle, left overnight for oviposition) to obtain large numbers of flies from each population. Their progeny, offspring of two generations of intermating, eclosed within population cages (36 cm × 36 cm × 20 cm), where they were fed live baker's yeast and held to maturity for egg collection.

Eggs were collected simultaneously from all six populations (three of each species), sterilised after 16 hours using calcium hypochlorite, which removes the chorion and any microbes which may be present on the eggs (procedures as in Krebs and Barker, 1991), and transferred to sterile agar plates. At 25° C, the mean duration of the egg stage is about one to two hours less for *D. aldrichi*. So that

newly hatched larvae of both species would be available at the same time, *D. buzzatii* eggs were held at 25° C until hatching, while those of *D. aldrichi* were held at 22° C prior to dechorionating, and then moved to 25° C until hatching. Larvae (< 1 hour old) were transferred to a growth medium of simulated necrotic cactus, 5 g autoclaved *Opuntia stricta* tissue per vial inoculated with naturally occurring yeasts and bacteria (procedures as in Krebs and Barker, 1991).

Experimental design

For each of the nine pair-wise combinations of the two *Drosophila* species (3 populations *D. aldrichi* × 3 populations *D. buzzatii*), 20 axenic larvae of each species were placed together in each vial. This density, 40 per vial, produced a moderate level of competition in previous interspecific tests, with viabilities about 50 per cent (Krebs and Barker, 1991). The vials then were transferred to 25°, 28° or 31° C. Two blocks of five replicate vials each were prepared (3 days apart) for each pair-wise species combination at each temperature, with all treatments otherwise prepared simultaneously, and with a single preparation of medium for each block.

Upon emergence, flies were collected daily, anaesthetised, sorted to species and sex, and dried in glass vials at 65° C. Viability (first instar larva to adult), mean developmental time (in days) and mean dry weight were recorded for each species and sex per vial, and these vial means were used for all statistical analyses. Data for developmental time and body weight were missing in some vials, where either no males or no females of one or the other species emerged (4 of 540 cells for *D. aldrichi* and 18 of 540 cells for *D. buzzatii*). Such missing cells were scored as the average of their replicate vials within that block, with the appropriate number of degrees of freedom removed from the error term.

Viability, developmental time and dry weight of each species were analysed by 5-way fixed factor analyses of variance (SAS Institute, 1985), with the main effects block, population of *D. aldrichi*, population of *D. buzzatii*, temperature and sex. In preliminary analyses including all interaction terms, seven of 90 interaction terms involving block (in the six ANOVA) were significant, but with only one or two in each ANOVA and no consistent pattern. Therefore, in the analyses presented, all variation due to interactions of treatment effects with block was included in the error term. Interactions between species were tested in a 4-way ANOVA, with population nested within species.

Allozyme analyses

Allozyme genotypes were determined for 20 loci by assaying wild caught *D. aldrichi* males and females from the three sites for a subset of the loci, and first generation offspring of the females for all loci. The wild caught females included those from which the isofemale lines were established, and the numbers that gave progeny for assay were seven from Roma, 13 from Planet Downs and 26 from Binjour. Assay of at least four offspring from each female (plus her own genotype

Table 1. Allozyme frequencies in three populations of *D. aldrichi*: Roma, Planet Downs and Binjour.

