Thermal adaptation in *Drosophila serrata* under conditions linked to its southern border: unexpected patterns from laboratory selection suggest limited evolutionary potential

ANDRÉA MAGIAFOGLOU and ARY HOFFMANN*

Centre for Environmental Stress and Adaptation Research, La Trobe University, Bundoora, Victoria 3086, Australia

Abstract

To investigate the ability of *Drosophila serrata* to adapt to thermal conditions over winter at the species southern border, replicate lines from three source locations were held as discrete generations over three years at either 19°C (40 generations) or temperatures fluctuating between 7°C and 18°C (20 generations). Populations in the fluctuating environment were maintained either with an adult 0°C cold shock or without a shock. These conditions were expected to result in temperature-specific directional selection for increased viability and productivity under both temperature regimes, and reduced development time under the fluctuating-temperature regime. Selection responses of all lines were tested under both temperature regimes after controlling for carry-over effects by rearing lines in these environments for two generations. When tested in the 19°C environment, lines evolving at 19°C showed a faster development time and a lower productivity relative to the other lines, while cold shock reduced development time and productivity of all lines. When tested in the fluctuating environment, productivity of the 7–18°C lines selected with a cold shock was relatively lower than that of lines selected without a shock, but this pattern was not observed in the other populations. Viability and body size as measured by wing length were not altered by selection or cold shock, although there were consistent effects of source population on wing length. These results provide little evidence for temperature-specific adaptation in *D. serrata*—although the lines had diverged for some traits, these changes were not consistent with a priori predictions. In particular, there was no evidence for life-history changes reflecting adaptation to winter conditions at the southern border. The potential for *D. serrata* to adapt to winter conditions may therefore be limited.

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Introduction

Adaptive constraints in natural systems can involve a number of factors, and the border of a species distribution provides an opportunity to investigate some of these constraints in nature (Mayr 1963; Hoffmann and Blows 1994; Hoffmann *et al*. 1995; Garcia-Ramos and Kirkpatrick 1997; Kirkpatrick and Barton 1997). Laboratory selection experiments, when combined with field information, provide a means of clarifying some of the factors that can be involved in these constraints (Blows 1993; Blows and Hoffmann 1993; Jenkins *et al*. 1997; Harshman and Hoffmann 2000; Scheiner 2002). For instance, laboratory natural selection of populations exposed to different temperatures can suggest a fitness trade-off among environments because populations perform relatively better in the environment where they evolved (Lenski and Bennett 1993; Partridge *et al*. 1994a; Partridge *et al*. 1995). Similarly, artificial selection experiments and correlated responses to selection highlight the costs that can occur when animals respond to abiotic conditions (Cohan and Graf 1985; Krebs and Loeschcke 1996; Gibbs *et al*. 1997; Jenkins *et al*. 1997; Baer and Travis 2000). Selection for traits closely

*For correspondence. E-mail: a.hoffmann@latrobe.edu.au.

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associated with fitness may also identify potential constraints to evolutionary change (Reeve and Fairbairn 1999; Foley and Luckinbill 2001; Kern *et al*. 2001; Mockett *et al*. 2001; Prasad *et al*. 2001). Nonetheless, inferences pertaining to natural systems based on laboratory selection responses can be misleading if selection regimes are not ecologically relevant, or if genetic variances of laboratory populations are not reflective of those in nature (Harshman and Hoffmann 2000; Hoffmann *et al*. 2001; Linnen *et al*. 2001; Scheiner 2002).

A number of causes may account for evolutionary constraints but one obvious cause is insufficient genetic variation for phenotypic change and adaptive evolution. Inbreeding, genetic drift, historical directional selection, and a low rate of mutation can all influence genetic variances (Cheverud 1984; Barton and Turelli 1989; Arnold 1992; Crespi 2000; Hoffmann and Hercus 2000). Furthermore, even if genetic variance is present, pleiotropic effects and epistatic interactions may prevent much adaptation (Cheverud 1984; Partridge and Sibly 1991; Blows 1993; Hoffmann *et al*. 1995; Blows and Hoffmann 1996). Gene flow leading to swamping of adapted genotypes may be particularly pertinent in natural populations where adapted gene complexes are continually being diluted through migration (Dhondt *et al*. 1990; Hoffmann and Blows 1994; Garcia-Ramos and Kirkpatrick 1997; Holt and Gomulkiewicz 1997; Kirkpatrick and Barton 1997; Case and Taper 2000; Hoffmann and Hercus 2000; Lenormand 2002).

In this study we test the response of *Drosophila serrata* collected from marginal and central locations to ecologically relevant selection regimes. *D. serrata* has been the subject of many field and laboratory-based investigations querying ecological and evolutionary constraints acting at species borders (Blows 1993; Blows and Hoffmann 1993; Jenkins and Hoffmann 1999, 2000, 2001; Magiafoglou *et al*. 2002; Magiafoglou and Hoffmann 2003). This species utilizes a variety of breeding sites, and resource utilization is unlikely to affect the position of the southern border (Jenkins and Hoffmann 2001; van Klinken and Walter 2001). Comparative studies of Australian drosophilids have shown that *D. serrata* has a greater susceptibility to cold stress compared to other species with more southerly (i.e. colder) distributions (Jenkins and Hoffmann 1999). Conspecific studies indicate clinal and seasonal variation for fitness-related traits including development time and viability (Magiafoglou *et al*. 2002). Clinal patterns were also observed for resistance to cold shock with winter collections of flies showing increased resistance towards the border. Microsatellite variation reveals low levels of genetic differentiation among southern populations (Magiafoglou *et al*. 2002). These findings suggest that natural selection is responsible for the observed geographic patterns and that extensive gene flow may prevent expansion of the southern border. Winter field cage trials, both within and beyond the distribution of *D. serrata*, support the negative impact of cold exposure on fitness under seminatural conditions; with increasing marginality, productivity and viability fitness measures declined concurrently, and larval development was extremely slow (Jenkins and Hoffmann 1999; A. Magiafoglou, unpublished data). Offspring of flies held in field cages located beyond the distribution (approximately 70 km) were unable to successfully develop until springtime conditions were encountered (A. Magiafoglou, unpublished data). Diapause has not been detected in this species but flies appear to exhibit quiescence, where reproduction is immediately resumed after conditions improve.

