1 **Evolution of reproductive traits have no apparent life-history associated cost in**
2 **populations of *Drosophila melanogaster* selected for cold shock resistance**

3 Karan Singh^{a*} and Nagaraj Guru Prasad^e

4
5

6 ^aCurrent Affiliations: NYU Langone Health, 650 Medical Science Building
7 550 First Ave, New York, NY 10016
8 E-mail: karan.iiser@gmail.com

9 Phone+1-267-881-3908
10

11 ^aPrevious Affiliations: Indian Institute of Science Education and Research Mohali,
12 Knowledge City, Sector 81, SAS Nagar, PO Manauli, Punjab 140306, India
13 Ph: 91 172 2240266, 2240124
14 Fax: 91 172 2240266, 2240124
15 E-mail: karan.iiser@gmail.com

16
17

18 ^bAffiliations:
19 Indian Institute of Science Education and Research Mohali,
20 Knowledge City, Sector 81, SAS Nagar, PO Manauli, Punjab 140306, India
21 Ph: 91 172 2240266, 2240124
22 Fax: 91 172 2240266, 2240124
23 E-mail: prasad@iisermohali.ac.in

24
25 *Corresponding author
26

27 **Keywords:** Reproductive traits, life-history evolution, trade-off, life time fecundity, egg
28 viability and mating number

29

30 **Abstract**

31 In insect species like *Drosophila melanogaster*, the ability to evolve greater resistance or
32 evolution of certain traits under specific environmental conditions leads to energy trade-offs
33 with other important life-history traits. A number of studies from multiple fields have
34 documented the life-history associated cost. However, no known studies have assessed the
35 life-history associated cost with evolved reproductive traits and increase egg viability in cold
36 shock selected population. To explore this, we used replicate populations of *D. melanogaster*
37 that have evolved reproductive traits and egg viability in response to increased resistance to
38 non lethal cold shock. To assess life-history cost; we measured longevity, life time fecundity,
39 Larvae to adults development time, and larvae to adults survival. We found that there were no
40 significant differences in longevity, life time fecundity, larvae to adults survival, and male
41 body weight between the selected and control populations. However, selected populations
42 have significantly longer pre adults developmental time compared to their control population.
43 Females from the selected populations were bigger in size compared to the control
44 populations. These findings suggest that there is no life-history cost associated with the
45 evolution of greater resistance in the aspect of faster recovery of egg viability and
46 reproductive traits post cold-shock. It quite possible the cost of the evolution of reproductive
47 traits and egg viability in response to cold shock resistant is paid in terms of reduced
48 resistance to other stresses

49

50 **INTRODUCTION**

51 A number of ecological factors including temperature are known to vary across time and
52 space and as a result, organisms experience different types of unfavorable environmental
53 conditions during their lifespan. These environmental stresses can be major drivers of
54 evolution of life-history of organisms in nature (Hoffman and Parsons, 1991, reviewed in
55 Parsons, 2005).

56
57 Temperature is one of the fundamental ecological features of an organism's environment.
58 Organisms can respond to extreme temperatures in various ways, like changes in their
59 behavior patterns and physiology or life-history traits (Hoffmann and Parsons, 1991,
60 Patton and Krebs, 2001, Fasolo and Krebs, 2004). Resources used for coping with stress
61 are unavailable for other functions under limited resource conditions, which can lead to
62 trade-offs across important life-history traits such as somatic maintenance and
63 reproduction (Stearns, 1992). For example, one important way in which organisms cope
64 with immediate changes in temperature (heat-shock and cold-shock) is by expressing
65 heat shock proteins (HSPs). Expression of these proteins is extremely costly and is
66 known to affect reproduction (Krebs and Loeschcke, 1994). Thus, temperature shock can
67 affect various important life-history traits (Huey and Berrigan, 2001, Hochachka and
68 Somero, 2002, Sinclair et al., 2003, Angilletta, 2009). Deviation from ambient temperature
69 (where absolute fitness of an organism is maximum) drastically affects various life-history
70 and related traits of insects (Lee and Denlinger, 1991, Voituron et al., 2002, Hoffmann et al.,
71 2003) such as fecundity, male fertility, lifespan (Denlinger and Yocum, 1998, Bubliy and
72 Loeschcke, 2005, Rohmer et al., 2004, reviewed in Hance, 2007, Lieshout et al., 2013,
73 Nguyen et al., 2013, Singh et al., 2016), reproduction (Singh et al., 2016, Singh and Prasad,
74 2016), mating ability (Singh et al., 2015), development time (Trotta et al., 2006, Austin and
75 Moehring, 2013) and motility (Angilletta et al., 2002)

76

77 Several studies have investigated the evolution of life-history traits in response to thermal
78 variation. *D. melanogaster* being widely distributed offers a great model to study the
79 evolution of life-history traits in response to temperature variation across latitudes and
80 altitudes. In general, a number of traits vary progressively across populations inhabiting
81 various latitudes. This pattern of results suggests that life-history evolution in populations of
82 *Drosophila* is primarily being driven by environmental differences and that the populations
83 are adapting to local environment, most probably, including temperature, which is an
84 important component of the environment. Latitudinal clines have been found in a number of
85 life-history traits such as development time, survivorship, larval competitive ability,
86 fecundity and body size (Stanley and Parsons, 1981, Bouletreau-Merle et al., 1982, James and
87 Partridge, 1995, 1998, reviewed in Hoffmann et al., 2003, Hangartner et al., 2015).

88

89 Some experimental evolution studies have investigated the evolution of life-history traits in
90 response to selection for cold stress tolerance (Tucic, 1979, Chen and Walker, 1993, Watson
91 and Hoffmann, 1996, Anderson et al., 2005, Bublly and Loeschcke 2005, MacMillan et al.,
92 2009). However, such studies are a few (Overgaard et al., 2010). Anderson et al. (2005)
93 found increased female fecundity and decreased male longevity in populations of *D.*
94 *melanogaster* selected for rapid chill-coma recovery. MacMillan et al. (2009) also
95 documented reduced longevity in females (but not in males) in populations selected for
96 increased resistance to freeze shock. However, Bublly and Loeschcke (2005) did not notice
97 any difference in longevity and development time in populations of *D. melanogaster* selected
98 for increased cold tolerance. Thus, the correlated evolution of life-history traits in response to
99 cold stress has been fairly variable.