Enzyme	Locus	Buffer	Allele	Roma		Binjour		Planet Downs	
				N[1]	Freq	N	Freq	N	Freq
Acid Phosphatase	Acph	a	A	28	0.000	144	0.000	74	0.014
			B		0.393		0.458		0.297
			C		0.607		0.528		0.689
			D		0.000		0.014		0.000
Alcohol dehydrogenase-2	Adh-2	a	B	30	1.00	144	1.00	76	1.00
Aldehyde oxidase	Aldox	b	B	26	0.039	104	0.106	58	0.017
			C		0.462		0.663		0.741
			D		0.500		0.202		0.224
			E		0.000		0.029		0.017
Aldolase	Ald	b	A	28	1.00	100	1.00	52	1.00
Esterase-2	Est-2	c	B	30	0.033	136	0.016	52	0.043
			C		0.100		0.336		0.217
			D		0.867		0.648		0.717
			E		0.000		0.000		0.022
Esterase-3	Est-3	c	A	30	1.00	136	1.00	52	1.00
Esterase-5[2]	Est-5	c	A	28	0.000	130	0.031	46	0.043
			B		0.036		0.123		0.109
			C		0.143		0.177		0.174
			D		0.214		0.200		0.174
			E		0.571		0.408		0.435
			F		0.000		0.015		0.022
			G		0.036		0.046		0.043
Fumarase	Fum	b	B	28	1.00	104	1.00	52	1.00
α-Glycerophosphate dehydrogenase	αGpdh	a	A	28	1.00	144	1.00	76	1.00
Hexosaminidase	Hex	b	A	28	0.036	108	0.000	66	0.000
			B		0.393		0.241		0.197
			C		0.571		0.759		0.803
β-Hydroxybutyrate dehydrogenase[3]	βHbdh	b	B	21	0.952	78	1.00	39	0.923
			C		0.048		0.000		0.077
Isocitrate dehydrogenase	Idh	b	A	28	0.000	104	0.058	50	0.080
			B		1.00		0.942		0.920
Leucine aminopeptidase	Lap	b	B	28	0.964	104	0.981	52	0.885
			C		0.036		0.019		0.096
			D		0.000		0.000		0.019
Malic enzyme	Me	a	A	28	1.00	100	1.00	52	1.00
Malate dehydrogenase	Mdh	a	A	28	0.786	100	0.780	52	0.923
			B		0.214		0.220		0.077
Octanol dehydrogenase	Odh	b	A	28	1.00	110	1.00	70	1.00
Phosphoglucomutase	Pgm	a	B	30	0.967	146	0.938	76	0.987
			C		0.033		0.055		0.013

Table 1. (*Continued*)

Enzyme	Locus	Buffer	Allele	Roma		Binjour		Planet Downs	
				N[1]	Freq	N	Freq	N	Freq
6-Phosphoglucose dehydrogenase	6Pgdh	b	A	26	1.00	104	1.00	46	1.00
Phosphoglucose isomerase	Pgi	b	A	28	1.00	100	1.00	52	1.00
Superoxide dismutase	Sod	b	A	16	1.00	100	1.00	34	1.00

The electrophoretic gel buffer systems used were (a) Tris Borate EDTA pH 8.0; (b) Tris Citrate pH 7.0; and (c) Tris Borate pH 8.5 discontinuous buffer systems.

[1] Number of genomes

[2] Expressed in males only

[3] Sex-linked

where known for some loci) allowed inference of the most probable genotypes for both parents for each locus. If parental genotypes remained ambiguous due to suspected multiple mating or lack of flies, the data were excluded from the analyses. For some wild caught females, too few offspring were obtained for analyses of all loci.

Flies were homogenised in 20 μl tris-citrate buffer, pH 7.0, in a 400 μl micro-centrifuge tube using a motor driven homogenising pestle. The homogenate was centrifuged at 16 000 rpm for 10 seconds, and the supernatant divided equally on to two 4 × 7 mm filter paper wicks for use on each of two independent horizontal starch gels. Enzymes were assayed according to previously described methods (Poulik, 1957; Shaw and Koen, 1968; Ayala et al., 1972; Barker and Mulley, 1976), with buffer systems as listed in Table 1.

Differences among populations in allozyme frequencies were analysed by a chi squared test of single locus allele frequencies (Nei, 1987, p. 227), and by F statistics (Weir and Cockerham, 1984) computed with BIOSYS-1 (Swofford and Selander, 1989), as recently modified (W. C. Black, pers. comm.). Genetic distances among populations were estimated using the method of Reynolds et al. (1983), which is based on the coancestry coefficient and accounts for small sample size. The unweighted pair group method with arithmetic means (UPGMA, Sneath and Sokal, 1973) was applied to the pair-wise distances to estimate average relationships among the populations. In all these analyses, female data only were used for the sex-linked locus (βHbdh).

Results

Viability

Temperature significantly affected viability ($P < 0.01$, Tab. 2), which was highest at 28° C in both species (Tab. 3). Viability of *D. aldrichi* was greater than that of

Table 2. Summary of ANOVA results for *D. aldrichi* and *D. buzzatii* from three populations of each species placed in interspecific larval competition at three temperatures (25°, 28° and 31° C). Differences were determined by 5-way ANOVA for the main effects listed, but only the two-way interactions are presented[1]: Temp, three rearing temperatures; Apop, three populations of *D. aldrichi*; Bpop, three populations of *D. buzzatii*, Sex and Block. Differences between *D. aldrichi* and *D. buzzatii* (Spec, Spec × Temp, Spec × Sex and Spec × Temp × Sex) were tested by a 4-way ANOVA with populations nested within species.

