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NO INBREEDING DEPRESSION FOR LOW TEMPERATURE DEVELOPMENTAL ACCLIMATION ACROSS MULTIPLE *DROSOPHILA* SPECIES

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Populations are from time to time exposed to stressful temperatures. Their thermal resistance levels are determined by inherent and plastic mechanisms, which are both likely to be under selection in natural populations. Previous studies on *Drosophila* species have shown that inherent resistance is highly species specific, and differs among ecotypes (e.g., tropical and widespread species). Apart from being exposed to thermal stress many small and fragmented populations face genetic challenges due to, for example, inbreeding. Inbreeding has been shown to reduce inherent resistance levels toward stressful temperatures, but whether adaptation to thermal stress through plastic responses also is affected by inbreeding is so far not clear. In this study, we test inherent cold resistance and the ability to respond plastically to temperature changes through developmental cold acclimation in inbred and outbred lines of five tropical and five widespread *Drosophila* species. Our results confirm that tropical species have lower cold resistance compared to widespread species, and show that (1) inbreeding reduces inherent cold resistance in both tropical and widespread species, (2) inbreeding does not affect the ability to respond adaptively to temperature acclimation, and (3) tropical species with low basal resistance show stronger adaptive plastic responses to developmental acclimation compared to widespread species.

KEY WORDS: **Cold stress resistance, ecotype, environmental sensitivity, homozygosity, plasticity.**

Tolerance to thermal stress is an important environmental factor dictating the distribution and abundance of organisms (Cossins and Bowler 1987; Hoffmann and Parsons 1991). Coping with thermal stress involves high inherent tolerance and also the capacity to respond to temperature variation by adaptive phenotypic plasticity (Levins 1968; Cossins and Bowler 1987; Angilletta 2009). The ability of organisms to be phenotypically plastic thus plays an important role in their lifetime fitness (Chown and Terblanche 2007; Ghalambor et al. 2007; Angilletta 2009; Chevin and Lande 2010), and plastic responses through physiological, behavioral,

and/or morphological changes are thought to be important in determining species distributional ranges (Angilletta 2009; Stillman and Tagmount 2009; Overgaard et al., in press).

Small isolated populations are exposed to increased levels of inbreeding and genetic drift. The deleterious effects of inbreeding have been demonstrated in a large number of species for life-history traits, and several studies have demonstrated that inbreeding depression in fitness-related traits is environment specific, and often more severe under harsh and stressful environmental conditions (Bijlsma et al. 1999; Armbruster and Reed 2005; Kristensen et al. 2008). However Schlichting and Levin (1986) found no evidence for a difference in plasticity in plant vigor and fecundity between inbred and outbred individuals of the annual plant *Phlox drummondii* grown in different environments. Despite some knowledge on fitness consequences of inbreeding by environment interactions little is known about the effect of inbreeding on adaptive phenotypic plasticity of physiological responses directly induced by specific biotic or abiotic environmental cues, such as temperature and pathogen/predator exposure. Auld and Relyea (2010) recently showed that inbreeding reduced the expression of predator induced adaptive plasticity in shell thickness in a hermaphroditic snail species (*Physa acuta*), but this is to our knowledge the only study reporting such evidence and general conclusions should not be drawn based on one study investigating one species.

Given the importance of inherent and plastic responses to thermal stress for species survival, and the increasing number of small populations exposed to genetic threats such as inbreeding, it is important to investigate on a multispecies level how species may differ in their ability to respond to temperature changes and how inbreeding affects these components of thermal tolerance. Recent studies indicate that tropical species of *Drosophila* have lower inherent cold tolerance compared to more widespread species (Kellermann et al. 2009; Overgaard et al., in press). It has also been suggested that a broader fundamental niche often observed in species living in variable thermal environments is partly due to larger thermal plasticity (Janzen 1967; Levins 1968; Hoffmann and Watson 1993; Ghalambor et al. 2006; Chown and Terblanche 2007). Furthermore, tropical species of *Drosophila*, in contrast to some widespread species, seem to have limited capacity to change evolutionarily their inherent cold and desiccation resistance (Hoffmann et al. 2003a; Kellermann et al. 2009). Thus the genetic architecture of inherent and induced cold tolerance may differ between widespread and tropical species, suggesting that inbreeding may impact differently on the two ecotypes.