100

In this study, our aim in probing the life-history costs, if any, of increased resistance to cold stress in terms of increased reproductive traits and egg viability (Singh et al., 2015, Singh et al. 2016, Singh and Prasad 2016) in populations of *D. melanogaster* selected for cold shock resistance. To investigate the underlying life-history cost to increased resistance to cold stress, we assayed various life-history (longevity and life time fecundity) and related traits such as development time, and body weight in the populations of *D. melanogaster* selected for increased resistance to cold stress. These experiments were performed over 24-33 generations of selection.

MATERIALS AND METHODS:

Experimental populations:

Details of the maintenance and derivation of the selected (FSB; Cold shock Selected population derived from BRB population) and their control (FCB; Cold shock Control derived from BRB population) populations has been explained previously (Singh et al. 2015). Briefly, after 35 generation of the laboratory adaption of BRB 1-5, one FSB population and one FCB population were established from each of the BRB populations, for example, FSB 1 and their corresponding control FCB 1 derived from the BRB 1, similarly FSB 2 and their control FCB 2 established from the BRB2 and so on. Hence, we had five replicate populations for the selected population and five replicate of control populations. Populations carrying the same numerical subscript have originated from same base line population (BRB) and are more close to each other than any other populations. For instance, FSB 1 and FCB 1 are more close (due to the origin from the same ancestral population) to each other than FSB 2 or FCB 2 or any other population. Hence, in our statistical data analysis FSB 1 and FCB 1

are included in block 1, similarly FSB 2 and FCB 2 are included in block 2 and so on. FSB and FCB populations are large outbred populations maintained under standard laboratory environment (25°C temperature, 50–60% relative humidity, 12 hour light: 12 hour dark cycle, on a 13-day discrete generation cycle). On day 12 post egg collection, flies (flies are roughly 2-3 days old as adults and mated) are moved into empty, clean, dry glass vials (30 mm diameter × 90 mm length). After that flies belonging to the FSB populations are subjected to -5°C temperature in ice-salt-water slurry for one hour. FCB populations, on the other hand, are held at 25°C for one hour. Subsequently, all populations are quickly moved into a separate Plexiglass cage (25 cm length × 20 cm width × 15 cm height) having a fresh food plate. After 24 hour a fresh food plate is given to flies in order to collect eggs to initiate next generation. For each population, 20 vials are collected at density of 70 eggs per vial containing ~ 6 ml of a fresh food.

Standardization

To account the non-genetic parental effects (Rose 1984), flies from the selected populations and from their controls were reared for one generation in common rearing environment. This method is referred to as standardization and these flies are known as standardized flies. A detail of the standardization of the protocol has been described earlier in Singh et al. (2015). Shortly, to control eggs density, for each selected and their control populations, 20 vials were established at density of 70 eggs per vials in ~6 ml food, reared at standard laboratory conditions (12 hours light:12 hours dark). On day 12 after egg collection, (roughly 2-3 days old as adult flies) ~ 1200-1400 flies of each population were transferred separately in a Plexiglass cage and provided a fresh food plate. These flies were further used for experiment egg collection.

151

152 **Cold shock treatment for experiments**

153 Detailed account of the cold shock protocol has been described in our previous study (Singh
154 et al., 2015). In short, on day 12 post egg collection, (by this time flies were roughly 2-3 days
155 old as adult and mated flies) 25 pairs of males and females were moved to clean, dry glass
156 vials under mild carbon dioxide anesthesia. The cotton plug was inserted deep into the vial
157 such that the flies were allowed to stay in a confined space in vial (1/3 of the vial). The flies
158 were kept in an incubator to recover from carbon dioxide anesthesia for half an hour. The
159 vials containing flies were then kept for one hour in ice-salt-water slurry maintained at -5°C.
160 Post cold shock, flies were quickly shifted to Plexiglass cages (14 cm length × 16 cm width ×
161 13 cm height. The cage was provided with a food Plate and was kept under standard
162 laboratory conditions (Singh et al., 2015). The control treatment flies were handled similar
163 way, except that the vials containing flies, were kept in a water bath that maintained at 25°C
164 for one hour.

165

166 **Experimental details**

167 **Experiment: 1.1: Longevity assay**

168 The longevity assay was performed after 24 generations of selection. Eggs were collected
169 from standardized flies at a controlled egg density of 70 eggs/vial provisioned with ~ 6 ml of
170 fresh banana-yeast-jaggery food (hereafter referred to as “food”). Twenty four such type of
171 vials were set up for each of the FSB (1-5) and FCB (1-5) populations. On day 12 after egg
172 collection, flies were sorted (25 mating pairs per vial) under mild carbon dioxide anesthesia.
173 After sorting, flies were divided into two sets: (a) set first for cold-shock treatment (both male
174 and female flies were exposed to cold shock for one hour) and (b) set second for no-shock
175 treatment (neither males and nor females were exposed to cold-shock).

176

(a) Cold-shock: For each population, flies contained in 12 vials (each vial contains 25 mating pairs of male and female) were imposed cold-shock (-5°C for one hour) as mentioned in the cold shock protocol. Quickly, after the cold shock, 12 vials were randomly divided into 3 sets referred to as a “replicate”. Each set having 4 vials of flies (100 mating pairs each) were moved into a Plexiglass cage and given a fresh food plate. Hence, each population (FSB 1-5 and FCB 1-5) had 3 replicates.

(b) No-shock: For each population, flies contained in 12 vials (each vial contain 25 mating pairs of male and female) were subjected to no-shock treatment (25°C for one hour). Post treatment, 12 vials were quickly randomly divided into three sets that were known as replicate. Each set having 4 vials containing total of 100 mating pairs of male and female flies were moved into Plexiglas cages and given a fresh food plate. Hence, each population (FSB 1-5 and FCB 1-5) had 3 replicates.

We established three replicate cages per selection × block × treatment combination (Except block 1 of the FCB population which had 2 replicates for both-shock treatment, due to accidental death of one of the replicates during the assay). Food plate was changed 48 hours internal and dead flies were aspirated out and computed. Sex of the dead flies was determined under microscope on the basis of sex combs. Mortality was recorded until the last fly died. Using the mortality data, for each cage, we measured mean longevity of males and females from the selection regime (FSB and FCB), treatment and block. For the analysis of mean longevity, cage means were used as the unit of analysis.

Experiment 1.2: Life time fecundity assay

Fecundity assay was performed along with longevity assay, using the same set of flies. We measured fecundity at every sixth day along with longevity. In order to measure fecundity,

fresh food plate was placed in the Plexiglas cage for 6 hours for oviposition. After that, total number of eggs on each plate was counted under the microscope. Subsequently, fecundity per female - number of eggs divided by total number of live females at that time point- was calculated. Average fecundity of the eleven time points was calculated for three replicates for each of the FSB 1-5 and FCB 1-5 populations, and treatments. We also computed median and maximum longevity for each cage populations. The aging rate data was analyzed using the Gompertz model.

