Natural selection on plasticity of thermal traits in a highly seasonal environment

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Abstract

Although plasticity has been proposed as an escape from climate change, beyond certain limits genetic adjustments may be required to persist in a warming world. Evolutionary adaptation depends on the amount of additive genetic (co)variances and on the strength of phenotypic selection. However, in spite of its paramount importance to prevent demographic extinction, it is unknown whether selection in nature targets thermal acclimation capacity itself. We addressed such an important gap in our knowledge by measuring survival, through mark recapture integrated into an information-theoretic approach, as a function of the plasticity of critical thermal limits for activity, behavioral thermal preference and the thermal sensitivity of metabolism in the northernmost population of the four-eyed frog *Pleurodema thaul*. Overall, our results indicate that thermal acclimation is a target of selection in nature. In particular, we found that survival strongly increases with body size, although models with directional selection on trait plasticity showed support (ca. 25% of cumulative Akaike weights) and suggest a rather complex fitness landscape where different high-fitness strategies are being favoured. The models including correlational, directional and stabilizing selection for more than one trait had very weak empirical support. One strategy favoured frogs that are able to tolerate the high temperatures that occur during the cold breeding season whilst the other favoured frogs that increase their activity levels during the warmer periods of the year.

Keywords: Climate change, amphibians, natural selection, physiological plasticity, acclimation, *Pleurodema thaul*, Atacama Desert

Introduction

The biodiversity of the Earth is undergoing an extraordinary transformation as a result of the effects of human activities on every terrestrial ecosystem [1,2]. Although it is clear the impact of global change drivers will depend on the region, ecosystem and species, without a doubt, global warming is projected to be the largest human-induced disturbance placed on natural ecosystems [3,4]. In the face of warming, a population (or a species) has four possible compensatory mechanisms to prevent demographic extinction. Mobile species can track their current climate envelope given the structure of the landscape or they can regulate their body temperature behaviourally if the thermal environment is heterogeneous [5]. However, when dispersal and behavioural thermoregulation are not options, a population should adjust to a warming climate by physiological plasticity and/or evolutionary adaptation under the force of natural selection [6,7].

Although plasticity has been proposed as an escape from climate change, beyond certain limits genetic adjustments may be required to persist in a warming world [6]. Evolutionary adaptation depends on the amount of additive genetic (co)variances and on the strength of phenotypic selection [8–11]. Recently, Logan and collaborators [12], showed that when lizards are transplanted to a warmer and more thermally variable site, thus mimicking future climate change, natural selection favored individuals that run faster at warmer temperatures and across a broader range of temperatures. However, in spite of its paramount importance to prevent demographic extinction, it is unknown whether selection in nature targets thermal acclimation capacity itself. We addressed such an important gap in our knowledge by measuring survival, through mark recapture integrated into an information-theoretic approach, as a function of the plasticity of four thermal key traits in the northernmost population of the four-eyed frog

Pleurodema thaul. At the limit of its distribution and inhabiting two small ponds in the oasis Carrera Pinto in the hyperarid Atacama Desert, this population does not have any dispersal opportunities. Furthermore, residing in such a thermally variable environment on both daily and seasonal basis, this population will have to face warming either by physiological plasticity, evolutionary adaptation or both. We have recently shown that this population will be able to endure the worst projected scenario of climate warming as it has not only the plasticity [13] but also the environmental opportunities to regulate its body temperature behaviourally [14]. However, we still do not know whether that physiological plasticity, which results from inhabiting a highly variable environment, is being targeted by natural selection. Therefore, we measured for the first time natural selection on plastic responses of thermal critical temperatures (CT_{Max} and CT_{Min}), preferred temperature (T_{Pref}) and thermal sensitivity of metabolism (Q_{10}) after acclimation to 10°C and 20°C. We tested three predictions regarding phenotypic selection and plasticity (i.e. $Trait_{20°C}$-$Trait_{10°C}$) that built up from previous findings showing that acclimation to warmer temperatures produces an increase in the upper but not in the lower limits of the thermal performance curve [14]. First, there is positive directional selection for plasticity of CT_{Max} and T_{Pref} as well as correlational selection among them. Second, there is stabilising selection on CT_{Min} plasticity. As energy inputs are limited, the energetic definition of fitness indicates that individuals with higher maintenance costs (i.e. resting metabolic rate) would have less energy available to allocate to growth, reproduction and/or performance The main prediction of this principle is that natural selection should maximize the residual available energy, and therefore, higher maintenance costs would be associated with lower fitness if no compensations in other functions were available [15,16]. Thus, our third prediction is that there is stabilising selection on Q_{10} plasticity.

METHODS

Study organism and laboratory maintenance

Eighty-three adults individuals of *P. thaul* were captured during September 2012 on two small ponds at Carrera Pinto (27º06'40.2'' S, 69º53'44.3'' W), a small oasis in the Atacama Desert that is known to be the northernmost population of the species [17]. In both ponds, we performed an exhaustive search across microhabitats (below rocks, in the vegetation and in the water). All individuals were transported to the laboratory (Universidad Austral de Chile, Valdivia) within 2 – 3 days of capture. Following capture all animals were marked by toe clipping and maintained in the laboratory for one month at a temperature of 20º ± 2ºC and with a photoperiod 12D:12L. Animals were housed (N = 5) in terrariums (length x width x height: 40 x 20 x 20 cm) provided with a cover of moss and vegetation and a small recipient filled with water. Individuals were fed once a week with mealworms (*Tenebrio sp*. larvae) and Mazuri® gel diets.