Current research therefore indicates that the southern border of *D. serrata* is associated with cool winter conditions and, hence, variation in cold resistance may be limiting further range expansion. Clinal variation for cold resistance is evident only after winter (Jenkins and Hoffmann 1999; Magiafoglou *et al*. 2002), suggesting that selection for increased cold resistance over winter leads to adaptive differences that are lost again in warmer months, perhaps through gene flow or tradeoffs with fecundity. Genetic variation in adult cold resistance does not appear to be limiting because parent–offspring regressions indicate heritable variation for this trait in the field (Jenkins and Hoffmann 1999). However, adult cold resistance measures only one component of the suite of traits likely to be under selection over winter, as winter field cage trials indicate that low productivity and very slow development can limit the ability of *D. serrata* populations to persist over winter.

Here we address the potential of *D. serrata* populations to evolve and increase adult reproductive ability and preadult fitness under cool conditions by undertaking a laboratory natural selection experiment where populations of *D. serrata* were maintained under two selection regimes. These were designed to reflect the temperature and photoperiod parameters typically experienced by *D. serrata* at the southern species border during winter. One of the regimes involved daily exposure to temperatures ranging from 7 to 18°C and a cold shock of 0°C, conditions typically experienced over winter at the southern border, along with the photoperiod experienced at this time. The other selection regime included the fluctuating temperatures but no cold shock. For comparison, replicate populations were also maintained under a constant 19°C temperature and light laboratory regime.

The following questions are considered:

(i) Can populations evolve to increase fitness after adult exposure to nonlethal cold shock? Laboratory investigations suggest reproductive ability can be adversely affected by exposure to nonlethal stress (Krebs and Loeschcke 1994a,b; Patton and Krebs 2001; Bubliy *et al*. 2002), while field investigations support a reduction in productivity after exposure to cool conditions (A. Magiafoglou,

unpublished data). Fitness effects may be mediated by carry-over effects in *D. serrata* impacting on viability and development time in progeny (Magiafoglou and Hoffmann 2003) as well as directly on the adults. This may be particularly important at the species border where parental cold exposure over winter could influence the fitness of offspring emerging in spring.

(ii) Can populations evolve to increase fitness under fluctuating temperatures and a photoperiod? In *D. serrata* preadult development is arrested at constant temperatures below 14°C (Jenkins 1999). Meteorological data indicate that this threshold is often exceeded in southern locations and development appears restricted over winter until conditions become warmer (A. Magiafoglou, unpublished data).

(iii) Do population differences exist for evolvability? Central and marginal populations of *D. serrata* may differ for evolvability in response to desiccation stress (Blows 1993; Blows and Hoffmann 1993) although microsatellite variation (Magiafoglou *et al*. 2002) and levels of variation for other traits (Jenkins and Hoffmann 1999, 2000) suggest that populations have similar overall levels of genetic variability.

Materials and methods

***Stocks and selection*:** *D. serrata* stocks were initiated using field females collected from three localities in April 1998: Wollongong (34º40′S, 150º56′E), the southern species border, and Forster (32º12′S, 152º32′E) and Coffs Harbour (30º01′S, 153º10′E), located approximately 300 km and 600 km north of the southern border respectively. To establish mass-bred populations for each locale, equal numbers of progeny from 25 inseminated field females were combined. These populations, designated as W (Wollongong), F (Forster) and C (Coffs Harbour), were held at 25°C ± 0.5°C for 20 generations at a census size of 1000 flies. While laboratory adaptation is known to occur in this species, population-specific responses for stress and life-history measures appear uncommon (Hoffmann *et al*. 2003; M. Schiffer, unpublished data). At this time, three replicate strains (i.e. W1, W2 and W3) were generated from each population and maintained independently within each selection environment. Prior to the present study, stocks from 19°C had undergone about 40 generations of 19°C culture compared to 20 generations in the fluctuating environment owing to a slower generation time.