	Viability		Devel. Time		Weight	
	ald	buzz	ald	buzz	ald	buzz
Blocks	*	*	*	***	ns	***
Temp	**	**	***	***	***	***
Apop	ns	ns	***	ns	***	ns
Bpop	ns	*	**	ns	**	*
Sex	**	***	**	*	***	***
Temp × Apop	ns	ns	ns	ns	ns	*
Temp × Bpop	ns	ns	ns	ns	ns	ns
Temp × Sex	ns	**	ns	ns	*	*
Apop × Bpop	$P - 0.07$	ns	ns	*	ns	ns
Apop × Sex	*	ns	ns	ns	ns	ns
Bpop × Sex	ns	ns	ns	ns	ns	ns
Spec	***		ns		***	
Spec × Temp	ns		*		ns	
Spec × Sex	$P = 0.06$		ns		ns	
Spec × Temp × Sex	*		ns		ns	

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$
[1] The three-way interaction Bpop × Temp × Sex was significant ($P < 0.01$) for developmental time of *D. buzzatii*.

Table 3. Preadult viability (numbers of adults emerging from 20 larvae of each species) of *Drosophila aldrichi* and *D. buzzatii* ($N = 90$ vials for each temperature), and the correlation of viability between species at each temperature and overall.

Temp	Number Aldrichi			Number Buzzatii			Ratio		Between species correlation/vial	
	males	females	total	males	females	total	(ALD/BUZ) Males × Fem		r	P
25	5.56	4.86	10.42	3.98	3.44	7.46	1.40	1.41	0.256	<0.05
28	5.53	5.01	10.54	4.88	4.01	8.79	1.13	1.25	0.356	<0.001
31	4.64	4.30	9.09	4.54	2.63	7.26	1.02	1.63	0.158	0.137
OVERALL	5.24	4.72	10.02	4.47	3.36	7.84	1.17	1.40	0.282	<0.001

D. buzzatii at all temperatures (Tab. 3). For both species, male viability was significantly greater than that of females, assuming that equal numbers of male and female first instar larvae were seeded initially. The viability of *D. aldrichi* relative to that of *D. buzzatii* (Tab. 3 – Ratio) differed among temperatures, and differentially for males and females (significant species × temperature × sex interaction – Tab. 2). The ratio of *D. aldrichi* males to *D. buzzatii* males was largest at 25° C, but the ratio for females was largest at 31° C (Tab. 3). Viabilities of *D. aldrichi* and *D. buzzatii* were positively correlated at all temperatures, with significant correlation coefficients at 25° and 28° C (Tab. 3).

For *D. aldrichi*, female viability for the Planet Downs population was less than that for Roma or Binjour (Apop × sex interaction, $P < 0.05$, Tab. 2, Fig. 2), but this difference was consistent across temperatures.

Viability differed significantly among populations of *D. buzzatii* ($P < 0.05$, Tab. 2, Fig. 2). Viability was highest for the Roma population and lowest for Planet Downs, with the flies of the Binjour population intermediate and not significantly different from the other two ($P < 0.05$, Scheffe's multiple range test, Tab. 4B). *Drosophila buzzatii* females showed a marked decrease in viability at 31° C (temperature × sex, $P < 0.01$, Tab. 2, Tab. 3). The Apop × Bpop interaction was near to significance ($P = 0.07$), as the viability of the Planet Downs population of *D. aldrichi* was lowest when cultured with Roma *D. buzzatii*, while the other two *D. aldrichi* populations varied less among *D. buzzatii* populations (Tab. 4A).

Developmental time

Drosophila aldrichi and *D. buzzatii* were not different in average developmental time, but the species × temperature interaction was significant ($P < 0.05$, Tab. 2).