Acclimation and thermal traits

After one month at maintenance conditions, in a split cross design frogs were acclimated to either 10°C or 20°C for two weeks before measuring thermal traits. Frogs were randomly assigned to the first acclimation temperature using a coin. Next they were acclimated to the other temperature and again measured thermal traits. We chose these acclimation temperatures because they are close to the mean minimum temperatures during the breeding season (August - October, 10ºC) and to the mean temperatures during the active period of the species (20ºC) at Carrera Pinto (www.cr2.cl). None of the investigators were blinded to the group allocation during the experiments.

Critical temperatures were determined as the environmental temperature at which an individual was unable to achieve an upright position within 1 minute [14]. Each individual was placed in a small chamber inside a thermo-regulated bath (WRC-P8, Daihan, Korea) at 30°C (CT_{Max}) and 5ºC (CT_{Min}) for 15 minutes, after which the bath temperature was increased (or decreased) at a rate of 0.8ºC per minute [18]. Every minute or at every change in 1ºC, the chamber was turned upside down and we observed if the animal was able to return to the upright position. When an animal was unable to achieve an upright position within 1 minute it was allowed to recover at ambient temperature (CT_{Min}) or for 30 minutes in a box with ice packs (CT_{Max}). Body mass (a proxy of body size) was obtained before each trial using a Shimadzu TX323L electronic balance.

Preferred temperature (T_{Pref}) was determined simultaneously for five individuals in five open-top terraria (length x width x height: 85 x 12 x 30 cm). Each terrarium had a thermal gradient between 10ºC and 30ºC produced by an infrared lamp overhead (250 W) on one end, and ice packs on the other. The organic gardening soil was moisturized at the beginning of each trial to prevent the desiccation of the frogs. Five individuals were placed at the centre of each one of the terraria and 45 minutes later we registered T_{Pref} as the dorsal body temperature (T_b) using a UEi INF155 Scout1 infrared thermometer. Dorsal and cloacal T_b are highly associated (r_P = 0.99) [see ,14 for details on the calibration procedure]. Body mass was obtained before each trial using a Shimadzu TX323L electronic balance.

Standard metabolic rate, measured through oxygen consumption at 20°C and 30°C was measured continuously using an infrared O_2 - CO_2 analyzer (LI-COR LI6262, Lincoln, NV, USA). The analyzer was calibrated periodically against a precision gas mixture. Although there was almost no difference between calibrations, baseline measurements were performed before and after each recording. Flow rates of CO2 – free air was maintained at 100 ml min^{-1} ± 1% by a Sierra mass flow controller (Henderson, NV, USA). We used cylindrical metabolic chambers (60 ml), covered by metal paper. O_2 consumption was recorded during 45 minutes per individual. Each record was automatically transformed by a macro program recorded in the ExpeData software (Sable Systems), to (1) transform the measure from % to mlO_2 min^{-1}, taking into account the flow rate and (2) to eliminate the first 5 min of recordings. For each individual, the metabolic sensitivity (Q_{10}) was calculated as the ratio between metabolic rate measured at 30ºC and metabolic rate measured at 20ºC.

Selection on thermal traits

After experiments, all frogs were put back to 20ºC for at least one month before releasing them. Marked frogs were released at Carrera Pinto in April 2013 and their survival was monitored on three separate recapture efforts (13th October 2013, 13th June and 9th September 2014). For each individual, we express plasticity as the difference in trait values between high versus low acclimation temperatures ($\Delta\text{Trait} = \text{Trait}_{20ºC}-\text{Trait}_{10ºC}$), hereafter referred to as ΔCT_{Max}, ΔCT_{Min}, ΔT_{Pref}, and ΔQ_{10}. As the desert surrounds these two small ponds dispersal was not a concern.

The relationship between trait plasticity and survival was analyzed using a Cormack-Jolly-Seber (CJS) framework in Program MARK. An overall goodness of fit test was run using U-Care to check for the presence of structure in the data which could be accommodated within the modeled parameters and to obtain a value for the over dispersion parameter (c-hat). The time interval between capture occasions (as a fraction of 1 year and considering also the original capture event) was included in the analysis to accommodate the unequal intervals. The resulting resighting and survival estimates were therefore corrected to annual estimates. Survival and resighting parameters were obtained in a two-stage process. First, the best-fit resighting model was identified from three candidate models (constant, time dependent and a linear trend). The fit of the three candidate resighting models was compared using survival modeled as both a constant rate and also as a time-dependent rate, to ensure that selection of the best-fit resighting model was not influenced by choice of survival model. Once the best-fit resighting model had been identified (using AICc) this was then retained for all candidate survival models. Survival rates were extracted as a function of the individual covariates. A model selection and an information-theoretic approach [19] was employed to contrast the adequacy of different working hypotheses (the candidate models) of selection on trait plasticity. To reduce the number of candidate models, thereby minimizing the likelihood of spurious results [19,20], we tested only for a null model, a model with body mass and models with directional and quadratic selection for each trait separately and also for correlational selection (interaction of trait combinations) among traits (Table 1). In total, 27 models were evaluated. Body mass was included in all models including physiological traits. All analyses were performed in R version 3.1.3 employing package RMark [21]. No transformation was required to meet assumptions of statistical tests.