***Selection regime I (S-I, fluctuating with cold shock)*:** This regime incorporates conditions designed to reflect average minimum and maximum temperatures and photoperiod parameters that *D. serrata* individuals experience at the southern species border over the coldest winter month at Wollongong (Australian Bureau of Meteorology, www.bom.gov.au). Temperature fluctuated daily from 7°C (12 h) to 18°C (12 h), with a photoperiod of LD 10 h : 14 h. The lower temperature consistently occurred during the dark cycle of the photoperiod regime. Adults were also subjected to a nonlethal cold shock, because at the southern border conditions may occasionally approach 0°C for a short period (Australian Bureau of Meteorology). Emerging adults were collected at the peak emergence period over 5–6 days and combined before they were distributed evenly across four 200-ml culture (yeast – sugar – agar – potato whip) bottles at densities of approximately 200 flies per bottle. Adults were then held at 19°C for 1–2 days prior to the cold shock, at which time they were transferred to empty 200-ml bottles and immersed for one hour in a 20% ethylene glycol solution cooled to 0°C. This cold-shock treatment was designed to prevent mortality but was severe enough to induce coma in the flies. After one hour, bottles were removed and placed at 19°C. Adults were subsequently transferred to fresh culture bottles until the following day when the process was repeated. After the cold-shock treatment, flies were serially transferred to fresh culture bottles every three days before flies were discarded at 11–16 days post-eclosion. After each transfer, bottles containing eggs and larvae were moved to the fluctuating-temperature environment (7–18°C) for development. Around 300 flies emerged per bottle, much less than the number that can be supported by this culture medium without overcrowding (> 500 flies without marked reduction in size). Flies were usually collected from one set of bottles (either the first or second set) but sometimes combined across both sets to increase numbers. Flies eclosing from these bottles were collected over a 5–6-day period from when adults first emerged, and the cycle was repeated. Because flies emerged over a longer time frame than the collection period, this regime was expected to select for faster development time. This selection regime was also expected to select directionally for increased viability and high productivity at the peak of fecundity (which occurs after 10 days at this temperature).

***Selection regime II (S-II, fluctuating without shock)*:** Stocks maintained under this environment act as a control to the adult cold shock selection procedure above. Temperature and photoperiod parameters were as for selection regime I (7°C 12 h, 18°C 12 h, LD 10 h : 14 h). All aspects of the maintenance of these stocks were identical to the above procedure (including being placed at 19°C, alongside flies from S-I), except that adults were not exposed to the cold-shock treatment. Eggs were collected via serial transfer in the method outlined above using adults of the same age range as for S-I (i.e. 8–16 days). Furthermore, larval densities were comparable to those in the S-I regime. As in the previous regime, increased viability, fast development and high productivity would have been favoured in this regime.

***Selection regime III (S-III, constant)*:** This regime is typical of *D. serrata* laboratory maintenance techniques. Strains were cultured at 19°C under constant light. Emerging progeny were collected over 2–3 days at the peak emergence period (when more than 95% of the flies had emerged). We set up a minimum of four replicate 200-ml culture bottles for each line, with each bottle housing approximately 150 flies. These flies were held for seven days and then used to establish the next generation by serially transferring adult flies to fresh culture bottles every three days until approximately 12 days post-eclosion. Parental age at reproduction therefore ranged between 7 and 12 days. This falls within the age range of peak production in this species when held at 19°C (7–14 days). Offspring were collected from all replicate bottles (from either the first or second set of culture bottles) and then randomly distributed across fresh culture bottles (approximately 150 individuals per bottle) to initiate the next generation. Larval densities were again well below the maximum number that could be supported by the culture medium without overcrowding. This regime was expected to select for increased viability and productivity under 19°C but selection on development time would have been weak.

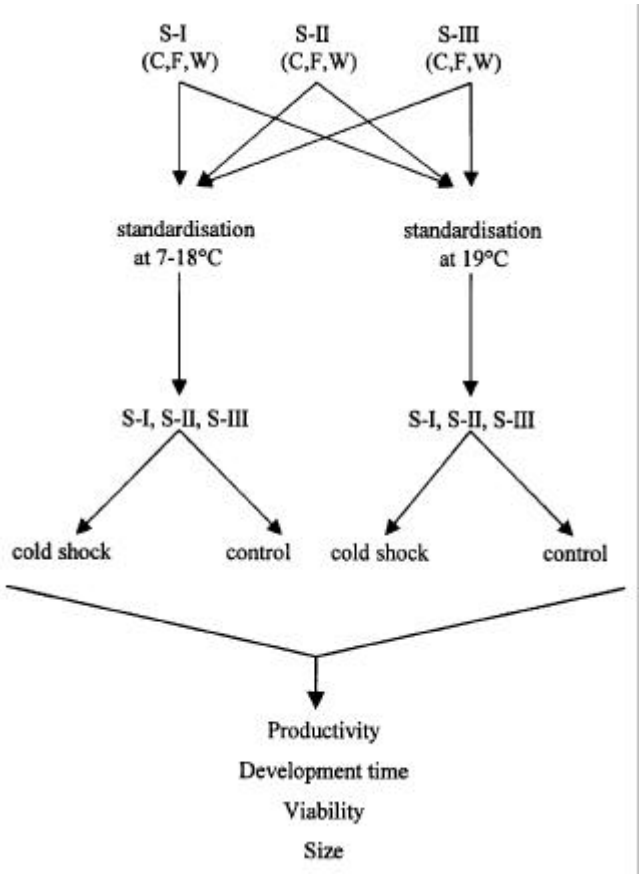

Figure 1. Experimental design testing selection response. Populations (C, F and W) from each selection regime (S-I, S-II and S-III) were standardized by rearing at 7–18°C 10L : 14D (for testing at 7–18°C 10L : 14D) and at 19°C 24L (for testing at 19°C 24L) for two generations. Productivity, development time, viability and size were then measured for all populations and selection regimes within each environment.

***Selection response*:** To test the selection response, development time, viability, productivity and wing size of all stocks were measured within each environment in which selection had occurred (i.e. 19°C (24L) and 7–18°C (10L : 14D) environments) (see figure 1). As cross-generation effects due to environment can influence fitness measures, all stocks were cultured for two generations at a constant density within each testing environment prior to testing.