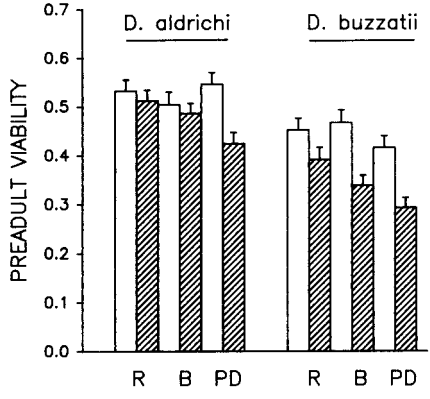

Fig. 2. Preadult viability, pooled across temperatures, of males (clear) and females (cross-hatched) of three populations of *D. aldrichi* and *D. buzzatii*: Roma (R), Binjour (B) and Planet Downs (PD).

Table 4. Means and standard errors for viability (viab), developmental time (dt) and dry weight (wt) of three populations of (A) *D. aldrichi* and (B) *D. buzzatii*, Roma, Binjour and Planet Downs, when cultured with each of the three populations of the other species (with data for each temperature and sex pooled within population, $N = 60$ for each population × population comparison).

A. *D. aldrichi*		Competing Population of *D. buzzatii*			
		Roma	Binjour	Planet Downs	Means
Roma	viab	0.523 ± 0.028	0.508 ± 0.029	0.535 ± 0.029	0.522 ± 0.016
	dt	12.52 ± 0.28	12.54 ± 0.25	12.21 ± 0.24	12.42 ± 0.15
	wt	0.433 ± 0.008	0.456 ± 0.010	0.453 ± 0.009	0.447 ± 0.005
Binjour	viab	0.503 ± 0.028	0.470 ± 0.027	0.512 ± 0.032	0.495 ± 0.017
	dt	12.53 ± 0.25	12.34 ± 0.29	12.17 ± 0.25	12.35 ± 0.15
	wt	0.419 ± 0.008	0.418 ± 0.008	0.422 ± 0.008	0.419 ± 0.005
Planet Downs	viab	0.408 ± 0.025	0.518 ± 0.030	0.528 ± 0.032	0.485 ± 0.017
	dt	12.32 ± 0.24	12.01 ± 0.24	12.05 ± 0.23	12.13 ± 0.14
	wt	0.399 ± 0.010	0.423 ± 0.009	0.414 ± 0.008	0.412 ± 0.005
Means	viab	0.478 ± 0.015	0.499 ± 0.017	0.525 ± 0.018	0.501 ± 0.010
	dt	12.46 ± 0.15	12.30 ± 0.15	12.14 ± 0.14	12.30 ± 0.08
	wt	0.417 ± 0.005	0.432 ± 0.005	0.430 ± 0.005	0.426 ± 0.003

B. *D. buzzatii*		Competing Population of *D. aldrichi*			
		Roma	Binjour	Planet Downs	Means
Roma	viab	0.392 ± 0.030	0.435 ± 0.032	0.438 ± 0.029	0.422 ± 0.018
	dt	12.29 ± 0.22	12.28 ± 0.25	12.53 ± 0.25	12.37 ± 0.14
	wt	0.461 ± 0.010	0.460 ± 0.010	0.469 ± 0.012	0.464 ± 0.006
Binjour	viab	0.368 ± 0.030	0.442 ± 0.028	0.397 ± 0.032	0.402 ± 0.018
	dt	12.70 ± 0.34	12.36 ± 0.29	11.94 ± 0.22	12.33 ± 0.17
	wt	0.442 ± 0.011	0.452 ± 0.012	0.451 ± 0.010	0.449 ± 0.006
Planet Downs	viab	0.338 ± 0.025	0.360 ± 0.032	0.365 ± 0.027	0.354 ± 0.016
	dt	12.30 ± 0.27	12.48 ± 0.26	12.14 ± 0.26	12.31 ± 0.15
	wt	0.451 ± 0.010	0.442 ± 0.011	0.453 ± 0.009	0.448 ± 0.006
Means	viab	0.366 ± 0.016	0.412 ± 0.018	0.400 ± 0.017	0.393 ± 0.010
	dt	12.43 ± 0.16	12.37 ± 0.15	12.20 ± 0.14	12.34 ± 0.09
	wt	0.452 ± 0.006	0.451 ± 0.006	0.458 ± 0.007	0.454 ± 0.004

Developmental time (Fig. 3A, B) of *D. buzzatii* was shorter than that of *D. aldrichi* at 25° C (14.6 days vs. 14.8), the same at 28° C (11.5 days), but longer than that of *D. aldrichi* at 31° C (10.9 vs. 10.6 days). The overall decrease in developmental time of *D. aldrichi* with the increase in temperature from 25° to 31° C was therefore significantly greater: 4.2 days as compared with 3.7 days for *D. buzzatii*.