Here, we consider ecotype and inbreeding effects on cold tolerance across five tropical and five widespread species of *Drosophila* using ecologically relevant temperature ramping assays in the laboratory. Cold resistance, assessed as the critical thermal minimum (CTmin), was first investigated in inbred and outbred flies from the 10 species developed at either 15°C or 20°C. We then examined whether inbreeding and ecotype (tropical vs. widespread) affected the ability to respond plastically to different temperatures during development (15°C or 20°C).

Materials and Methods

COLLECTION OF FLIES

Ten *Drosophila* species were collected in December 2007 to April 2008 at different locations along the east coast of Australia (Table 1). Five species are tropical specialists restricted to the Australasian region (particularly rainforests in northern Queensland, Australia and Papua New Guinea). Another five species are widespread cosmopolitan species whose range includes temperate regions of Australia and elsewhere. Inseminated field collected females were placed singly into vials for one generation at 25 ± 1°C

Table 1. Species used in the study, their distribution (tropical/widespread), collection sites and dates and number of inbred and outbred lines investigated. For further details on collection sites and distribution of the investigated species see Mitchell and Hoffmann (2010) and Overgaard et al. (in press).

Species	Distribution (restricted/ widespread)	Location and date of collection	Number of inbred/outbred lines
Drosophila melanogaster	widespread	Melbourne, Vic. (Dec 2007)	6/3
Drosophila simulans	widespread	Melbourne, Vic. (Dec 2007)	3/3
Drosophila hydei	widespread	Melbourne, Vic. (Dec 2007)	8/3
Drosophila repleta	widespread	Townsville, Qld (Apr 2008)	10/3
Drosophila serrata	widespread	Finch Hatton, Qld (April 2007)	10/3
Drosophila birchii	tropical	Lake Eacham, Qld (Apr 2008)	4/3
Drosophila bunnanda	tropical	Kirrama, Qld (Apr 2008)	5/3
Drosophila bipectinata	tropical	Gordonvale, Qld (Feb 2008)	7/3
Drosophila sulfurigaster	tropical	Gordonvale, Qld (Feb 2008)	10/3
Drosophila pseudoananassae	tropical	Gordonvale, Qld (Feb 2008)	9/3

with constant light to generate isofemale lines. Mass bred populations were established from 18 to 22 of these isofemale lines for each species, and maintained for 15 generations in the laboratory at 20 ± 1°C and a 12/12 h light/dark cycle before the inbred and outbred lines investigated in this study were established. In each generation, prior to establishing inbred and outbred lines flies were maintained at a minimum population size of 1000 individuals in bottles on Leeds medium which is a nutritionally balanced artificial diet composed of sucrose (40 g/L water), yeast (60 g/L water), agar (16 g/L water), oatmeal (30 g/L water), nipagen (12 mL/L water), and acetic acid (1.2 mL/L water).

BREEDING REGIMES

Inbred lines were established from the mass bred populations for all 10 species by four consecutive generations of full sibling mating. The inbreeding procedure was performed in a climate room at 20 ± 1°C on Leeds medium. When initiating the inbreeding procedure, 20 replicates of one male and one female (virgins) from each species were collected from the mass bred populations and placed in each of 20 vials with food. In the next generation, a male and female (virgins) were collected from each vial. This procedure was followed until all lines had expected equivalent levels of inbreeding ($F = 0.56$). After reaching the desired level of inbreeding, lines were flushed to a minimum of 500 individuals. The number of inbred lines generated per species varied between three and 10 (Table 1). The reason for this unbalanced number of lines was that a different number of lines went extinct through the inbreeding procedure in the different species. Three outbred lines assumed to be noninbred were set up per species and originating from the same mass bred population as the inbred lines. Outbred lines were established by sampling approximately 750 individuals from the base population. The inbred and outbred lines were established in such a way that all lines reached the expected level of inbreeding (0 and 0.56, respectively) at the same time. All lines were subsequently maintained in bottles with 35 mL of Leeds medium in a climate room (20°C, 50% relative humidity, 12/12 h light/dark cycle) and kept at population sizes of approximately 750 individuals per line per generation. Experiments were performed in the second generation after inbreeding had been completed.