The experimental protocol within each testing environment was similar to that used for selection. After being reared for two generations to control for carry-over effects, flies were collected over five days within the 7–18°C testing environment, and over three days within the 19°C environment, reflecting the selection procedure. At an average of 3 days of age (2–4 days post-eclosion for 19°C testing environment or 1–5 days post-eclosion for 7–18°C testing environment), flies from each strain were sexed under CO_2 and sorted into four 200-ml culture (yeast – sugar – agar – potato whip) bottles, each containing 50 pairs. These were held at 19°C until two bottles from each strain had undergone the cold-shock treatment.

Cold shock (0°C ± 0.5°C for 1 h) involved exposure on two consecutive days from approximately 7 days of age (or 6–8 days post-eclosion at 19°C or 5–9 days post-eclosion at 7–18°C). Flies from two replicate bottles of each strain were transferred to two empty bottles and exposed to the same cold-shock treatment outlined in selection regime I.

To score productivity, three replicate 40-ml vials (containing 15 ml of culture media) each holding two pairs of adults were set up per replicate bottle. Hence, a total of six replicate productivity vials were set up per strain for each treatment. Productivity was measured over six days, beginning 48 h after exposure to the second cold-shock (or control) treatment. Flies were sexed without CO_2 anaesthesia to prevent confounding effects. To avoid density effects within these vials, adults were serially transferred to fresh vials every two days. At this time the vials, containing eggs and some larvae, were transferred to the appropriate testing environment to allow development of progeny. Productivity was then scored as the average number of offspring emerging from the vials.

To score viability and development time, eggs were collected from flies on the third day after the cold-shock or control treatment exposure. Two replicate bottles of flies (each with at least 35 adult pairs) from each treatment group (cold shock and control) were set up on spoons containing treacle–semolina–yeast–agar medium to stimulate oviposition. Eggs were collected over a 19-hour period

and transferred to vials each containing 15 ml of medium (yeast – sugar – agar – potato whip) (10 eggs per vial and four vials per treatment). Vials were then randomly arranged in a container and held within each testing environment until fully developed. To determine development time, flies emerging from vials were collected every eight hours (19°C testing environment) or every 24 h (7–18°C testing environment). Vials were scored until no new adults emerged for >48 h.

Size was scored as wing length. Wings of individuals emerging from the viability vials were mounted on slides to measure adult wing length. Images of wings were captured using a Wild M38 microscope attached to a Panasonic WV-GP460 digital camera. Wing length was determined by placing landmarks at the intersection of the anterior cross vein with the third longitudinal vein and at the wing tip of the third longitudinal vein, using the computer program tpsDig version 1.2 written by F. James Rohlf. This program expresses landmarks as *x* and *y* Cartesian coordinates, from which wing length was calculated.

***Statistical analyses*:** Prior to analysis, data were tested for normality, presence of outliers and equality of variances through the Kolmogorov–Smirnov, Grubbs and Scheffe–Box tests respectively.

Statistical analysis of development time, size and productivity data was via ANOVAs. Cold-shock treatment (CS), selection regime (SEL) and population (POP) were treated as fixed main factors with strain (STR) treated as a random factor nested within both selection regime and population. To determine appropriate variance components and error terms for the ANOVA design, expected mean squares were calculated following the rules outlined in Hicks (1973).

Viability data across both testing environments showed skewed distributions and transformation did not fully overcome this problem. Despite this, ANOVAs (based on arcsine-transformed data) were undertaken as the validity of these analyses is not greatly affected by skewed distributions (Zar 1996). Nevertheless we did also undertake nonparametric analyses of the data (Kruskal–Wallis tests) to verify any significant differences among groups (data not presented).

Development time and productivity measures can be sensitive to changes in viability and size respectively. To determine if these variables influenced the results, analyses of covariance were used to correct for differences in viability among vials and size among individuals. However, inclusion of these covariates did not ultimately change the results and ANOVAs are therefore presented without inclusion of covariates.

Results

Selection might lead to temperature-independent or temperature-dependent responses or both. If responses were temperature independent, we did not necessarily anticipate changes in viability or productivity unless the additional generations of culture experienced by the 19°C lines had resulted in further selection responses. However, for development time we anticipated selection for rapid development in the fluctuating environment but not in the constant environment. For temperature-specific responses, we predicted that the lines from the fluctuating-selection environment would have a higher productivity and viability at 7–18°C relative to the S-III lines but that the opposite result should be evident at 19°C. The flies from the fluctuating-selection environment were also expected to show more rapid development but only under 7–18°C. The results indicate that these predictions were generally not met.

19°C testing environment

Exposure to cold shock decreased the development time of females and males (figure 2), although only the effect for females was significant (table 1). An effect of selection regime was evident for both sexes, with S-III flies having a faster development time than those from either fluctuating-selection regime. An interaction between cold-shock exposure and strain was evident for females, while for male development time there was a significant effect of the nested strain term (table 1). This appeared to be mainly due to variation among the Forster strains from S-III. The relatively rapid development of the 19°C strains (S-III) suggests a temperature-dependent response to selection.

For viability there were no significant effects of treatment, selection regime or population (table 1). Therefore there was no regime-specific response to selection for this trait.

Neither cold-shock exposure nor selection regime had an effect on the wing length of either sex. Population differences were found (table 1, figure 3) owing to the relatively larger size of flies from the Coffs Harbour population. Strain effects were also evident for both sexes. Female strain effects were mostly due to variation in the Forster strains under both selection regimes I and II, while the Coffs Harbour and Forster populations from S-I contributed to strain variation for male size.

Cold-shock exposure significantly affected productivity (table 1). This effect was due to a decrease in productivity following cold shock in populations from the S-I and S-II regimes but not the constant-selection environment (figure 4). Selection significantly affected productivity (table 1), owing to a reduced productivity of flies from the 19°C selection environment (S-III) compared to the other regimes (figure 4). The ANOVA also showed a significant interaction between cold-shock treatment and selection regime, as productivity was not reduced in the S-III lines. The overall reduction in productivity in the 19°C selection lines was contrary to expectations.