Experiment 2: Development time (first instar larva to eclosion)

Development time was assayed after 33 generations of selection. Followed by one generation of common rearing environment or standard laboratory condition (no selection was imposed on FSB and FCB population), 12 vials each were set up for FSB 1-5 and FCB 1-5 populations at a density of 70 eggs per vial. On day 12 after egg collection, vials containing flies were randomly divided into two sets for - (a) cold-shock (b) no-shock treatment. For both ‘cold-shock’ and ‘no-shock’ treatments, flies were transferred into empty glass vials at density of ~70 flies and the cotton plug was pushed deep up to the bottom one-third of the vial. After that, the flies were subjected to cold shock or no shock treatments, following the protocol as mentioned above. Immediately after cold-shock treatment, flies (200 males and 200 females) were transferred to Plexiglass cage and provided with a fresh food plate. Twenty four hours post cold-shock, fresh - food plates were given to each cage for 1 hour to lay stored eggs. After that another set of fresh plates were given for four hours. The second set of plates containing eggs were then incubated at standard laboratory conditions for 18 hours to allow eggs to hatch and first instars larvae to emerge. The larvae were collected (using a moist brush) into vials with 6 ml of a fresh food. For each population and treatment combination, 10 replicate vials were set up (each containing 30 larvae in 6 ml of food). The vials were incubated at standard laboratory conditions. The positions of the vials were

randomized and moved daily within the incubator. Once pupae formed, each vial was manually scanned every 2 hours. Freshly eclosed flies were transferred into empty glass vials, sexed and counted. The flies were then flash frozen using liquid nitrogen and then transferred to -80°C for storage used to assess dry body weight.. Mean larva to eclosion development time was computed for each vial and this vial mean time was considered as the unit of analysis.

Experiment 3: Measurement of dry body weight of male and female flies

In order to measure the dry body weight, we used the same flies from the development time assay (mentioned above). Freshly eclosed flies were flash frozen using liquid nitrogen and stored at -80°C until dry body weight measurement. Five flies of a given sex were grouped together, dried in a hot air oven at 65°C for 48 hours and weighed. For each population, treatment and sex combination, ten such sets were weighed. Thus, a total of 50 males and 50 females per population and treatment were used for body weight measurement. Body weight of each group of five flies was considered as the unit of analysis.

Experiment 4: Larvae to adults survival:

To investigate larvae to adults survival, we monitored total number of flies eclosed from the cultured larval vial at density of 30 larvae/vial. We calculated percentage of larvae to adult survival using the equation in given a bracket (percentage of larvae to adults survival = (number of eclosed flies in a vial/total number of larvae cultured in a vial)*100).

Statistical analysis

Mean longevity, development time, dry body weight of males and females, and larvae to adults survival were analyzed using a three-factor mixed model analysis of variance (ANOVA) treating selection regime (FSB vs. FCB), treatment (cold-shock vs. no-shock) as a fixed factors crossed with a random block (1-5). The sexes were analyzed separately.

Fecundity per female was analyzed using a three-factor mixed model ANOVA treating selection regime (FSB vs. FCB) and treatment (cold-shock vs. no-shock) as fixed factors crossed with block as a random factor. All the analyses were done at $\alpha=0.05$ level of significance using Statistica (for Windows, version 10, Statsoft). Multiple comparisons were carried out employing Tukey's HSD.

Rates of aging

Age dependent and age independent rate of aging was measured using the method used by Mueller et al. (1995) and Jafari et al. (2007). Raw survivorship data were used to calculate 'proportion survival' values with subsequent calculation of running average of the proportion survival data, r_x .

$$r_x = (p_x + p_{x+2})/2 \quad (1)$$

Where, p_x is the proportion of individuals surviving at a given age x . Since mortality was monitored every alternate day, x and $x+2$ are two successive age intervals noticed. The hazard rate that is the probability of death per unit time, μ_x at age x was computed employing the following equation:

$$\mu_x = (r_x - r_{x+2})/r_x \quad (2)$$

According to the Gompertz equation, the mortality rate at age x is given by,

$$\mu_x = ae^{bx} \quad (3)$$

Where, a and b represent age-independent and age-dependent rate of aging respectively. Log-hazard rate was regressed against age intervals; the intercept and the least square slope gave the estimates of *Gompertz a* and *Gompertz b* respectively. The derived parameters were analyzed using three factor mixed model ANOVA with selection regime (FSB vs. FCB), treatment (cold shock vs. no shock) as fixed factor crossed with random blocks (1-5).

RESULTS

Experiment 1.1: Longevity assay for male and female

Male and female longevity was assessed in terms of mean, median and maximum longevity. Analyses revealed that the results were similar regardless of the measure used. After 24 generations of selection, there was no significant effect of selection, treatment or selection $\times$ treatment interaction on male or female mean longevity (Table 1a, b Figure 1a, b, c, and d). Interestingly, the absence of any significant effect of treatment indicated that flies subjected to cold-shock treatment as well as the flies that were not subjected to cold-shock, which proved that cold shock had no direct effect on mean longevity.

We found a significant effect of treatment on the *Gompertz a* (age independent mortality rate) and *b* (age dependent mortality rate) parameters among males. The FSB and FCB males subjected to cold-shock showed significantly higher age independent mortality but a significantly lower age dependent mortality compared to the males not subjected to cold-shock (Table 1c). The net effect of these two factors was that the average (and median) lifespan of the males subjected to cold-shock and those not subjected to cold-shock was not different. There was no effect of selection or a selection $\times$ treatment interaction on the Gompertz parameters. Among the females, none of the factors affected the Gompertz parameters (Table 1d). Thus, we found no evidence for any significant change in mean longevity or rates of aging as a correlated response to selection for increased resistance to cold-shock.

Experiment 1.2: Life time fecundity

The mean number of eggs laid per female in each of the FSB and FCB populations and treatments were computed by averaging across the 11 time points of fecundity measurement and used it as the unit of analysis. We did not observe significant effects of selection,

treatment or selection $\times$ treatment interaction on female fecundity (Table 2, Figure 2a, and 2b). Just like longevity, the absence of any significant effect of treatment on fecundity revealed that cold-shock treatment had no direct effect on lifetime fecundity.