ACCLIMATION TREATMENTS AND REARING OF EXPERIMENTAL ANIMALS

To examine the influence of developmental acclimation at different temperatures and the influence of breeding regime on resistance to thermal extremes, eggs (density controlled with ∼20 eggs per vial) from all species and lines were placed at either 15°C or 20°C to complete development. Eggs from all species and lines were set up to synchronize adult emergence within each acclimation treatment. Simultaneous experimental tests were conducted using three- to eight-day-old adult virgin males and females. Until the time of testing, flies were kept at their respective acclimation regimes (15°C or 20°C). As developmental time was much slower at 15°C compared to 20°C, flies developed at the two temperatures were not tested at the same time.

RAMPING KNOCK DOWN TEMPERATURE ASSAY

We estimated the critical thermal minimum (CTmin) under gradual cooling of flies developed at 15°C and 20°C respectively. CTmin was scored by placing individual flies in empty 5-mL glass vials that were subsequently submerged in a water bath at 20°C. The temperature of the water bath was then gradually decreased from 20°C to 0°C at a rate of 0.1°C/min and CTmin was recorded as the temperature at which the flies were knocked down by cold and became unable to move any body part (for details on the assay see Mitchell and Hoffmann 2010).

ASSESSMENT OF CTmin AND THE ACCLIMATION RESPONSE

CTmin was assessed in all inbred and outbred lines developed at, respectively, 15°C and 20°C using the ramping knock down temperature assay. The effect of breeding regime (inbred vs. outbred) on developmental acclimation was assessed by comparing CTmin of flies that developed at 15 and 20°C. The acclimation response of all lines was quantified as the difference in CTmin of flies developed at 20°C minus CTmin of flies developed at 15°C, respectively. A positive value indicates that flies developed at 15°C tolerate lower temperatures than flies developed at 20°C. The effect of breeding regime (inbred or outbred) was assessed by comparing acclimation responses of inbred and outbred lines. Inbreeding depression for plasticity occurs if inbred flies gain less, compared to outbred flies, in increased cold tolerance by developing at 15°C compared to 20°C (CTmin 20°C outbred − CTmin 15°C outbred > CTmin 20°C inbred − CTmin 15°C inbred).

STATISTICS

CTmin data from flies developed at, respectively, 15°C and 20°C were tested using a restricted maximum likelihood (REML) approach testing effects of breeding regime, species, developmental temperature, and sex (all fixed effects). Line was nested within species and breeding regime as a random effect. To investigate the effect of breeding regime, species, and sex on the acclimation response (developmental acclimation), we first calculated the difference in degrees Celsius between CTmin of flies developed at 15°C and at 20°C, respectively, for each line from all species. Impact of the fixed effects breeding regime, species, and sex on these line estimates were tested using a standard least square ANOVA. Effects of breeding regime on the acclimation response were subsequently tested for each species individually

with one-way ANOVAs. Ecotype (tropical vs. widespread) effects on inbreeding depression for induced CTmin were tested with a full-factorial ANOVA with species nested within ecotype and sex and ecotype as fixed effects. Ecotype effects on inbreeding depression for innate CTmin were tested with a full-factorial ANOVAs with species nested within ecotype and with sex, developmental temperature and ecotype as fixed effects. Finally, ecotype effects on basal levels of CTmin of outbred lines only (including inbred lines and a treatment effect [inbred vs. outbred] in the model did not change the conclusions drawn) were tested separately for the two developmental temperatures with a full-factorial ANOVA with species nested within ecotype and with the fixed factors sex and ecotype. All data were treated as normally distributed although deviations from normality were observed in a few cases. Transformations solved the problem in some cases but this then led to increased skewness and kurtosis in others. All analyses were performed on both nontransformed and transformed data and in all cases results were qualitatively similar so only the analyses on nontransformed data are shown. All models were reduced in complexity until only significant interactions were included in the final model. Analyses were performed using JMP for Windows version 7 (SAS Institute Inc., Cary, NC).