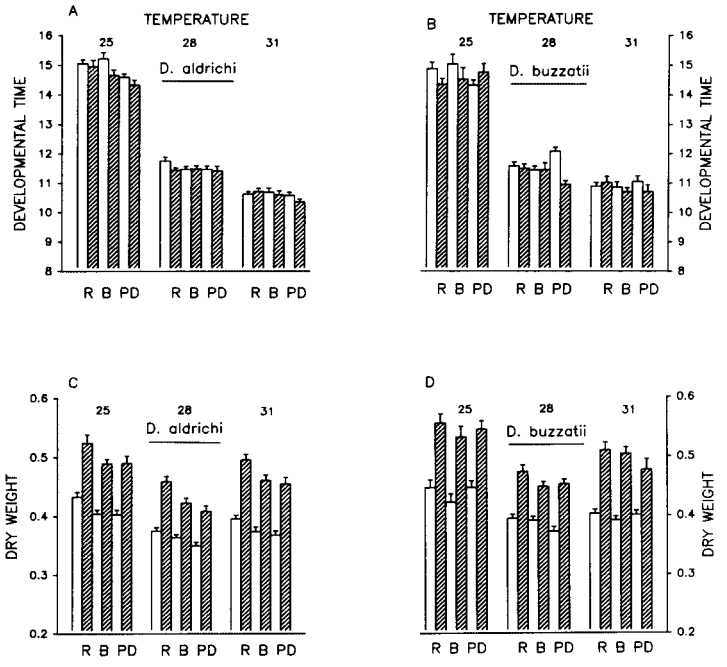

Fig. 3. Developmental time (A & B in days) and dry weight (C & D in milligrams) for males (clear) and females (cross-hatched) of three populations of *D. aldrichi* and *D. buzzatii*, Roma (R), Binjour (B) and Planet Downs (PD), at three temperatures.

Populations of *D. aldrichi* varied in developmental time, with the Planet Downs population emerging faster than either Roma or Binjour ($P < 0.05$, Scheffe's test, Fig. 3A). Population of *D. buzzatii* affected developmental time of *D. aldrichi* (Bpop, $P < 0.01$, Tab. 2). Averaging over the three populations, *D. aldrichi* emerged significantly faster when cultured with Planet Downs *D. buzzatii* than with Roma *D. buzzatii* ($P < 0.05$, Scheffe's test, Tab. 4A). Developmental time with Binjour *D. buzzatii* was not significantly different from that with the other two populations. Overall, the developmental time of males was significantly longer than that of females ($P < 0.01$, Tab. 2, Fig. 3A).

Populations of *D. buzzatii* did not differ in developmental time (Tab. 2, Fig. 3B), nor were there any average differences due to populations of the competing species, *D. aldrichi* (Tab. 4B). However, the Apop × Bpop interaction was significant. Roma *D. buzzatii* flies emerged fastest when paired with either Roma or Binjour *D. aldrichi* (Tab. 4B), while Binjour *D. buzzatii* emerged fastest against Planet Downs and Binjour *D. aldrichi*, and Planet Downs *D. buzzatii* emerged fastest against Planet Downs *D. aldrichi*. As observed for *D. aldrichi*, the developmental time of males was significantly longer than that of females, but the Bpop × temperature × sex interaction was significant ($P < 0.01$), caused primarily by the longer developmental time for Planet Downs females at 25° C (Fig. 3B).

Dry weight

Drosophila buzzatii were significantly larger than *D. aldrichi* ($P < 0.001$, Tab. 2), for all population × temperature × sex combinations (Fig. 3C, D). Flies of both species were largest at 25° C, and smallest at 28° C (Fig. 3C, D). The significant temperature × sex interaction in both species ($P < 0.05$) was due to a greater relative decrease in female size than in male size at 28° C as compared with the other temperatures.