Experiment 2: Development time (first instar larva to adult eclosion)

Unlike longevity and fecundity, selection did affect mean development time. Mean development time of males showed a significant effect of selection. (Table 3a, Figure 3a). Starting as first instar larvae, FSB male took about 2-4 hours more to emerge as adults compared to FCB males (Figure 3a). Female mean development time analysis showed that there was significant effect of selection (Table 3b, Figure 3b). However, none of the other effects were significant. Just like the males, FSB females also took ~3-6 hours more to emerge as adults compared to FCB females (Figure 3b). Again, the cold shock experienced by the parents had no effect on offspring development time (no significant treatment effect).

Experiment 3: Dry body weight

Male mean dry body weight analysis revealed that there was no significant effect of selection, treatment or selection $\times$ treatment interaction (Table 4a, Figure 4a). In case of female dry body weight, found significant main effect of selection (Table 4b). However, there was no significant effect of treatment or selection $\times$ treatment interaction (Table 4b). Mean body weight of FSB females was about ~0.01 mg higher than that of FCB females (Figure 4b).

Experiment 4: larvae to adults survival

Mean larvae to adult survivals analysis shown that there was no significant effect of selection or selection $\times$ treatment interaction (Table 5, Figure 5).

DISCUSSION

In this study, we assessed mean longevity, rates of aging, developmental time and dry body weight in the FSB and FCB populations with and without cold shock. Neither longevity nor fecundity was different between the FSB and FCB populations. However, we found that males and females from the FSB populations took significantly more time to develop (from first instar larvae to adult) relative to the FCB populations. Females from the FSB populations were heavier than females from the FCB populations. However, there was no difference in male body size between the FSB and FCB populations. Taken together, our finding suggests there is no evidence for a trade-off between the ability to resist cold stress and important life-history traits.

The correlation between cold-shock resistance and longevity is variable across studies. MacMillan et al. (2009), using a selection protocol very similar to the present study found that females of the cold-shock selected populations had decreased longevity compared to females of the control populations whereas no such difference was visible in the males. According to Anderson et al. (2005) populations selected for faster chill-coma recovery had reduced lifespan compared to controls. In the contrary Norry and Loeschcke (2002) observed that cold adapted populations lived longer at 14°C and shorter at 25°C compared to control populations. Bubliy and Loeschcke (2005) found no change in female longevity between populations selected for cold resistance and their controls. In populations directly selected for increased lifespan, increased cold resistance evolved as a correlated response in adults and pupae of *D. melanogaster* (Luckinbill, 1998). In contrast to all these studies, we found that selection for resistance to cold-shock had no effect on lifespan or rates of aging. There were several possible differences including the base population used for selection, the definition of ‘cold stress’, the assay protocols, *etc.* between these studies that preclude a direct comparison of results. More importantly, other studies, typically selected for increased survivorship post

cold-shock. However, in our study there was very little cold induced mortality. This is further strengthened by the fact that the lifespan of the FSB and FCB populations that were subjected to cold-shock were not different from the longevity of those populations not subjected to cold shock. Thus, it is not surprising that longevity did not evolve in the FSB compared to FCB populations.

In several previous studies, fecundity has responded to selection for cold resistance. Anderson et al. (2005) found that at least two of the three replicates in their selection regime evolved lower fecundity. Watson and Hoffmann (1996) found that cold selected populations had lower fecundity. However, we found no difference in the life-time fecundity of in the FSB relative to the FCB populations. This is in agreement with our earlier, short-term measurement of fecundity in these two populations (Singh et al., 2015). Thus, we found no evidence of a trade-off between evolved cold stress resistance and fecundity.

Increased development time can potentially be a cost in species like *D. melanogaster* that inhabit ephemeral habitats and have to complete their development before the habitat disappears. We did find that the FSB males and females had increased developmental time. However, the magnitude of the increase was very small (~3-4 hours) and hence we are not sure whether this represented a cost. Increased development time represented an adaptation to increase resource storage that helped in coping stressful conditions. During the late third larval instar stage, *D. melanogaster* larvae feed rapidly and increased their weight exponentially (reviewed in Prasad and Joshi, 2003). An increase of ~3-4 hours of feeding time during this period drastically increased the amount of resources stored by the larvae. Accordingly, populations of *D. melanogaster* selected for increased starvation and desiccation stress resistance are known to show increased development time and increased

body size (Chippindale et al., 1996, 1998). In this study, increased development time represented an adaptation to acquire necessary resources to cope with cold stress.

Body weight at eclosion is often used as a proxy for the amount of resources stored by the larvae. Anderson et al., (2005) and Watson and Hoffmann (1996) found no difference in body size of flies selected for increased cold resistance. In this study, FSB females were heavier at eclosion compared to FCB females. This indicated that FSB females were storing extra/specific nutrients to survive cold-shock. However, there was no difference in body weight between FSB and FCB males. Taken together, this indicated that at least in females, increased development time was likely to be beneficial in aspect of increased resource acquisition. It is also to be noted that in our previous study, females suffered more mortality post cold shock relative to males (Singh et al., 2015).

Absence of any change in lifespan and fecundity of the FSB populations could be because of many reasons. Firstly, the evolved cold-shock resistance ability of the FSB populations might be very cheap. Thus, the resources required to combat the effects of cold stress in our selection regime might be very low. It is a known fact that the flies in our population need to produce active gametes and mate in order to increase egg viability post cold shock. Accordingly, the FSB populations mate more often than the FCB populations post cold-shock (Singh et al., 2015, 2016, Singh and Prasad, 2016). Courtship and mating carry a substantial cost to both males and females (Wedell, 2010). Thus, the costs of evolved cold-shock resistance are expected to be substantial in our selection regime. A second alternative is that the resources are abundant and the FSB populations are able to acquire them as adults. The food used in our selection regime was indeed rich. The larval and adult densities were low. Therefore, it was possible that our flies inhabited resource-rich environment. If this is true,

then assays under resource depleted condition should lead to different results. Finally, it is quite possible that the cost of increased cold resistance is paid in a different currency. While we did not find any difference in adult longevity or fecundity, other traits that we have not measured here might have been reduced in the FSB populations. The possible set of such traits include starvation and desiccation resistance.

CONCLUSIONS

Our findings revealed that there is no life-history trade-offs between increased resistance to cold-shock (in aspect of increased reproductive traits and egg viability post cold shock) with life history traits i.e. the longevity, life time fecundity, larvae to adults survival, and larvae to pre adults developmental time, which indicated that evolved cold stress resistance need not come at a cost of life-history traits. It is quite possible that the cost of increased cold stress resistance is paid in terms of reduced resistance to other stresses.