7–18°C testing environment

Female development time was extended in this environment and differed significantly among populations (table 2). Generally flies from the Coffs Harbour population had a faster development time than flies from Forster or Wollongong (figure 2). A significant interaction between selection regime and population was also evident (table 2), suggesting that selection responses depend partly on population. For Wollongong development was slower in the S-I regime, but this pattern was not evident in the other populations. The effect of cold shock was negligible although this treatment did show an interaction with selection regime and population (table 2) owing to the response of the Coffs Harbour population (figure 2), which showed a reduction in female development time after parental exposure to cold shock.

For male development time, the only significant main effect was due to cold-shock treatment (table 2) because of a reduction in development in offspring from shocked parents (figure 2). As in females, this effect was particularly evident in the Coffs Harbour strains under the S-I selection regime.

As in the 19°C testing environment, viability did not differ statistically among any of the treatment groups, selection regimes or populations (table 2), even though strains from the fluctuating environment (S-I and S-II) were expected to have a relatively higher viability than those held at 19°C (S-III) if temperature-specific adaptation had occurred.

Both males and females showed significant population differences for wing length (table 2), which had increased in this environment compared to the 19°C testing environment (figure 3). Consistent with results from the 19°C testing environment, Coffs Harbour flies of both sexes tended to be relatively larger, while flies from Wollongong tended to be the smallest (figure 3). For male wing length, the ANOVA also showed an effect of strain nested within population and selection (table 2). This effect is mainly due to a strain of the Coffs Harbour population (S-II) being larger than other strains from this population.

Productivity was sharply reduced in the fluctuating environment (figure 4) and there was an interaction between cold shock and selection regime (table 2). We had anticipated that lines selected in the presence of a shock would have shown a relatively higher productivity in the

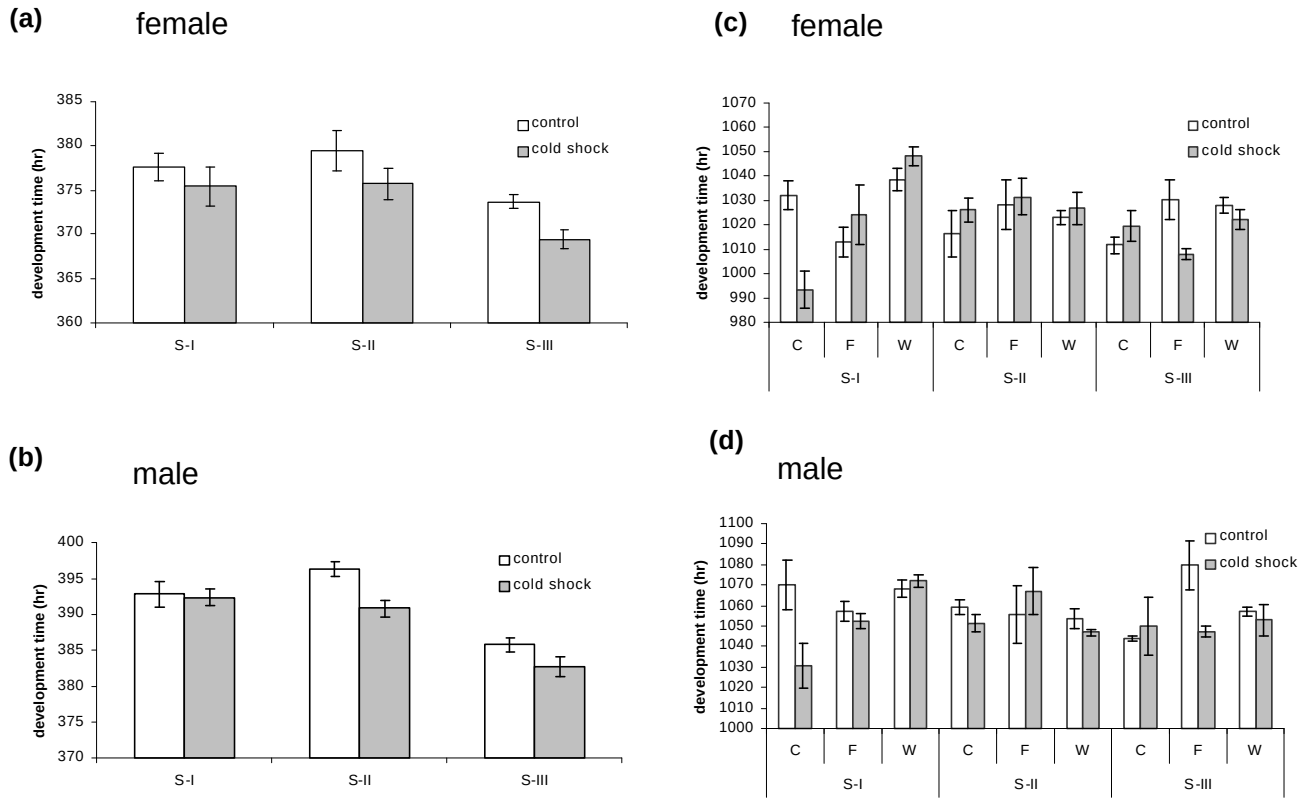

Figure 2. Female (a) and male (b) development time tested within 19°C 24L: development time data are pooled across populations for lines tested within 19°C 24L; error bars are based on population means within selection background and represent ± 1 SE. Female (c) and male (d) development time tested within 7–18°C 10L : 14D: error bars are based on strain means within population and selection background and represent ± 1 SE. C, Coffs Harbour; F, Forster; W, Wollongong; S-I, S-II and S-III refer to selection regimes.

presence of the shock; there was some support for this prediction, as differences between the shock and non-shock treatments tended to be smaller in the S-I lines compared to the S-II lines (figure 4). There was also an unexpected increase in productivity following a cold shock in the 19°C lines.