Results

CTmin

When developing at 15°C, inbred flies had on average a CTmin that was 0.2°C higher compared to outbred flies, whereas when developing at 20°C inbred flies had on average a CTmin that was 0.6°C higher compared to outbred flies (Fig. 1). Although this difference suggests that inbreeding depression is more severe for flies developed at 20°C, the interaction term developmental temperature by breeding regime was nonsignificant ($F_{1,3829} = 0.59$, NS); thus we find no evidence that across multiple species inbreeding effects on CTmin depend on developmental temperature. This result is supported by significant positive correlations between levels of inbreeding depression for flies developed at 15°C and 20°C, respectively (females: Spearman's $\rho = 0.52$, $P < 0.0001$; males: Spearman's $\rho = 0.30$, $P < 0.05$). CTmin was significantly affected by line nested within species (variance

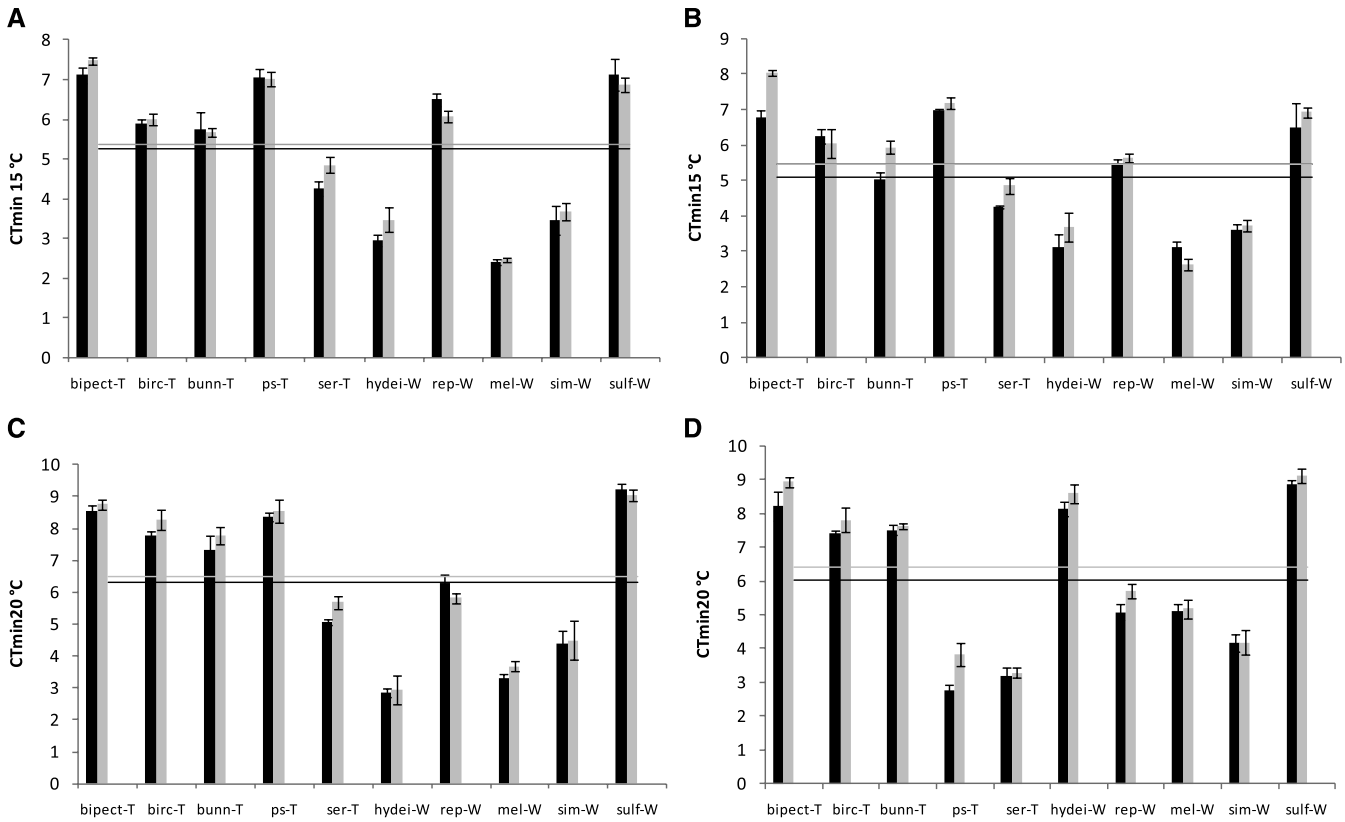

Figure 1. CTmin (mean ± SE) for females (A and C) and males (B and D) of five widespread (W) and five tropical (T) species of inbred (gray) and outbred (black) *Drosophila* developed at 15°C (A and B) and 20°C (C and D). The species are: ***D. bipectinata*** (bipect), ***D. birchii*** (birc), ***D. bunnanda*** (bunn), ***D. hydei*** (hydei), ***D. melanogaster*** (mel), ***D. pseudoananassae*** (ps), ***D. repleta*** (rep), ***D. serrata*** (ser), ***D. simulans*** (sim), and ***D. sulfurigaster*** (sulf). CTmin is obtained from a ramping assay where flies are exposed to gradually decreasing temperatures going from 20°C to 0°C at 0.1°C/min. Differences between inbred and outbred flies are interpreted as inbreeding depression for inherent cold resistance. Horizontal lines represent means for inbred (gray) and outbred (black) lines, respectively.

component = 0.11, lower 95% confidence limit = 0.06, upper 95% confidence limit = 0.17), species ($F_{9,79.1} = 181.84$, $P < 0.001$), breeding regime ($F_{1,176.9} = 4.12$, $P < 0.05$), sex ($F_{1,3826} = 5.63$, $P < 0.05$) and developmental temperature ($F_{9,3668} = 338.45$, $P < 0.001$). Furthermore, the interaction between species and developmental temperature significantly affected CTmin ($F_{9,3786} = 29.37$, $P < 0.001$). CTmin was significantly lower in outbred flies from widespread species compared to outbred flies from tropical species at both developmental temperatures (15°C: $F_{1,45} = 348.04$, $P < 0.0001$; 20°C: $F_{1,48} = 1322.84$, $P < 0.0001$).