RESULTS

All measured traits including critical thermal limits (CT_{Max}, CT_{Min}), thermal preference (T_{Pref}) and sensitivity of metabolic rate to temperature (Q_{10}) and their norms of reaction for acclimation plasticity (ΔCT_{Max}, ΔCT_{Min}, ΔT_{Pref}, ΔQ_{10}) showed high variance among individuals (Fig. 1). In addition, for all traits some individuals shifted their thermal traits to higher values when acclimated to high temperatures, but other individuals showed the reverse response, that is their traits shifted to lower values after acclimation at higher temperatures (Fig. 2). Body size showed a positive relationship with ΔCT_{Max} ($b = 0.59 \pm 0.18$ SE, $F_{1,86} = 10.99$, $P = 0.0013$) which indicates that larger individuals had positive delta values of CT_{Max} (i.e. the values at 20ºC were higher than at 10ºC). Body size was not associated with any other trait plasticity (results not shown).

The overall goodness of fit measure for the CJS model indicated a moderate level of over-dispersion (c-hat = 2.65, $P = 0.103$), however with only 3 recapture occasions it was not possible to identify an alternative starting model and the basic CJS model was adopted as the basis for subsequent model fitting, with unexplained over-dispersion controlled using the c-hat adjustment. A constant resighting rate was the best-fit model irrespective of whether survival was modeled as a constant or time dependent rate (Table 1). Consequently, the constant rate-resighting model was retained for subsequent modeling of survival. The model selection procedure indicated that from the 27 candidate models tested, there was not a single best-fit one. In particular, the null model and the one containing only body size had a relative strong support (ca. 60% of cumulative Akaike weights), whilst a remaining 35% was split among models including simple directional selection (ca. 26% of cumulative Akaike weights) and those including directional and non-linear selection on the plasticity of each trait (ca. 9%, Table 1). The models including correlational, directional and stabilizing selection for more than one trait had very weak empirical support (Table 1). Strong support for the simpler models may in part have been due to the relatively high value of c-hat, which penalizes models on the basis of parameter number. Survival in relation to each covariate was obtained as the model averaged value across all candidate models (Table 1), weighted by individual model probability. In particular, survival increased with body mass, ΔCT_{Min} and ΔQ_{10} and decreased with ΔCT_{Max} and ΔT_{Pref} (Fig. 3).

DISCUSSION

To persist in a warming world evolutionary adaptation might be required when acclimatisation responses reach their limit [6]. As both the strength and shape of selection are key elements that impact the speed at which populations can evolve, determining whether selection in nature targets plasticity itself is of paramount importance. Here, to the best of our knowledge for the first time, we studied natural selection on thermal acclimation capacity of performance (ΔCT_{Max} and ΔCT_{Min}), metabolism (ΔQ_{10}) and behaviour (ΔT_{Pref}). Our results indicate that thermal acclimation is a target of selection in nature, although the pattern of phenotypic selection evidences a complex fitness landscape where different high-fitness strategies are being favoured. Summarising, we found that survival increased in individuals: (i) with larger body size, (ii) with higher CT_{Max} when cold acclimated, (iii) with higher CT_{Min} when warm acclimated, (iv) that selected higher temperatures (T_{Pref}) when cold acclimated and (v) that increase their Q_{10} when warm acclimated.

Acclimation, particularly from the point of view of environmental or comparative physiologists, has long been thought to be adaptive (usually *post hoc*), although that claim clearly does not represent a test for it [22]. However, most of the empirical tests of this beneficial acclimation hypothesis (i.e. BAH, acclimation to a higher temperature should enhance performance at those temperatures) have offered little support for it [22,23]. In fact, it has been shown that physiological traits can evidence a wide repertoire of responses to acclimation [23,24]. Here we show an adaptive benefit of the BAH in terms of improved survival for CT_{Min}

and Q_{10}. In addition, CT_{Max} and T_{Pref} show that acclimation to a low temperature enhances performance at those low temperature, which is known as the cold is better with complete temperature compensation hypothesis [23]. Furthermore, although we did not use an experimental framework to isolate a particular agent of selection [12,25], we consider that our results strongly suggest that the thermal environment is responsible for the patterns we found. First, this population inhabits two highly isolated ponds were the presence of other potentials competitors (anurans) has not been observed, although there might be a risk of predation by herons (*L.D.B. personal observation*). Second, survival was monitored during a complete year on three separate recapture efforts encompassing specific phases related to the breeding season (August – October). After measurements, animals were released in April 2013 (non-breeding), the first recapture occurred at the end of that breeding season (October 2013), the second occurred almost at the onset (mid-June 2014) and during the following breeding season (September 2014). In this context, although our survival estimates have been averaged out through that year (see Methods), they incorporate that within year variation associated with clearly different thermal regimens during the breeding season and the active period. Third, using biophysical models at Carrera Pinto, we have determined that mean operative temperature during daytime was only affected by sun exposure (shade – sunshine) but not by hydric (dry – wet) conditions [14].