Conflict of Interest

All the authors declare “No Conflict of Interest”

References

Hoffmann, A.A. and Parsons, P.A., 1991. *Evolutionary genetics and environmental stress*. Oxford University Press.

- 445 Parsons, P.A., 2005. Environments and evolution: interactions between stress, resource
446 inadequacy and energetic efficiency. *Biological Reviews*, 80(4), pp.589-610.
- 447 Patton, Z.J. and Krebs, R.A., 2001. The effect of thermal stress on the mating behavior of
448 three *Drosophila* species. *Physiological and Biochemical Zoology*, 74(6), pp.783-788.
- 449 Fasolo, A.G. and Krebs, R.A., 2004. A comparison of behavioural change in *Drosophila*
450 during exposure to thermal stress. *Biological Journal of the Linnean Society*, 83(2),
451 pp.197-205.
- 452 Stearns, S.C., Oxford: Oxford University Press; 1992. *The evolution of life histories*, 264.
- 453 Krebs, R.A. and Loeschcke, V., 1994. Effects of exposure to short-term heat stress on
454 fitness components in *Drosophila melanogaster*. *Journal of Evolutionary*
455 *Biology*, 7(1), pp.39-49.
- 456 Huey, R.B. and Berrigan, D., 2001. Temperature, demography, and ectotherm fitness. *The*
457 *American Naturalist*, 158(2), pp.204-210.
- 458 Hochachka, P.W. and Somero, G.N., 2002. *Biochemical adaptation: mechanism and process*
459 *in physiological evolution*. Oxford University Press.
- 460 Angilletta Jr, M.J. and Angilletta, M.J., 2009. *Thermal adaptation: a theoretical and*
461 *empirical synthesis*. Oxford University Press.
- 462 Lee, R.E., 1991. Principles of insect low temperature tolerance. In *Insects at low*
463 *temperature* (pp. 17-46). Springer, Boston, MA. Voituron, Y., Mouquet, N., de
464 Mazancourt, C. and Clobert, J., 2002. To freeze or not to freeze? An evolutionary

465 perspective on the cold-hardiness strategies of overwintering ectotherms. *The*
466 *American Naturalist*, 160(2), pp.255-270.

467 Hoffmann, A.A., Sørensen, J.G. and Loeschke, V., 2003. Adaptation of *Drosophila* to
468 temperature extremes: bringing together quantitative and molecular
469 approaches. *Journal of thermal Biology*, 28(3), pp.175-216.

470 Denlinger, D.L., Yocum, G.D. and Hallman, G.J., 1998. Thermal sensitivity in insects and
471 application in integrated pest management. *Physiology of heat sensitivity*, pp.11-18.

472 Bubliy, O.A. and Loeschke, V., 2005. Correlated responses to selection for stress resistance
473 and longevity in a laboratory population of *Drosophila melanogaster*. *Journal of*
474 *evolutionary biology*, 18(4), pp.789-803.

475 Rohmer, C., David, J.R., Moreteau, B. and Joly, D., 2004. Heat induced male sterility in
476 *Drosophila melanogaster*: adaptive genetic variations among geographic populations
477 and role of the Y chromosome. *Journal of Experimental Biology*, 207(16), pp.2735-
478 2743.

479 Hance, T., van Baaren, J., Vernon, P. and Boivin, G., 2007. Impact of extreme temperatures
480 on parasitoids in a climate change perspective. *Annu. Rev. Entomol.*, 52, pp.107-126.

481 Van Lieshout, E., Tomkins, J.L. and Simmons, L.W., 2013. Heat stress but not inbreeding
482 affects offensive sperm competitiveness in *Callosobruchus maculatus*. *Ecology and*
483 *evolution*, 3(9), pp.2859-2866.

484 Nguyen, T.M., Bressac, C. and Chevrier, C., 2013. Heat stress affects male reproduction in a
485 parasitoid wasp. *Journal of Insect Physiology*, 59(3), pp.248-254.

486 Singh, K., Samant, M.A., Tom, M.T. and Prasad, N.G., 2016. Evolution of pre-and post-
487 copulatory traits in male *Drosophila melanogaster* as a correlated response to
488 selection for resistance to cold stress. *PloS one*, 11(4).

489 Singh, K. and Prasad, N.G., 2016. Evolution of pre-and post-copulatory traits in female
490 *Drosophila melanogaster* as a correlated response to selection for resistance to cold
491 stress. *Journal of insect physiology*, 91, pp.26-33.

492 Singh, K., Kochar, E. and Prasad, N.G., 2015. Egg viability, mating frequency and male
493 mating ability evolve in populations of *Drosophila melanogaster* selected for
494 resistance to cold shock. *PloS one*, 10(6).

495 Trotta, V., Calboli, F.C., Ziosi, M., Guerra, D., Pezzoli, M.C., David, J.R. and Cavicchi, S.,
496 2006. Thermal plasticity in *Drosophila melanogaster*: a comparison of geographic
497 populations. *BMC evolutionary biology*, 6(1), p.67.

498 Austin, C.J. and Moehring, A.J., 2013. Optimal temperature range of a plastic species,
499 *Drosophila simulans*. *Journal of animal ecology*, 82(3), pp.663-672.

500 Angilletta MJ Jr, Niewiarowski PH, Navas CA. The evolution of thermal physiology in
501 ectotherms. *J Therm Biol*. 2002; 27: 249-268.

502 Stanley, S.M. and Parsons, P.A., 1981. The response of the cosmopolitan species, *Drosophila*
503 *melanogaster*, to ecological gradients. In *Proc Ecol Soc Aust* (Vol. 11, pp. 121-130).

504 Boulétreau-Merle, J., Allemand, R., Cohet, Y. and David, J.R., 1982. Reproductive strategy
505 in *Drosophila melanogaster*: significance of a genetic divergence between temperate
506 and tropical populations. *Oecologia*, 53(3), pp.323-329.

507 James, A.C. and Partridge, L., 1998. Geographic variation in competitive ability in
508 *Drosophila melanogaster*. *The American Naturalist*, 151(6), pp.530-537.

509 James, A.C. and Partridge, L., 1995. Thermal evolution of rate of larval development in
510 *Drosophila melanogaster* in laboratory and field populations. *Journal of Evolutionary*
511 *Biology*, 8(3), pp.315-330.