DEVELOPMENTAL ACCLIMATION

The capacity to increase cold resistance (lower CTmin) through developmental acclimation was not affected by inbreeding ($F_{1,155} = 0.34$, NS) or sex ($F_{1,155} = 1.37$, NS; Fig. 2). However species differed strongly in acclimation response ($F_{9,155} = 31.20$, $P < 0.001$; Fig. 2). Although inbreeding did not affect the ability to respond to developmental temperature across all species, we tested whether inbreeding did affect adaptive plasticity in each of the two ecotypes. This was not the case ($F_{1,98} = 1.15$, NS). Investigating the outbred lines only revealed a strong ecotype effect on the plastic response with tropical species increasing their CTmin more by developing at 15°C than at 20°C compared to the widespread species ($F_{1,45} = 82.25$, $P < 0.0001$; Fig. 3). The same conclusion was drawn when plasticity of inbred lines was contrasted with basal CTmin levels of inbred flies developed at 20°C (results from the statistical analysis not shown, Fig. 3).

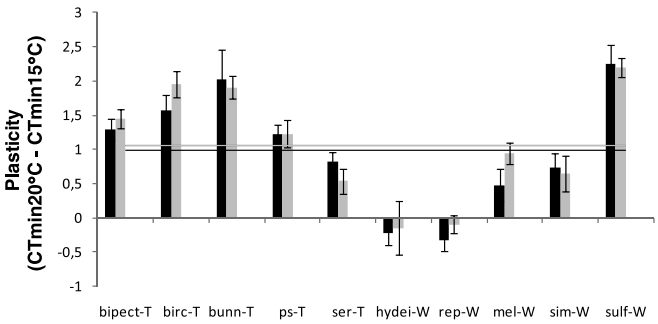

Figure 2. Plasticity/acclimation response quantified in five widespread (W) and five tropical (T) species of inbred (gray) and outbred (black) *Drosophila* as the difference between CTmin of flies developed at 15°C and 20°C (mean ± SE). Male and female data are pooled for ease of interpretation. The species are: *D. bipectinata* (bipect), *D. birchii* (birc), *D. bunnanda* (bunn), *D. hydei* (hydei), *D. melanogaster* (mel), *D. pseudoananassae* (ps), *D. repleta* (rep), *D. serrata* (ser), *D. simulans* (sim), and *D. sulfurigaster* (sulf). Positive values indicate a lower CTmin (higher cold resistance) for flies developed at 15°C compared to flies developed at 20°C whereas negative values indicate that flies developed at 20°C had lower CTmin (higher cold resistance). Horizontal lines represent means for inbred (gray) and outbred (black) lines, respectively.