Selection favored bigger individuals, something that have been previously reported in the literature [26–29]. This is somewhat unsurprising, given that body mass is known to be positively associated with several physiological traits that enhance performance [30–34] including plasticity itself [35]. Furthermore, bigger individuals showed positive delta values of CT_{Max}, that is their CT_{Max} increased when warm acclimated. This might seem puzzling as we also shown that survival increased in individuals with higher CT_{Max} when cold acclimated (i.e. the opposite pattern in directional selection on ΔCT_{Max}). We believe these two different high-fitness strategies are probably related to *Pleurodema thaul's* natural history. These frogs are active and aboveground 365 days a year, only retreating to the pond to breed, cool off, or to hydrate. They breed during August – October where they experience an average minimum temperature of 10ºC but where temperatures reach an average maximum of 25ºC. In addition during the breeding season temperatures have been recorded to fluctuate from below 0ºC (minimum -6.2ºC) up to above 30ºC (maximum 37.5ºC) (1993 – 2014; www.cr2.cl). The non-breeding season (November – July) has higher averages of minimum and maximum temperatures, but less extreme records of minimum temperatures. In this context, one strategy exhibits increased survival in individuals that are able to tolerate high temperatures during the breeding season. That is, they showed higher CT_{Max} and T_{Pref} when cold acclimated (Fig. 3). However, the higher tolerance to high temperatures when cold acclimated, came at a cost of lower tolerance to cold temperatures in that cold season (i.e. higher CT_{Min}, Fig. 3). The alternative strategy exhibits higher survival in individuals that, when warm acclimated (i.e. 20ºC mean temperature during the whole year), increase their Q_{10} and reduce their investment in cold tolerance mechanisms. In addition, it might be possible that bigger individuals, who also have higher values of CT_{Max}, are able to tolerate better the high temperatures during the non-breeding months. Nevertheless, further work is needed to evaluate whether selection operates differently on and off the breeding period for body size.

The difference between habitat temperature and CT_{Max} is thought of as an index warming tolerance [36,37]. Here we construct an analogous metric between T_{pref} and CT_{Max} as a thermal safety margin. A frog that has a large difference between its thermal preference and CT_{Max} will maintain a larger safety margin than one with a small difference. We computed plasticity in the thermal safety margin under acclimation, $\Delta[CT_{Max} – T_{Pref}]$, and correlated it with ΔQ_{10} for metabolism (b= -0.00256 ± 0.00124 S.E., $F_{1,82}$=4.26, P =0.04), controlling for effects of frogs with higher growth plasticity that also have higher Q_{10} plasticity (b= 0.0705±0.0295 S.E., $F_{1,82}$ = 5.71, P =0.02). That is, frogs with a positive value for ΔQ_{10} (and also higher survival when warm acclimated) have negative values for $\Delta[CT_{Max} – T_{Pref}]$ and thus, lose some of their safety margin during acclimation. Therefore, the high plasticity in Q_{10} involves a change in preference that brings the body temperatures of thermoregulating frogs closer to CT_{Max} when they move from lower to higher temperatures. In addition, frogs that increased they T_{Pref} when warm acclimated showed a decrease in survival (Fig. 3) suggesting that the gains in metabolic capacity when warm acclimated might be offset by the costs of being active at higher temperatures. Summarizing, one strategy favored frogs that are able to tolerate the high temperatures that occur during the cold breeding season whilst the other favored frogs that increase their activity levels during the warmer periods of the year.

It is important to mention though, that we have measured plasticity in just one life stage. It is likely that other ecological and physiological traits might also be plastic, their responses to acclimation might be different and they might even be different between different life stages and thus, only further work in other traits and stages might disentangle these possibilities. Nevertheless, we still consider our results show a strong signal and provide the first evidence of phenotypic plasticity as an actual target of selection in nature, and therefore to evaluate the potential of evolutionary adaptation to prevent demographic extinction from climate change [38].

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Competing interests. We declare we have no competing interests

Author Contributions. L.D.B conceptualized the study, designed the experimental procedures and carried out the experiment with A.M.B., A.G.M., M.R.A. and J.D.G.E; B.S., M.T. and L.D. B. analyzed the data and L.D.B. and B.S. wrote the paper with input from A.M.B and J.D.G.E.

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Ethics. This study did not involve endangered or protected species and was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Comisión Nacional de Investigación Científica y Tecnológica de Chile (CONICYT). All experiments were conducted according to current Chilean law. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Universidad Austral de Chile.