512 Hangartner, S.B., Hoffmann, A.A., Smith, A. and Griffin, P.C., 2015. A collection of
513 Australian *Drosophila* datasets on climate adaptation and species
514 distributions. *Scientific data*, 2(1), pp.1-8.

515 Tucić, N., 1979. Genetic capacity for adaptation to cold resistance at different developmental
516 stages of *Drosophila melanogaster*. *Evolution*, pp.350-358.

517 Chen, C.P. and Walker, V.K., 1993. Increase in cold shock tolerance by selection of cold
518 resistant lines in *Drosophila melanogaster*. *Ecological Entomology*, 18(3), pp.184-
519 190.

520 Watson, M.J. and Hoffmann, A.A., 1996. Acclimation, cross-generation effects, and the
521 response to selection for increased cold resistance in *Drosophila*. *Evolution*, 50(3),
522 pp.1182-1192.

523 Anderson, A.R., Hoffmann, A.A. and McKECHNIE, S.W., 2005. Response to selection for
524 rapid chill-coma recovery in *Drosophila melanogaster*: physiology and life-history
525 traits. *Genetics Research*, 85(1), pp.15-22.

526 Bubliy, O.A. and Loeschke, V., 2005. Correlated responses to selection for stress resistance
527 and longevity in a laboratory population of *Drosophila melanogaster*. *Journal of*
528 *evolutionary biology*, 18(4), pp.789-803.

529 MacMillan, H.A., Walsh, J.P. and Sinclair, B.J., 2009. The effects of selection for cold
530 tolerance on cross-tolerance to other environmental stressors in *Drosophila*
531 *melanogaster*. *Insect Science*, 16(3), pp.263-276.

532 Overgaard, J., Sørensen, J.G. and Loeschcke, V., 2010. Genetic variability and evolution of
533 cold-tolerance. In *Low temperature biology of insects* (pp. 276-296). Cambridge
534 University Press.

535 Rose, M.R., 1984. Laboratory evolution of postponed senescence in *Drosophila*
536 *melanogaster*. *Evolution*, 38(5), pp.1004-1010.

537 Mueller, L.D., Nusbaum, T.J. and Rose, M.R., 1995. The Gompertz equation as a predictive
538 tool in demography. Jafari, M., Khodayari, B., Felgner, J., Bussel, I.I., Rose, M.R. and
539 Mueller, L.D., 2007. Pioglitazone: an anti-diabetic compound with anti-aging
540 properties. *Biogerontology*, 8(6), pp.639-651.

541 Norry, F.M. and Loeschcke, V.R., 2002. Longevity and resistance to cold stress in
542 cold-stress selected lines and their controls in *Drosophila melanogaster*. *Journal of*
543 *Evolutionary Biology*, 15(5), pp.775-783.

544 Luckinbill, L.S., 1998. Selection for longevity confers resistance to low-temperature stress in
545 *Drosophila melanogaster*. *The Journals of Gerontology Series A: Biological Sciences*
546 *and Medical Sciences*, 53(2), pp.B147-B153.

547 Prasad, N.G. and Joshi, A., 2003. What have two decades of laboratory life-history evolution
548 studies on *Drosophila melanogaster* taught us?. *Journal of genetics*, 82(1-2), pp.45-76.

549 Chippindale, A.K., Chu, T.J. and Rose, M.R., 1996. Complex trade-offs and the evolution of
550 starvation resistance in *Drosophila melanogaster*. *Evolution*, 50(2), pp.753-766.

Chippindale, A.K., Gibbs, A.G., Sheik, M., Yee, K.J., Djawdan, M., Bradley, T.J. and Rose, M.R., 1998. Resource acquisition and the evolution of stress resistance in *Drosophila melanogaster*. *Evolution*, 52(5), pp.1342-1352.

Wedell, N., 2010. Variation in male courtship costs in butterflies. *Behavioral ecology and sociobiology*, 64(9), pp.1385-1391.

Table 1a. Effect of cold-shock on the mean longevity of males (Experiment 1.1). Summary of results from a three-factor mixed model ANOVA on the male mean longevity using selection (FCB and FSB) and treatment (cold-shock and no-shock) as fixed factors crossed with random block (1-5).

Effect	SS	MS Num	DF Num	DF Den	F ratio	p
Selection (Sel)	33.439	33.439	1.000	4.006	1.348	0.310
Treatment (Trt)	14.549	14.549	1.000	4.008	0.752	0.435

Block (Blk)	478.234	119.559	4.000	6.177	3.024	0.107
Sel × Trt	9.169	9.169	1.000	4.032	1.972	0.232
Sel × Blk	99.283	24.821	4.000	4.000	5.350	0.067
Trt × Blk	77.398	19.349	4.000	4.000	4.170	0.098
Sel × Trt × Blk	18.559	4.640	4.000	39.000	0.422	0.792

Table 1b. Effect of cold-shock on the mean longevity of females (Experiment 1.1). Summary of results from a three-factor mixed model ANOVA on the female mean longevity using selection (FCB and FSB) and treatment (cold-shock and no-shock) as fixed factors crossed with random block (1-5). *p*-values in bold are statistically significant.

Effect	SS	MS Num	DF Num	DF Den	<i>F</i> ratio	<i>p</i>
Selection (Sel)	56.044	56.044	1.000	4.007	2.756	0.172
Treatment (Trt)	1.581	1.581	1.000	4.004	0.047	0.839
Block (Blk)	91.967	22.992	4.000	7.012	0.443	0.775
Sel × Trt	0.160	0.160	1.000	4.071	0.084	0.786
Sel × Blk	81.399	20.350	4.000	4.000	10.751	0.020
Trt × Blk	133.984	33.496	4.000	4.000	17.697	0.008
Sel × Trt × Blk	7.571	1.893	4.000	39.000	0.192	0.941

Table 1c. Effect of cold shock on the age independent and age dependent mortality rates of males (Experiment 1.1). Summary of results from a three-factor mixed model ANOVA on (a) age independent and (b) age dependent mortality rate among males using Selection (FCB and FSB) and Treatment (cold-shock and no-shock) as fixed factors crossed with random block (1-5). *p*-values in bold are statistically significant.