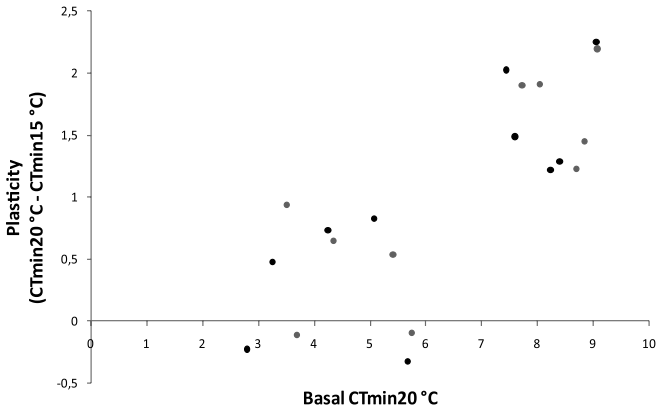

Figure 3. Plasticity/acclimation response quantified in five widespread (W) and five tropical (T) species as a function of CTmin of outbred or inbred flies developed at 20°C. Sexes and lines are pooled within the outbred and inbred groups, respectively. The species are—from left to right on the figure: *D. hydei*, *D. melanogaster, D. simulans, D. serrata, D. replete, D. bunnanda, D. birchii, D. pseudoananassae, D. bipectinata* and *D. sulfurigaster*. Positive values indicate a lower CTmin (higher cold resistance) for outbred and inbred flies developed at 15°C compared to flies developed at 20 °C whereas negative values indicate that flies developed at 20°C had lower CTmin (higher cold resistance). ● represent outbred lines and ● represent inbred lines.

Discussion

We tested multiple replicate inbred and outbred lines of five widespread and five tropical restricted species of *Drosophila* for their inherent cold resistance and the ability to respond plastically to developmental temperatures (developmental acclimation). Our results revealed (1) significant inbreeding depression for basal cold resistance, (2) no effects of inbreeding on the ability to induce an adaptive plastic response, (3) no ecotype effect on inbreeding depression for CTmin and plasticity, and (4) strong species and ecotype effects on basal cold tolerance and plasticity, with widespread species being more cold resistant but less plastic compared to the tropical specialists.

Our finding of lower cold tolerance (higher CTmin) in inbred compared to outbred individuals is in accordance with other studies of cold resistance in species of *Drosophila* (Kristensen et al. 2008) and suggests that inbred populations will suffer more than outbred populations during cold thermal conditions. However the lack of significant interactions between breeding regime and other fixed effects on CTmin suggests that inbreeding effects are independent of species, developmental temperature, and sex under the conditions, and for the traits that we have studied. The difference between inbred and outbred individuals in CTmin may

be due to a difference in innate basal cold resistance but also due to a different hardening ability during ramping between inbred and outbred individuals. We cannot rule out this possibility but find it unlikely that hardening responses would be sufficiently strong at the rather fast ramping rate employed in our study to explain this result alone (see also Sgrò et al. 2010).

For life-history traits, there is ample empirical evidence that inbreeding depression is environment dependent (Armbruster and Reed 2005 and references therein). For example Bijlsma et al. (1999) and Kristensen et al. (2008) found strong evidence for temperature-dependent inbreeding depression for fitness characters with increased inbreeding depression observed at stressful compared to at benign temperatures in *D. melanogaster*, suggesting that inbred individuals suffer more than outbred individuals if temperature conditions deviate from optimal. Such results suggest that inbreeding impacts the ability to respond through adaptive plastic responses to changes in the environment and may be due to inbreeding depression for adaptive plasticity as proposed by the recent finding of inbreeding depression for predator-induced adaptive plasticity in a hermaphroditic snail (Auld and Relyea 2010). However to test whether observations of more inbreeding depression in stressful compared to benign environments is a consequence of inbreeding depression for the ability to induce an adaptive plastic response more studies are needed that test the ability to react adaptive plastically to a specific environmental parameter. Our experiment is designed to do so and shows that inbred flies on average gained as much as outbred flies in terms of increased cold resistance when they developed at a lower compared to a higher temperature. Developmental acclimation for low temperature resistance therefore does not seem to be affected by inbreeding. This suggests that the effect of inbreeding on adaptive phenotypic plasticity is trait and/or species specific and depends on the physiology of the trait investigated. Further, this indicates that inbreeding by environment interactions are caused by environment-specific genetic architecture of the investigated traits and not by inbreeding depression for plasticity.