Discussion

Drosophila species frequently show thermal evolution under laboratory conditions (Cavicchi *et al*. 1989, 1991; Azevedo *et al*. 1996; Hercus and Hoffmann 1999; Matos *et al*. 2002). Thermal selection influences a string of pre-

Table 1. Summary of ANOVAs for the 19°C environment. Stocks were standardized at 19°C before being tested within that environment for development time (DT), viability, size and productivity. The effects of cold shock (CS), selection (SEL), population (POP), and strain (STR) nested within population and selection were tested.

	Female DT		Male DT		Viability		Female size		Male size		Productivity	
Model terms	MS	d.f.	MS	d.f.	MS	d.f.	MS	d.f.	MS	d.f.	MS	d.f.
CS	544.15*	1	479.50	1	10.49	1	93.24	1	21.89	1	611.25***	1
SEL	729.3**	2	1886.44**	2	63.51	2	220.28	2	76.82	2	574.96***	2
POP	72.69	2	157.13	2	8.22	2	379.39*	2	891.09**	2	127.66	2
STR (POP*SEL)	77.80	18	264.02***	18	198.80	18	234.00**	18	208.45**	18	56.48	18
CS*SEL	13.14	2	109.89	2	183.52	2	51.20	2	293.42	2	148.67*	2
CS*POP	212.99	2	31.30	2	477.44	2	58.35	2	90.44	2	50.93	2
SEL*POP	86.31	4	33.91	4	288.61	4	115.85	4	491.85	4	154.67	4
CS*SEL*POP	88.33	4	40.17	4	92.21	4	90.03	4	35.68	4	28.59	4
CS*STR (POP*SEL)	105.52*	18	117.12	18	159.02	18	107.44	18	100.17	18	31.21	18
Error	62.07	157	90.73	160	206.26	162	101.30	316	92.57	316	44.02	259

MS, Mean square. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

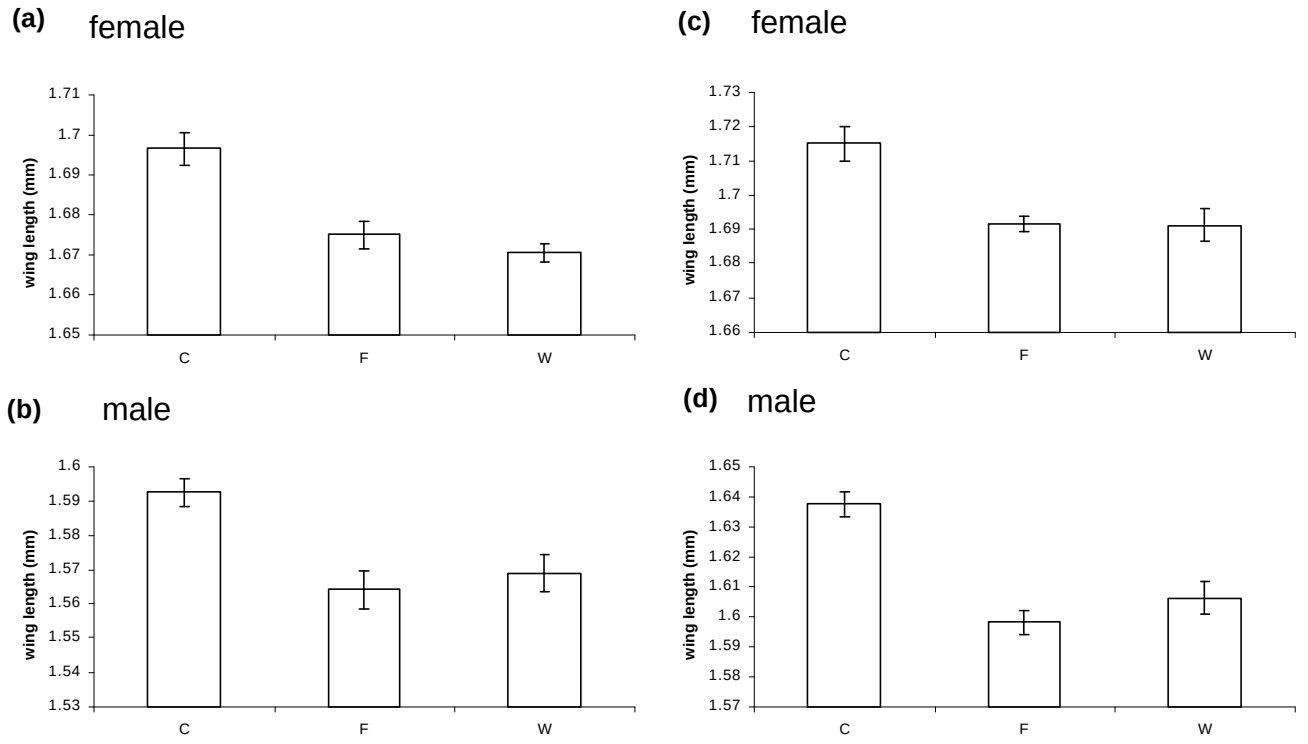

Figure 3. Female (a) and male (b) size tested within 19°C 24L; female (c) and male (d) size tested within 7–18°C 10L : 14D. Data are pooled across selection background; error bars are based on selection regime means and represent ± 1 SE. C, Coffs Harbour; F, Forster; W, Wollongong.

adult measures including larval critical weight, development time and survival (Huey *et al*. 1991; Partridge *et al*. 1994a; James and Partridge 1995; Gilchrist *et al*. 1997). Adult characteristics may also be responsive to thermal evolution, with changes demonstrated in life-history, morphological and physiological measures (Partridge *et al*. 1994b, 1995; Azevedo *et al*. 1996; Gilchrist *et al*. 1997). Thermal sensitivity of preadult and adult characters in *D. melanogaster* can be altered by laboratory natural selection using temperatures within the thermal range of this species (16.5–28°C).