Mean dry weight of *D. aldrichi* populations differed significantly, with Roma flies larger than those of Planet Downs and Binjour ($P < 0.05$, Scheffe's test, Fig. 3C, Tab. 4A). However, dry weight of *D. aldrichi* was also influenced by the competing population of *D. buzzatii* (Bpop, $P < 0.01$, Tab. 2). Flies in competition with the *D. buzzatii* Roma population were significantly smaller than those paired against either Binjour or Planet Downs *D. buzzatii* ($P < 0.05$, Scheffe's test, Tab. 4A).

Populations of *D. buzzatii* also were significantly different in mean dry weight (Tab. 2), with Roma adults significantly larger than those of Binjour and Planet Downs ($P < 0.05$, Scheffe's test, Tab. 4B).

In addition, for *D. buzzatii* dry weight, the temperature × Apop interaction was significant ($P < 0.05$). For all populations, *D. buzzatii* were largest at 25° C when paired against *D. aldrichi* from Planet Downs (viz. 0.507 ± 0.011 with Planet Downs, 0.497 ± 0.012 with Binjour, 0.474 ± 0.013 with Roma), but largest against Roma *D. aldrichi* at 28° C and 31° C (at 28° C, 0.430 ± 0.008, 0.420 ± 0.008 and 0.416 ± 0.008

Table 5. Electrophoretic variation among populations of *D. aldrichi*: *F* statistics for each locus (Weir and Cockerham, 1984), single locus genetic distances between populations using the coancestry coefficient (Reynolds et al., 1983), and pair-wise significance testing by a chi squared test (Nei, 1987) for each locus (PD is Planet Downs).

Locus	F_{IS}	F_{ST}	F_{IT}	Genetic distances between populations		
				Roma Binjour	Roma PD	Binjour PD
Aceph	−0.011	0.024	0.013	0.010	0.005	0.041*
Aldox	0.037	0.048	0.082	0.095*	0.126*	0.001
Est-2	0.117	0.037	0.150	0.089*	0.018	0.006
Est-5	0.133	−0.010	0.124	0.002	0.015	0.016
Hex	−0.054	0.026	−0.027	0.050*	0.090*	0.007
βHbdh	−0.050	0.061	0.014	0.000	0.008	0.087*
Idh	0.159	−0.001	0.159	0.008	0.039	0.013
Lap	−0.064	0.037	−0.025	0.017	0.006	0.064*
Mdh	0.156	0.026	0.178	0.028	0.055	0.054*
Pgm	0.151	0.002	0.152	0.018	0.013	0.012
	Jackknife				Jackknife[1]	
Mean	0.0679	0.0209	0.0872	Mean 0.032	0.037	0.010
SE	0.0324	0.0106	0.0270	SE 0.021	0.026	0.011

[1] Genetic distances include monomorphic loci
* $P < 0.05$

for Roma, Binjour and Planet Downs respectively, and at 31° C, 0.453 ± 0.009, 0.437 ± 0.009 and 0.451 ± 0.011 for Roma, Binjour and Planet Downs respectively).

Allozyme analyses

Variation was found for 10 of 20 enzyme loci, with the most common allele present at 95% or less in at least one population (Tab. 1). The most common allele was the same in all populations for all loci except Aldox, and genotypes at all loci were in Hardy-Weinberg equilibrium (except Idh at Binjour, $P < 0.05$). The Roma population differed from Planet Downs and Binjour with a mean genetic distance of 0.034, and Planet Downs differed by 0.010 from Binjour (Tab. 5). The chi squared test of single locus allele frequencies showed that seven loci contributed significantly to these differences among the three populations, and these loci also had the largest F_{ST} values (Tab. 5). The jackknife mean F_{ST} over all loci was significantly different from zero ($P < 0.05$), also indicating divergence among these populations, although the F_{IS} estimate explained most of the total inbreeding (F_{IT}).

Discussion

Differences among populations

There were significant differences among populations of *D. aldrichi* for developmental time and dry weight (Tab. 4A), and among populations of *D. buzzatii* for viability and dry weight (Tab. 4B). These differences among populations for these traits can be summarised conveniently in a composite value which, although not all inclusive, we designate as the relative performance index (RPI). Ruiz and Heed (1988) used a very similar index which they called "fitness".

$$\text{RPI} = \frac{(\text{Number emerged}) \times (\text{Dry weight})}{(\text{Developmental time})}$$

Because developmental time decreased rapidly with increased temperature, this index is most applicable for comparisons among populations within each temperature, or for comparisons among populations pooled over all temperature treatments.