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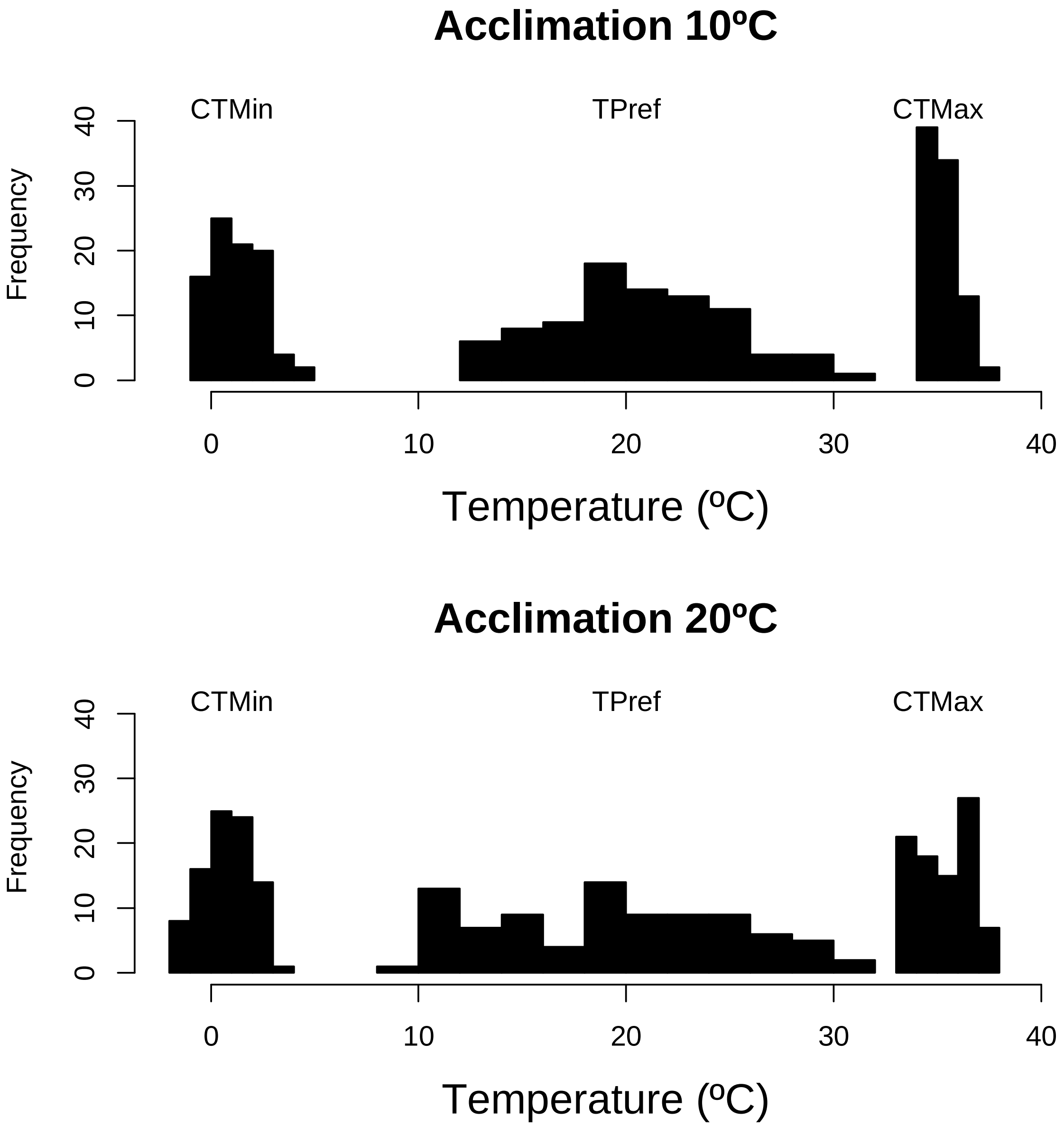
Figure Legends

Figure 1: Frequency distribution of CT_{Min}, T_{Pref} and CT_{Max} of the four-eyed frog when acclimated to 10ºC and 20ºC.