Inbreeding depression for a specific trait is a function of inbreeding and directional dominance levels and of allele frequencies at loci affecting the trait. It has been suggested (e.g., Schlichting and Pigliucci 1993; Li et al. 2006) that there are genes for the ability to react to environmental variation through adaptive phenotypic plasticity. If temperature-sensitive "plasticity genes" segregate in our populations, our data suggest that the level of dominance variance associated with such genes is low as no inbreeding depression for plasticity is observed. Alternatively, adaptive plasticity in a trait may be determined by the same genes that affect the mean of a character in different environments (Via et al. 1995 and references therein). Then selection on loci that change the mean will also change plasticity. The discrepancy between the effect of inbreeding on developmental acclimation and basal cold resistance suggests that "plasticity genes" affecting CTmin do exist, as our results cannot be explained solely by the assumption that it is the same genes that affect the mean and the plastic response in different environments. If the same genes were affecting developmental acclimation and inherent cold resistance, we would have expected to observe that inbreeding affected developmental acclimation and CTmin qualitatively in the same direction, which was not the case. On the other hand the observation that species with higher basal CTmin (tropical species, Fig. 3) induced a stronger acclimation response implies some shared genetic background for basal cold tolerance and developmental acclimation (plasticity). This indicates that correlated or independent selection patterns as well as genetic correlations impact inherent and induced cold tolerance. Thus our results show that some genes not only influence both traits, but also that there is genetic independence allowing some different patterns among species and traits (Auld and Relyea 2010).

Our results revealed that the two ecotypes, tropical and widespread species, responded similarly to inbreeding with respect to basal CTmin and adaptive plastic response to developing temperatures. This is in contrast to our hypothesis suggesting that a different genetic architecture of cold resistance in tropical and widespread species (Kellermann et al. 2009) leads to a different impact of inbreeding on the two ecotypes. Tropical species were less cold tolerant and induced a stronger adaptive acclimation response compared to widespread species. The latter result is in contrast to suggestions by, for example, Janzen (1967) and Levins (1968), hypothesising that a broader fundamental niche sometimes observed in species living in variable thermal environments can be partly explained by a larger thermal plasticity. We propose that tropical species of *Drosophila* that have lower innate cold resistance might actually compensate for this by inducing a stronger adaptive plastic response—that is, developing at 15°C is more stressful for tropical compared to widespread species and therefore they induce a stronger physiological response. The ecological importance of this result needs further testing as tropical species in contrast to widespread species rarely experience 15°C in their natural habitat.

Interactions between environmental conditions and the expression of inbreeding depression on the physiology of organisms are not well understood. However recent data show that inbred *D. melanogaster* differ from outbred flies in their transcriptional response to a temperature change; many more genes are differentially expressed between inbred and noninbred flies at stressful compared to at benign temperatures (Kristensen et al. 2010). A stronger response to temperature changes in inbred flies may explain why inbred flies gain as much in increased cold tolerance by developing at 15°C compared to at 20°C in this study (Fig. 2). Assuming that inbred flies are generally more sensitive to environmental changes and react to such changes through physiological

adjustments (Hoffmann et al. 2003b; Kristensen et al. 2010; Paige 2010), the net benefits/costs of acclimation in inbred and outbred individuals are difficult to predict. However based on our results we conclude that inbreeding reduces the capacity to cope with low stressful temperatures but not the ability to respond plastically to temperature changes during development. Furthermore, inbreeding effects on innate and induced cold resistance do not differ between the investigated tropical and widespread species of *Drosophila*; thus increased levels of inbreeding in these species will affect them to the same extent.

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LITERATURE CITED

Angiletta, M. J. 2009. Thermal adaptation: a theoretical and empirical analysis. Oxford Univ. Press, New York.

Armbruster, P., and R. H. Reed. 2005. Inbreeding depression in benign and stressful environments. Heredity 95:235–242.

Auld, J. R., and R. A. Relyea. 2010. Inbreeding depression in adaptive plasticity under predation risk in a freshwater snail. Biol. Lett. 6:222–224.

Bijlsma, R., J. Bundgaard, and W. F. van Putten. 1999. Environmental dependency of inbreeding depression and purging in *Drosophila melanogaster*. J. Evol. Biol. 12:1125–1137.