The rank order of the performance index was the same for the two species (Fig. 4), viz. Roma > Binjour > Planet Downs, suggesting that both species may be responding to differences in local environmental conditions, although in different ways. As noted above, the traits that differ among populations are different for the two species – developmental time and body size for *D. aldrichi*, and viability and body size for *D. buzzatii*. The lack of significant variation among *D. aldrichi* populations for viability, or among *D. buzzatii* populations for developmental time largely parallels the effects found for increasing larval density in intraspecific cultures (Krebs and Barker, 1991). We suggested then that the differences between

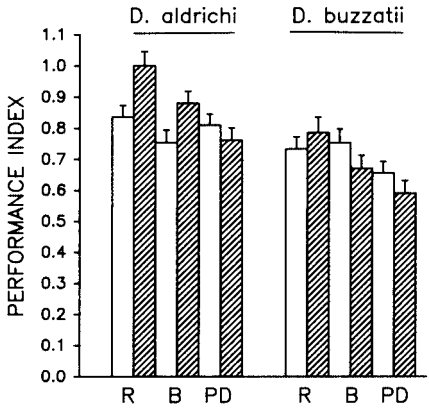

Fig. 4. Performance index of males (clear) and females (cross-hatched), pooled across temperatures, for three populations of *D. aldrichi* and *D. buzzatii*: Roma (R), Binjour (B) and Planet Downs (PD). Results were scaled such that the largest value shown is unity.

the two species in their responses to increasing larval density indicated differences in their life history evolution – for *D. aldrichi*, the capacity for maintaining preadult viability and/or body size being of most importance, while fast development was most important for *D. buzzatii*. The present results provide further support for this hypothesis.

Variation in competitive effects on the other species was found, but only among the three *D. buzzatii* populations. The Roma *D. buzzatii* population reduced adult weight and lengthened developmental time of *D. aldrichi* significantly more than did either Binjour or Planet Downs *D. buzzatii*. Roma *D. buzzatii* also had the highest RPI. Thus this *D. buzzatii* population had both the largest effect on *D. aldrichi* and the best performance when cultured with it; an association between effects and responses that has been found in other analyses of competition in *Drosophila* (Hemmat and Eggleston, 1988). Distinguishing effects and responses, and defining the genetic basis for each, is not simple as competitive effects are exerted by all individuals on every one of their number, i.e. effects are a property of the group, while the response to those effects is a property of the individual itself (Caligari and Mather, 1984).

The link between competitive effects and responses is most likely due to larval feeding efficiency (i.e. exploitative competition, with passive effects on the competitor due to resource reduction). Larvae from a population with higher average feeding efficiency would be more resistant to competitor effects and also, would deprive the competitor of resources. On average, the performance of *D. aldrichi* larvae against each *D. buzzatii* population was related inversely to the RPI of that competitor population (average relative performance index with Planet Downs *D. buzzatii*, 1.0, with Binjour, 0.95, with Roma, 0.88). The lack of a similar relationship for *D. buzzatii* populations is not surprising, as intraspecific competition is greater than interspecific competition due to *D. aldrichi* (Krebs and Barker, 1991).

Electrophoretic variation

The electrophoretic results showed a relative divergence among populations similar to that for the quantitative traits. Based on genetic distance comparisons, Roma *D. aldrichi* differed from both Binjour and Planet Downs (Tab. 5). Most alleles were shared among the three populations, and the same allele was generally present at the highest frequency in all populations, as expected where divergence time has been short. Quantitative differences in less common alleles, however, led to significant differences among populations for some allele frequencies.

Significant differences for some loci and positive F_{ST} estimates showed that these three populations have become differentiated since colonisation in Australia (probably about 400 generations). Multivariate analyses on the much larger data available for *D. buzzatii* have shown clines for frequencies of some alleles (Sokal et al., 1987) or major zones of differentiation at the periphery (Barbujani et al., 1989). The differences in allele and genotype frequencies among populations of *D. buzzatii* have been considered as most likely due to natural selection, with selection at different loci operating at different spatial scales (Mulley et al., 1979; Sokal et al., 1987).

The three populations analysed here are separated by similar geographic distances, but their localities differ climatically, with Binjour and Planet Downs more similar to each other than either is to Roma (Tab. 6). Thus selective effects that relate to climatic variables may contribute to the divergence among populations of *D. aldrichi* in allozyme frequencies, and in the quantitative traits of viability, developmental time and body size.