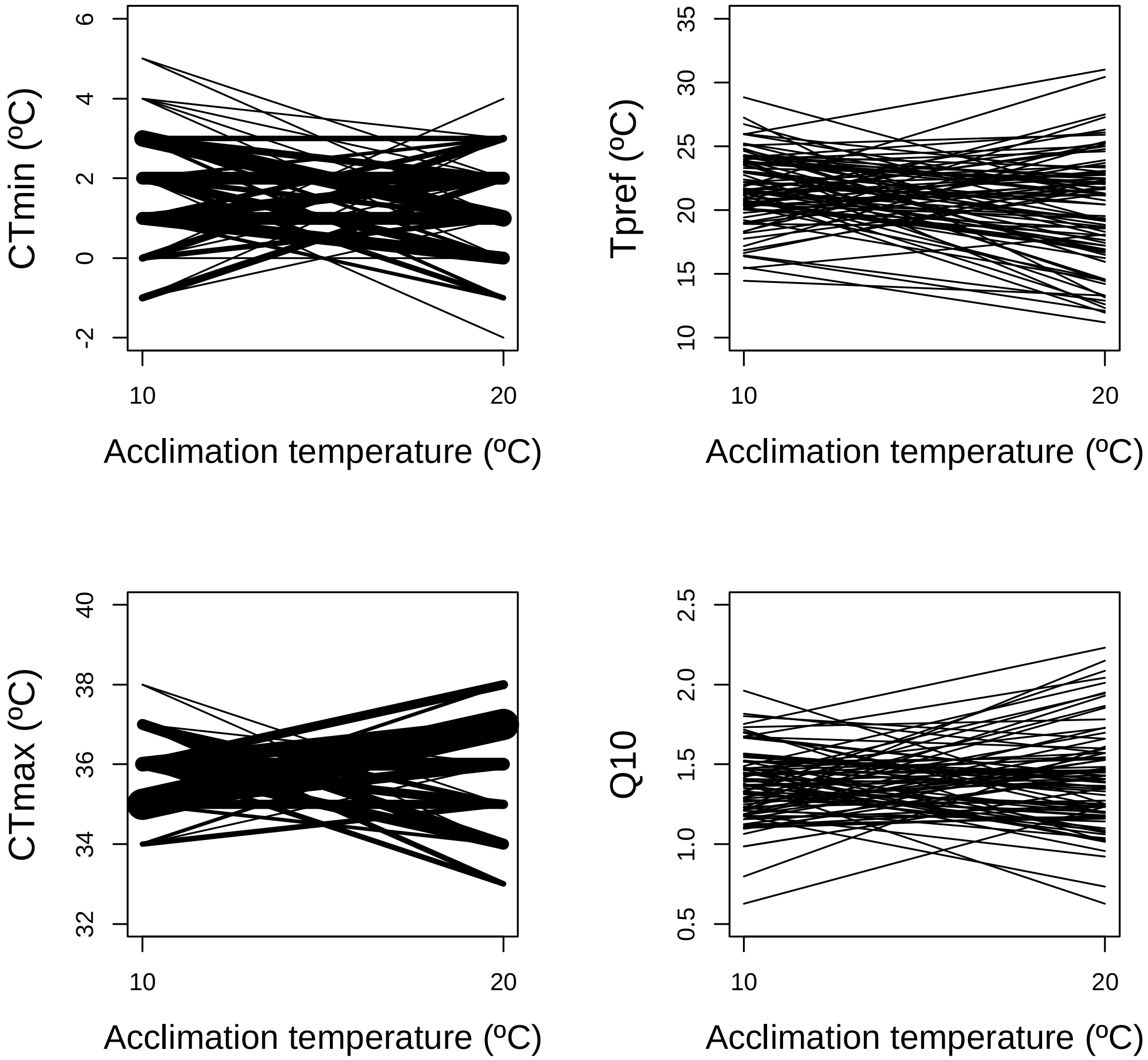
Figure 2: Individual plasticity in CT_{Min}, T_{Pref}, CT_{Max} and Q_{10} to 10 and 20ºC acclimation treatments. Each line represents the individual value of the specific traits at each temperature. For CT_{Min} and CT_{Max} the width of the line is directly proportional to the number of individuals that showed that specific response.

Figure 3: Survival estimates of directional selection as a function of individual plasticity, expressed as Δ (i.e. $Trait_{20ºC}$-$Trait_{10ºC}$).

Figure 1


Acclimation 10ºC
CTMin
TPref
CTMax
Frequency
0
10
20
30
40
0
10
20
30
40
Temperature (ºC)
Acclimation 20ºC
CTMin
TPref
CTMax
Frequency
0
10
20
30
40
0
10
20
30
40
Temperature (ºC)