Trait	Effect	SS	MS Num	DF Num	DF Den	<i>F</i> ratio	<i>p</i>
-------	--------	----	--------	--------	--------	----------------	----------

(a) Male	Selection (Sel)	0.479	0.479	1.000	4.021	2.920	0.162
age	Treatment (Trt)	9.979	9.979	1.000	4.021	61.398	0.001
independent	Block (Blk)	8.791	2.198	4.000	0.008	126.988	0.960
Mortality	Sel × Trt	0.127	0.127	1.000	4.011	0.410	0.557
	Sel × Blk	0.656	0.164	4.000	4.000	0.531	0.723
	Trt × Blk	0.649	0.162	4.000	4.000	0.525	0.726
	Sel × Trt × Blk	1.236	0.309	4.000	39.00	1.220	0.318
(b) Male	Selection (Sel)	3.9×10 ⁻⁵	3.9×10 ⁻⁵	1.000	4.017	0.340	0.591
age	Treatment (Trt)	7.3×10 ⁻³	7.3×10 ⁻³	1.000	4.030	112.215	<0.001
dependent	Block (Blk)	1.4×10 ⁻³	3.5×10 ⁻⁴	4.000	9×10 ⁻⁶	3470.59	1.000
Mortality	Sel × Trt	1.8×10 ⁻⁹	1.8×10 ⁻⁹	1.000	4.011	1×10 ⁻⁵	0.998
	Sel × Blk	4.6×10 ⁻⁴	1.1×10 ⁻⁴	4.000	4.000	0.638	0.663
	Trt × Blk	2.6×10 ⁻⁴	6.4×10 ⁻⁵	4.000	4.000	0.362	0.825
	Sel × Trt × Blk	7.1×10 ⁻⁴	1.8×10 ⁻⁴	4.000	39.00	1.251	0.305

Table 1d. Effect of cold-shock on the age independent and age dependent mortality rates of females (Experiment 1.1). Summary of results from a three-way mixed model ANOVA on (c) age independent and (d) age dependent mortality rate among females using selection (FCB and FSB) and treatment (cold-shock and no-shock) as fixed factors crossed with random block (1-5).

Trait	Effect	SS	MS Num	DF Num	DF Den	F ratio	P
-------	--------	----	--------	--------	--------	---------	---

(c) Female	Selection (Sel)	0.005	0.005	1.000	4.008	0.014	0.913
age	Treatment (Trt)	0.240	0.240	1.000	4.007	0.597	0.483
independent	Block (Blk)	0.600	0.150	4.000	1.491	0.366	0.822
Mortality	Sel × Trt	0.092	0.092	1.000	4.008	0.245	0.647
	Sel × Blk	1.534	0.384	4.000	4.000	1.019	0.493
	Trt × Blk	1.611	0.403	4.000	4.000	1.07	0.475
	Sel × Trt × Blk	1.505	0.376	4.000	39.000	1.74	0.161
(d) Female	Selection (Sel)	1.4×10^{-4}	1.5×10^{-4}	1.000	4.008	0.904	0.395
age	Treatment (Trt)	5.2×10^{-4}	5.1×10^{-4}	1.000	4.012	4.646	0.097
dependent	Block (Blk)	6.9×10^{-4}	1.7×10^{-4}	4.000	1.536	1.204	0.532
Mortality	Sel × Trt	1.3×10^{-4}	1.3×10^{-4}	1.000	4.010	1.038	0.366
	Sel × Blk	6.4×10^{-4}	1.6×10^{-4}	4.000	4.000	1.265	0.413
	Trt × Blk	4.4×10^{-4}	1.1×10^{-4}	4.000	4.000	0.871	0.552
	Sel × Trt × Blk	5.1×10^{-4}	1.3×10^{-4}	4.000	39.000	1.355	0.267

Table 2. Effect of cold shock on life time fecundity (Experiment 1.2). Summary of results from a three-factor mixed model ANOVA on the life time fecundity using selection (FCB and FSB) and treatment (cold-shock and no-shock) as fixed factors crossed with random block (1-5).

Effect	SS	MS Num	DF Num	DF Den	F ratio	p
Selection (Sel)	0.006	0.006	1	4.001	0.004	0.955
Treatment (Trt)	4.264	4.264	1	4.009	24.427	0.007

Block (Blk)	13.135	3.284	4	1.650	2.639	0.328
Sel × Trt	0.017	0.017	1	4.002	0.023	0.886
Sel × Blk	7.161	1.790	4	4	2.485	0.199
Trt × Blk	0.698	0.174	4	4	0.242	0.901
Sel × Trt × Blk	2.881	0.720	4	39	5.699	0.001

Table 3a. Effect of cold-shock on parents on the male developmental time (larvae to adult eclosion) (Experiment 2). Summary of results from a three-factor mixed model ANOVA on the mean larva to adult development time of males using selection (FCB and FSB) and treatment (cold-shock and no-shock) as fixed factors crossed with random block (1-5). *p*-values in bold are statistically significant.

Effect	SS	MS Num	DF Num	DF Den	<i>F</i> ratio	<i>p</i>
Selection (Sel)	758.240	758.240	1.000	4.000	17.449	0.014
Treatment (Trt)	214.335	214.335	1.000	4.000	0.601	0.482
Block (Blk)	141.786	35.446	4.000	3.993	0.098	0.977
Sel × Trt	54.776	54.776	1.000	4.000	1.404	0.302
Sel × Blk	173.816	43.454	4.000	4.000	1.114	0.460
Trt × Blk	1427.513	356.878	4.000	4.000	9.150	0.027
Sel × Trt × Blk	156.012	39.003	4.000	180.000	1.111	0.353

Table 3b. Effect of cold-shock on parent on the female developmental time (larvae to adult eclosion) (Experiment 2). Summary of results from a three-factor mixed model ANOVA on mean larva to adult development time of females using selection (FCB and FSB) and treatment (cold-shock and no-shock) as fixed factors crossed with random block (1-5). *p*-values in bold are statistically significant.

Effect	SS	MS Num	DF Num	DF Den	<i>F</i> ratio	<i>p</i>
Selection (Sel)	907.888	907.888	1.000	4.000	8.374	0.044

Treatment (Trt)	206.835	206.835	1.000	4.000	0.453	0.538
Block (Blk)	950.196	237.549	4.000	1.019	0.855	0.658
Sel × Trt	204.729	204.729	1.000	4.000	0.712	0.446
Sel × Blk	433.683	108.421	4.000	4.000	0.377	0.816
Trt × Blk	1828.026	457.007	4.000	4.000	1.590	0.332
Sel × Trt × Blk	1149.930	287.483	4.000	180.000	1.840	0.123

Table 4a. Effect of cold-shock on the dry body weight of male (Experiment 3). Summary of results from a three-factor mixed model ANOVA on the mean dry body weight of males using selection (FCB and FSB) and treatment (cold-shock and no-shock) as fixed factors crossed with random block (1-5). *p*-values in bold are statistically significant.