In contrast to these results, we found that selection in *D. serrata* failed to increase fitness in response to temperature and photoperiod conditions experienced at the southern border. We had anticipated that the selection response would include temperature-specific shifts in fitness leading to higher productivity and increased viability in the environment where selection had occurred. Development times of both males and females were expected to be shorter in the fluctuating environment. On the basis of other *Drosophila* studies, we also expected that lower temperatures might lead to a larger body size (Partridge *et al*. 1994b).

While the results do indicate changes due to selection, these generally ran counter to expectations. In the 19°C environment, the flies selected at 19°C showed an unexpected lower productivity. In this environment, there was also an unexpected decrease in productivity in the S-I lines following exposure to cold shock, compared to an absence of cold-shock effects in the S-III lines. At 7–18°C, flies selected in the fluctuating environment failed to show an increase in fitness for any of the traits. Therefore no temperature-specific adaptive shift was evident in this environment.

How do we explain the absence of changes specific to temperature regimes in these lines? One possibility is that there was insufficient time for much adaptive differentiation to develop in the lines. In other experiments with *Drosophila* maintained under different temperature regimes, differences were characterized after flies had been

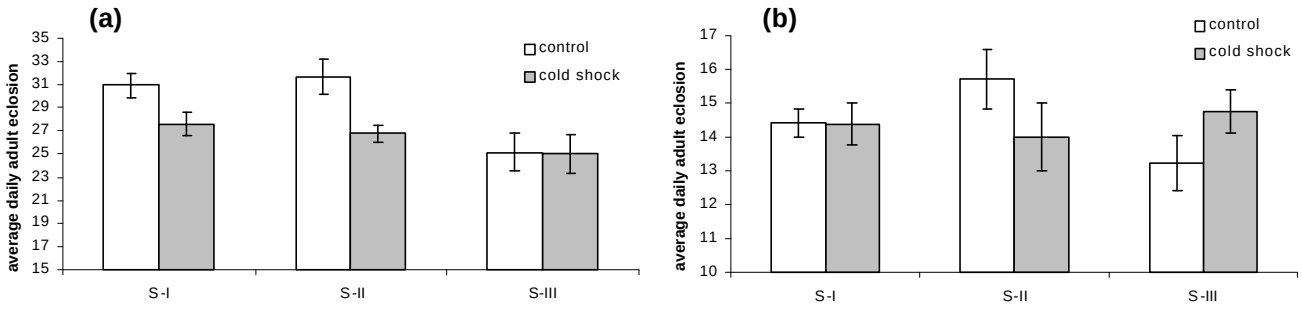

Figure 4. Average productivity of lines tested within 19°C 24L (a) and 7–18°C 10L : 14D (b). Productivity represents average daily adult eclosion from eggs laid over six days. Productivity data are pooled across populations within selection background. Error bars are based on population means within selection regime and represent ± 1 SE. S-I, S-II and S-III refer to selection regimes.

Table 2. Summary of ANOVAs for the 7–18°C environment. Stocks were standardized at 7–18°C before being tested within that environment for development time (DT), viability, size and productivity. The effects of cold shock (CS), selection (SEL), population (POP), and strain (STR) nested within population and selection were tested.

	Female DT		Male DT		Viability		Female size		Male size		Productivity	
Model terms	MS	d.f.	MS	d.f.	MS	d.f.	MS	d.f.	MS	d.f.	MS	d.f.
CS	288.20	1	3460.87**	1	522.04	1	0.61	1	11.31	1	0.48	1
SEL	586.68	2	214.44	2	230.70	2	534.77	2	89.67	2	19.19	2
POP	3649.46**	2	1556.77	2	110.95	2	288.69**	2	521.94***	2	61.71	2
STR (POP*SEL)	557.95	18	1033.44	18	293.04	18	175.43	18	221.65*	18	20.37	18
CS*SEL	792.88	2	673.12	2	382.51	2	5.94	2	107.03	2	66.52**	2
CS*POP	405.09	2	542.54	2	253.00	2	110.09	2	142.81	2	27.06	2
SEL*POP	1681.86*	4	1517.70	4	128.62	4	360.33	4	458.07	4	19.9	4
CS*SEL*POP	2758.39**	4	2727.38**	4	163.43	4	24.42	4	82.62	4	22.73	4
CS*STR (POP*SEL)	391.14	18	397.09	18	124.80	18	163.02	18	241.54*	18	10.44	18
Error	453.86	152	633.32	153	227.56	155	136.64	326	121.65	329	16.99	259

MS, Mean square. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

reared at constant temperatures for several years (Cavicchi *et al*. 1989; Huey *et al*. 1991; Partridge *et al*. 1995; Gilchrist *et al*. 1997). Although we maintained lines under the different regimes for more than two years, that may have been insufficient time for much divergence to develop. However, we did observe differences among the selection regimes (development time and productivity in the 19°C environment), suggesting that this explanation does not apply to all the traits. Moreover, changes in laboratory populations held under different conditions can occur extremely rapidly, as recently demonstrated in *D. melanogaster* (Kennington *et al*. 2003).