453 **Figure 2**



454

455

Figure 3

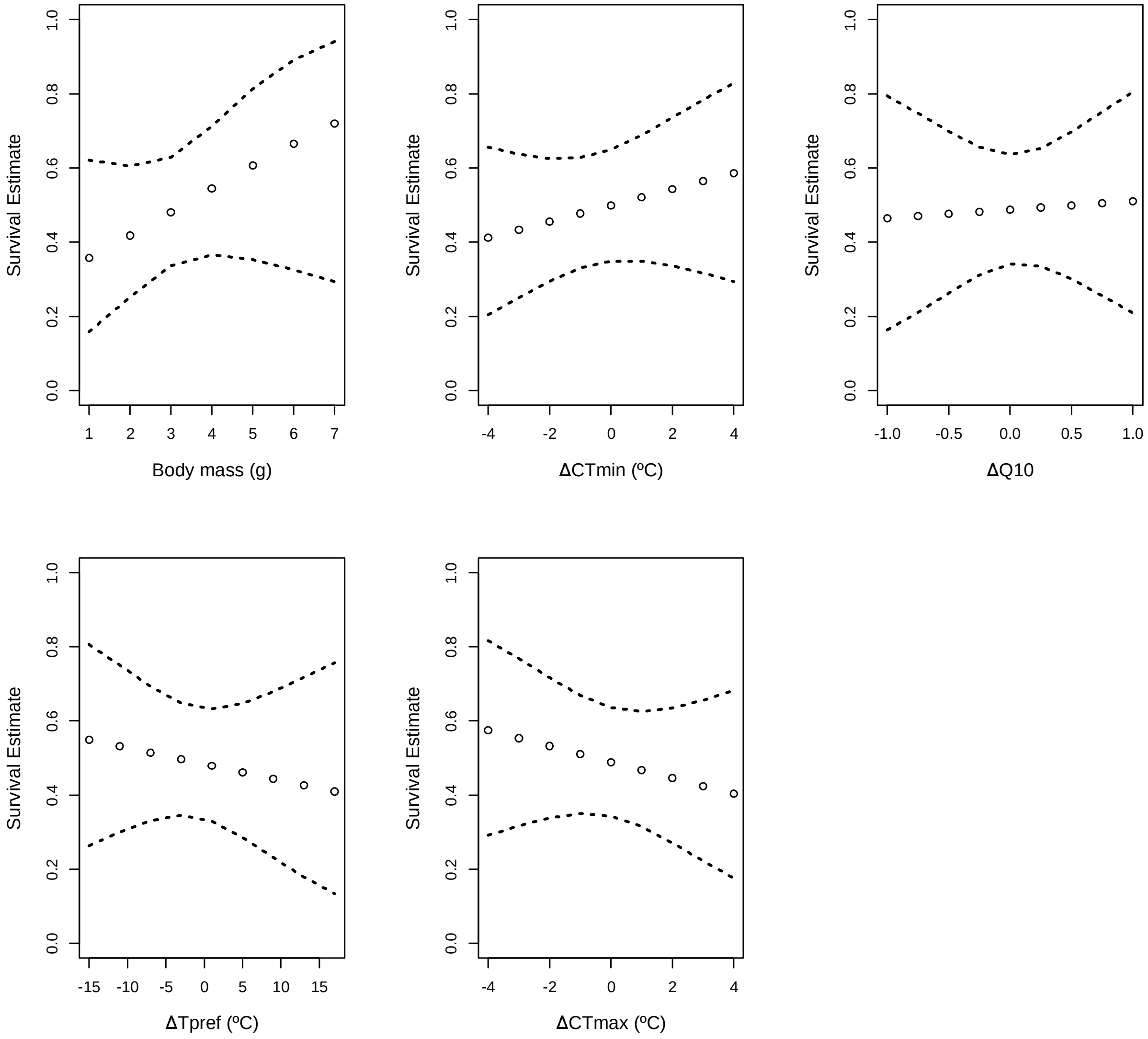
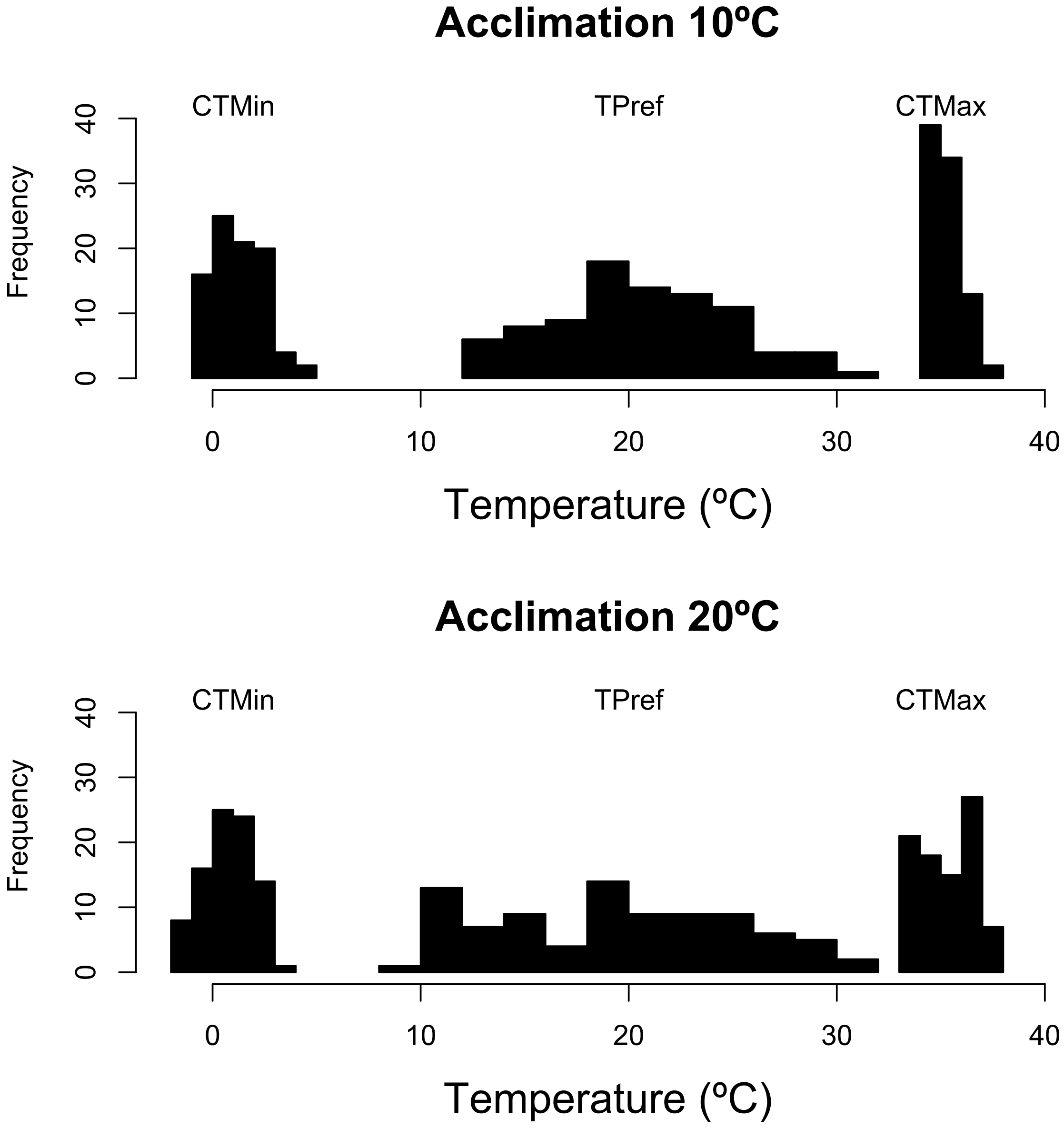