Effect	SS	MS Num	DF Num	DF Den	<i>F</i> ratio	<i>p</i>
Selection (Sel)	8.2×10 ⁻⁵	8.2×10 ⁻⁵	1.000	4.000	0.178	0.694
Treatment (Trt)	7×10 ⁻⁴	7×10 ⁻⁴	1.000	4.000	0.701	0.450
Block (Blk)	6×10 ⁻³	1.5×10 ⁻³	4.000	6.553	1.050	0.450
Sel × Trt	1.2×10 ⁻⁵	1.2×10 ⁻⁵	1.000	4.000	0.240	0.650
Sel × Blk	1.8×10 ⁻³	4.6×10 ⁻⁴	4.000	4.000	9.550	0.025
Trt × Blk	4×10 ⁻³	1×10 ⁻³	4.000	4.000	20.974	0.006
Sel × Trt × Blk	2×10 ⁻⁴	4.8×10 ⁻⁵	4.000	180.000	0.145	0.965

Table 4b. Effect of cold shock on the dry body weight of female (Experiment 3). Summary of results from a three-factor mixed model ANOVA on the mean dry body weight of females using selection (FCB and FSB) and treatment (cold-shock and no-shock) as fixed factors crossed with random Blocks (1-5). *p*-values in bold are statistically significant.

Effect	SS	MS Num	DF Num	DF Den	<i>F</i> ratio	<i>p</i>
Selection (Sel)	6.7×10 ⁻³	6.7×10 ⁻³	1.000	4.000	32.942	0.005

Treatment (Trt)	3×10^{-4}	3×10^{-4}	1.000	4.000	0.287	0.621
Block (Blk)	1.4×10^{-2}	3.4×10^{-3}	4.000	3.756	3.620	0.128
Sel $\times$ Trt	2×10^{-4}	2×10^{-4}	1.000	4.000	1.199	0.335
Sel $\times$ Blk	8×10^{-4}	2×10^{-4}	4.000	4.000	1.059	0.479
Trt $\times$ Blk	3.7×10^{-3}	9×10^{-4}	4.000	4.000	4.828	0.078
Sel $\times$ Trt $\times$ Blk	8×10^{-4}	2×10^{-5}	4.000	180.000	0.491	0.743

Table 5. Effect of cold shock on the larvae to adults survivals (Experiment 4). Summary of results from a three-factor mixed model ANOVA on the mean larvae to adults survivals considering selection (FCB and FSB) and treatment (cold-shock and no-shock) as fixed factors crossed with random Blocks (1-5). *p*-values in bold are statistically significant.

Effect	SS	MS	DF Num	DF den	F Ratio	Prob > F
		Num				
Selection (Sel)	37.556	37.556	1	4	2.449	0.193
Treatment (Trt)	128.000	128.000	1	4	3.578	0.132
Block (Blk)	1270.889	317.722	4	1.918	10.417	0.096
Sel $\times$ Trt	107.556	107.556	1	4	5.218	0.084
Sel $\times$ Blk	61.333	15.333	4	4	0.744	0.609
Trt $\times$ Blk	143.111	35.778	4	4	1.736	0.303
Sel $\times$ Trt $\times$ Blk	82.444	20.611	4	180	1.013	0.402

Figure 1a: Mean longevity of the FSB and FCB males after being subjected to cold-shock or no-shock treatment (Experiment 1.1). Selection, treatment or selection $\times$ treatment interaction did not have significant effect on male mean longevity. Open bars represent the FSB and closed bars represent the FCB populations.

Figure 1b: Male survivorship across ages (Experiment 1.1). Longevity was assayed after adult flies were subjected to cold-shock or no-shock treatment. There was no difference in the mean, median and maximum longevity of the FSB and FCB males. There was no significant difference in the *Gompertz* parameters between the FSB and FCB populations.

Figure 1c: Mean longevity of the FSB and FCB females after being exposed to cold shock or no shock treatment (Experiment 1.1). Selection, treatment or selection $\times$ treatment interaction did not have significant effect on female mean longevity. Open bars represent the FSB and closed bars represent the FCB populations.

Figure 1d: Female survivorship across ages (Experiment 1.1). Longevity was assayed after adult flies were subjected to cold-shock or no-shock treatment. There was no difference in the mean, median and maximum longevity of the FSB and FCB females. There was no significant difference in the *Gompertz* parameters between FSB and FCB populations.

Figure 2a: Mean life time fecundity per female (Experiment 1.2). Fecundity was measured at eleven time points once in every 6 days and mean of eleven time points for fecundity was computed. Selection, treatment or selection $\times$ treatment interaction did not have significant effect on fecundity. Open bars represent the FSB populations and closed bars represent the FCB populations.

Figure 2b: Life time fecundity per female (Experiment 1.2). Fecundity was measured at eleven time points once in every 6 days. Mean fecundity per female for each population and treatment was computed for eleven time points. Results indicate that fecundity reduces with age. However, none of the other effects were significant.

Figure 3a: Mean development time (larva to adult) of the FSB and FCB males when their parents were subjected cold-shock or no-shock treatments (Experiment 2). We found a significant effect of selection regime with the FSB males developing 3-4 hours slower than FCB males. Treatment had no significant effect. Open bars represent the FSB populations and closed bars represent the FCB populations.

741

742

743

744

745

746

747

Figure 3b: Mean development time (larva to adult) of the FSB and FCB females when their parents were subjected cold-shock or no-shock treatments (Experiment 2). We found a significant effect of Selection regime with FSB females developing 3-4 hours slower than FCB females. Treatment had no significant effect. Open bars represent the FSB populations and closed bars represent the FCB populations.

Figure 4a: Dry weight at eclosion of males from the FSB and FCB populations (Experiment 3). Selection, treatment or selection $\times$ treatment interaction did not have significant effect on mean dry body weight. Open bars represent the FSB and closed bar represent the FCB populations.

Figure 4b: Dry weight at eclosion of females from the FSB and FCB populations. Selection had significant effect on mean dry body weight (Experiment 3). However, treatment or selection × treatment did not have significant effects on mean dry body weight. Open bars represent the FSB and closed bar represent the FCB populations.

Figure 5: Larvae to adults survival from the FSB and FCB populations (Experiment 4). Selection, treatment or selection $\times$ treatment did not have any significant effects on mean larvae to adult survivals. Open bars represent the FSB and closed bar represent the FCB populations