Chevin, L. M., and R. Lande. 2010. When do adaptive plasticity and genetic evolution prevent extinction of a density regulated population. Evolution 64:1143–1150.

Chown, S. L., and J. S. Terblanche. 2007. Physiological diversity in insects: ecological and evolutionary contexts. Adv. Insect Physiol. 33: 50–152.

Cossins, A. R., and K. Bowler. 1987. Temperature biology of animals. Chapman and Hall, New York.

Ghalambor, C. K, J. K. Mckay, S. P. Carrol, and D. N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct. Ecol. 21:394–407.

Hoffmann, A. A., and P. A. Parsons. 1991. Evolutionary genetics and environmental stress. Oxford Univ. Press, Oxford, UK.

Hoffmann, A. A., and M. Watson. 1993. Geographical variation in the acclimation responses of *Drosophila* to temperature extremes. Am. Nat. 142:S93–S113.

Hoffmann, A. A., R. J. Hallas, J. A. Dean, and M. Schiffer. 2003a. Low potential for climatic stress adaptation in a rainforest *Drosophila* species. Science 301:100–102.

Hoffmann, A. A., J. G. Sørensen, and V. Loeschcke. 2003b. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. J. Therm. Biol. 28:175–216.

Janzen, D. H. 1967. Why mountain passes are higher in tropics. Am. Nat. 101:233–249.

Kellermann, V., B. van Heerwaarden, C. M. Sgró, and A. A. Hoffmann. 2009. Fundamental evolutionary limits in ecological traits drive *Drosophila* species distributions. Science 325:1244–1246.

Kristensen, T. N., J. S. F. Barker, K. S. Pedersen, and V. Loeschcke. 2008. Extreme temperatures increase the deleterious consequences of inbreeding under laboratory and semi-natural conditions. Proc. R. Soc. Lond. B 275:2055–2061.

Kristensen, T. N., K. S. Pedersen, C. J. Vermeulen, and V. Loeschcke. 2010. Research on inbreeding in the 'omic' era. Trends Ecol. Evol. 25:44–52.

Levins, R. 1968. Evolution in changing environments, Princeton Univ. Press, Princeton, NJ.

Li, Y., O. A. Álvarez, E. W. Gutteling, M. Tijsterman, J. Fu, J. A. G. Riksen, E. Hazendonk, P. Prins, R. H. A. Plasterk, R. C. Jansen, et al. 2006. Mapping determinants of gene expression plasticity by genetical genomics in *C. elegans*. PLoS Genet. 2:e222.

Mitchell, K. A., and A. A. Hoffmann. 2010. Thermal ramping rate influences evolutionary potential and species differences for upper thermal limits in *Drosophila*. Funct. Ecol. 24:694–700.

Overgaard, J., T. N. Kristensen, K. A. Mitchell, and A. A. Hoffmann. Thermal tolerance in widespread and tropical *Drosophila* species: does phenotypic plasticity predict latitudinal range? Am. Nat.: *In press*.

Paige, K. N. 2010. The functional genomics of inbreeding depression: a new approach to an old problem. BioScience 60:267–277.

Schlichting, C. D., and D. A. Levin. 1986. Effects of inbreeding on phenotypic plasticity in cultivated *Phlox*. Theor. Appl. Genet. 72:114–119.

Schlichting, C. D., and M. Pigliucci. 1993. Control of phenotypic plasticity via regulatory genes. Am. Nat. 142:366–370.

Sgrò, C. M., J. Overgaard, T. N. Kristensen, K. A. Mitchell, F. E. Cockerell, and A. A. Hoffmann. 2010. A comprehensive assessment of geographic variation in heat tolerance and hardening capacity in populations of *Drosophila melanogaster* from eastern Australia. J. Evol. Biol. 11:2484–2493.

Stillman, J. H., and A. Tagmount. 2009. Seasonal and latitudinal acclimatization of cardiac transcriptome responses to thermal stress in porcelain crabs, *Petrolisthes cinctipes*. Mol. Ecol. 18:4206–4226.

Via, S., R. Gomulkiewicz, G. de Jong, S. M. Scheiner, C. D. Schlichting, and P. H. van Tienderen. 1995. Adaptive phenotypic plasticity: consensus and controversy. Trends Ecol. Evol. 10:212–217.

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