Table 6. Climatic variables, yearly mean temperature (TMEAN), mean summer temperature (TSUMMER), mean winter temperature (TWINTER), mean summer maximum (SUMMAX), mean winter minimum (WINMIN), average temperature range per day (AVTRY), and average annual rainfall (RAIN) for the three populations, determined from the 30 year normals at the nearest meteorological site: Roma (itself), Planet Downs (the mean of Biloela and Springsure) and Binjour (Gayndah). Seasonality indices were measured as the difference between mean January and July temperatures (Seas-T) and between mean January and July rainfall (Seas-R).

	Roma	Binjour	Planet Downs
TMEAN	20.2 °C	20.5 °C	20.8 °C
TSUMMER	26.8	25.7	26.4
TWINTER	12.9	14.5	14.4
SUMMER	34.2	32.4	33.2
WINMIN	4.9	6.1	6.2
AVTRY	15.6	15.6	15.4
RAIN	520 mm	760 mm	680 mm
SEAS-T	15.5 °C	12.3 °C	13.5 °C
SEAS-R	29.2 mm	75.2 mm	71.6 mm

Coevolution among populations

Coevolution would be indicated if populations of the two species from the same locality both had higher performance when paired with each other than when paired with any other population. Although the two species appear to have responded to local environmental differences with respect to the quantitative traits, there is little evidence for coevolution resulting from competition with the other species, as indicated for some other *Drosophila* (Budnik and Brncic, 1974). However, viability and weight of Binjour and Planet Downs *D. buzzatii* were largest when each was paired with the *D. aldrichi* population from the same locality, and developmental time of Planet Downs was least when paired with Planet Downs *D. aldrichi*. Also, viability of the Planet Downs *D. aldrichi* population was highest when paired with Planet Downs *D. buzzatii*. Although these comparisons may be confounded by the parallel relative performances of the populations of both species, Roma > Binjour > Planet Downs, the results are suggestive that local adaptation of populations of different species to each other may develop.

Temperature, competition and the natural distribution of the species

Typically, *Drosophila* are larger at lower temperatures and, as temperature decreases, males increase more in absolute size than do females (Robertson, 1987). Thus the smaller adult size of both species at the intermediate temperature, 28° C, and the proportionately greater decrease in female body size at this temperature suggest that factors other than temperature were important. One likely possibility is resource availability. If larval growth rate increased more than microbial growth when temperature was increased from 25° C to 28° C, resources would have become limited more quickly at the higher temperature. Food limitation would then force larvae to pupate while small, and females, being much larger under optimal conditions, would decrease more in body size than males (Gebhardt and Stearns, 1988). At 31° C, viability may have decreased because of greater temperature stress on larvae, with more deaths unrelated to limitations in resources. The resulting reduced densities at 31° C would have increased the available food per surviving larva, so that adults would be larger than those reared at 28° C.

We have shown that rearing temperature affects the interactions between these species. Viability of both species was lowest at the highest temperature, but effects on *D. buzzatii* were more severe (Tab. 3). If reared at 31° C, some *D. buzzatii* males, but no *D. aldrichi* males, are infertile, and remain so until transferred to a lower temperature (unpubl.). The developmental time of *D. aldrichi* was longer than that of *D. buzzatii* at 25° C, but shorter at 31° C (Fig. 3A, B). Clearly *D. aldrichi* is superior for some fitness components at the highest temperature used in these experiments. Summer maximum temperatures in the northern part of the cactus distribution where both species are found commonly exceed 31° C (Tab. 6). Thus temperature variation throughout the cactus distribution may contribute to the different ranges of these two *Drosophila* species: with competitive exclusion of *D. aldrichi* in the cooler south, but coexistence in the warmer north.

In addition, the observed decrease in preadult viability in both species at the highest temperature may facilitate coexistence in the warmer north. If the larval resources that are shared by the two species are in fact limiting at lower temperatures, then the decreased viability at high temperatures will increase resource availability and reduce the intensity of interspecific interactions. Similar relationships between other species, especially where there are growth stages that are subject to environmental extremes, could lead to coexistence where physiological, if not ecological, differences are present.

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Corresponding Editor: V. Loeschcke