While the experimental design does not allow determination of the exact nature of constraints, the results can be related to factors limiting adaptation to the southern border of *D. serrata*. Within this context, we discuss the importance of the results to hypotheses on evolutionary constraints acting at species borders (Hoffmann and Blows 1994). Constraints include a lack of genetic variation, antagonistic pleiotropy and cross-generation effects, with the former often being considered as important (Brussard 1984; Hoffmann and Blows 1994; Lammi *et al*. 1999; Hoffmann and Hercus 2000). Several *D. serrata* studies have attempted to address the genetic variation issue (Blows 1993; Blows and Hoffmann 1993; Jenkins and Hoffmann 1999, 2000; Magiafoglou *et al*. 2002). Blows and Hoffmann (1993) showed that *D. serrata* can respond to selection for desiccation resistance, while Jenkins and Hoffmann (1999) undertook parent–offspring comparisons (field and laboratory) to demonstrate heritable variation for cold resistance, albeit at lower temperatures than normally experienced in the natural environment of this species. Further, clinal investigations indicate *D. serrata* can respond to selection for cold resistance and life-history traits in the face of extensive gene flow (Magiafoglou *et al*. 2002). Microsatellite markers indicate low levels of genetic differentiation among populations across the southern range, providing no evidence that marginal populations are genetically isolated from central groups. Further, changing selection pressures across seasons appear to influence clinal patterns in development time, viability and cold resistance (Magiafoglou *et al*. 2002). Hence, it seems that low overall levels of genetic variation in southern populations cannot account for the position of the border or the inability of laboratory populations to respond to conditions experienced at the border.

Low genetic diversity within lines, as a consequence of inbreeding or genetic drift throughout selection, can limit selection responses. In the present study, independent strains were maintained in excess of 600 individuals, and differences among strains within population and selection regime were few and inconsistent, suggesting that drift is an unlikely cause of the patterns observed in this study. Furthermore, in both *D. serrata* and its sibling species *D. birchii*, we have found that maintenance of populations under these conditions leads to no detectable loss of genetic variation based on microsatellite markers (Hoffmann *et al*. 2003; R. Hallas, unpublished data).

Trade-offs resulting from pleiotropy may constrain evolutionary change within both field and laboratory environments (Barton and Turelli 1989; Partridge and Sibly 1991; Hoffmann *et al*. 1995). Correlated responses to selection often reveal the presence of trade-offs. For example, Watson and Hoffmann (1996) found a reduction in female fecundity that was associated with increased cold-shock resistance in *D. simulans* and *D. melanogaster*. Previous research in *D. serrata* suggests a negative association between cold resistance and fecundity (Jenkins and Hoffmann 1999), while other, recent research suggests genetic associations between preadult development and adult size (Magiafoglou *et al*. 2002). It is possible that pleiotropy involving traits not measured in this study could have led to the unexpected selection response patterns, particularly as pleiotropy can depend on the testing environment (Hoffmann *et al*. 1995). For instance, trade-offs with an unknown trait may account for the low productivity of the S-III lines in the 19°C testing environment. Productivity is a complex measure incorporating many components, and any of these may have interacted with an unknown selected trait to generate the unexpected changes. Productivity may be affected by indirect selection on development time, as there is a negative relationship between fecundity and larval development time in some species (Krebs and Loeschcke 1999), consistent with the development-time and productivity patterns of the S-III lines when tested at 19°C. The additional generations of culture at 19°C compared to the fluctuating environment probably do not account for the reduction in productivity as laboratory culture tends to select for increased rather than reduced early fecundity (Sgrò and Partridge 2001). Differences in photoperiod may also have contributed to the selection response as fitness traits may be affected by photoperiod (Sheeba *et al*. 2000).

Finally, the responses to selection may have been influenced by carry-over effects as environmental variation experienced in the parental generation can impact on progeny phenotype (Krebs and Loeschcke 1994a; Zamudio *et al*. 1995; Sgrò and Hoffmann 1998) and on responses to selection for thermal conditions (Watson and Hoffmann 1996). In *D. serrata*, exposure to nonlethal cold shock can affect viability, development time and productivity in progeny and these effects have the potential to persist across multiple generations (Magiafoglou and Hoffmann 2003). Selection for increased performance after exposure to cold shock may therefore influence other traits, and perhaps the detection of a selection response.

Previous selection experiments have suggested differences among *D. serrata* populations in evolvability for desiccation resistance (Blows and Hoffmann 1993). How-

ever, while population differences were found for size traits, and, in one instance, female development time, there is not much evidence that the population background affected the selection response (only one interaction between selection and population was significant). Prior investigations indicate low genetic differentiation and extensive gene flow among southern groups (Magiafoglou *et al*. 2002). Border populations are therefore as likely to respond to selection as more central populations.

In conclusion, the reasons why the lines responded unexpectedly are not clear, but they do suggest that the potential of *D. serrata* to evolve to thermal conditions experienced at the border is limited. While there is evidence that this species can adapt to local thermal pressures and respond to changing conditions across seasons (Magiafoglou *et al*. 2002), unknown factors appear to limit the evolution of increased performance in response to abiotic conditions typically experienced at the southern species border. The inhibiting factors do not appear to be associated with low genetic diversity at the border, and further research is required to determine the possible role of antagonistic pleiotropy and cross-generation effects in range expansion.

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