Survival Estimate
0.0
0.2
0.4
0.6
0.8
1.0
1
2
3
4
5
6
7
Body mass (g)
-4
-2
0
2
4
ΔCTmin (ºC)
-1.0
-0.5
0.0
0.5
1.0
ΔQ10
-15
-10
-5
0
5
10
15
ΔTpref (ºC)
ΔCTmax (ºC)

Table 1. Candidate models ordered accordingly to their Akaike weights. Single term models represent directional (e.g. ΔCT_{Max}) and/or stabilizing selection when a square term is included (e.g. $\Delta CT_{Max} + (\Delta CT_{Max})^2$) and correlational selection when different traits plasticity are included (e.g. $\Delta CT_{Max} * \Delta CT_{Min}$).

Models		K	AICc	ΔAICc	w_i
1	Null model	2	106.28	0.00	0.41
2	MB	3	107.98	1.70	0.17
3	ΔCT_{Min}	4	109.89	3.61	0.07
4	ΔCT_{Max}	4	109.93	3.65	0.07
5	ΔT_{Pref}	4	110.02	3.74	0.06
6	ΔQ_{10}	4	110.09	3.81	0.06
7	$\Delta CT_{Min} + (\Delta CT_{Min})^2$	5	112.04	5.76	0.02
8	$\Delta CT_{Max} + (\Delta CT_{Max})^2$	5	112.08	5.80	0.02
9	$\Delta T_{Pref} + (\Delta T_{Pref})^2$	5	112.17	5.89	0.02
10	$\Delta Q_{10} + (\Delta Q_{10})^2$	5	112.24	5.96	0.02
11	$\Delta CT_{Max} * \Delta CT_{Min}$	6	113.14	6.86	0.01
12	$\Delta CT_{Max} * \Delta T_{Pref}$	6	113.21	6.93	0.01
13	$\Delta CT_{Max} * \Delta Q_{10}$	6	113.89	7.61	0.01
14	$\Delta CT_{Min} * \Delta T_{Pref}$	6	114.16	7.88	0.01
15	$\Delta CT_{Min} * \Delta Q_{10}$	6	114.21	7.93	0.01
16	$\Delta Q_{10} * \Delta T_{Pref}$	6	114.26	7.98	0.01
17	$\Delta CT_{Max} + (\Delta CT_{Max})^2 + \Delta CT_{Min} + (\Delta CT_{Min})^2$	7	115.88	9.60	0.00
18	$\Delta CT_{Max} + (\Delta CT_{Max})^2 + \Delta T_{Pref} + (\Delta T_{Pref})^2$	7	116.37	10.09	0.00
19	$\Delta CT_{Min} + (\Delta CT_{Min})^2 + \Delta T_{Pref} + (\Delta T_{Pref})^2$	7	116.39	10.11	0.00
20	$\Delta CT_{Min} + (\Delta CT_{Min})^2 + \Delta Q_{10} + (\Delta\ Q_{10})^2$	7	116.43	10.15	0.00
21	$\Delta CT_{Max} + (\Delta CT_{Max})^2 + \Delta Q_{10} + (\Delta\ Q_{10})^2$	7	116.44	10.16	0.00
22	$\Delta Q_{10} + (\Delta\ Q_{10})^2 + \Delta T_{Pref} + (\Delta T_{Pref})^2$	7	116.55	10.27	0.00
23	$\Delta CT_{Max} + (\Delta CT_{Max})^2 + \Delta CT_{Min} + (\Delta CT_{Min})^2 + \Delta Q_{10} + (\Delta Q_{10})^2$	9	120.32	14.04	0.00
24	$\Delta CT_{Max} + (\Delta CT_{Max})^2 + \Delta CT_{Min} + (\Delta CT_{Min})^2 + \Delta T_{Pref} + (\Delta T_{Pref})^2$	9	120.34	14.06	0.00
25	$\Delta CT_{Max} + (\Delta CT_{Max})^2 + \Delta T_{Pref} + (\Delta T_{Pref})^2 + \Delta Q_{10} + (\Delta\ Q_{10})^2$	9	120.83	14.55	0.00
26	$\Delta CT_{Min} + (\Delta CT_{Min})^2 + \Delta T_{Pref} + (\Delta T_{Pref})^2 + \Delta Q_{10} + (\Delta Q_{10})^2$	9	120.90	14.62	0.00
27	$\Delta CT_{Max} + (\Delta CT_{Max})^2 + \Delta CT_{Min} + (\Delta CT_{Min})^2 + \Delta Q_{10} + (\Delta\ Q_{10})^2 + \Delta T_{Pref} + (\Delta T_{Pref})^2$	11	124.87	18.59	0.00

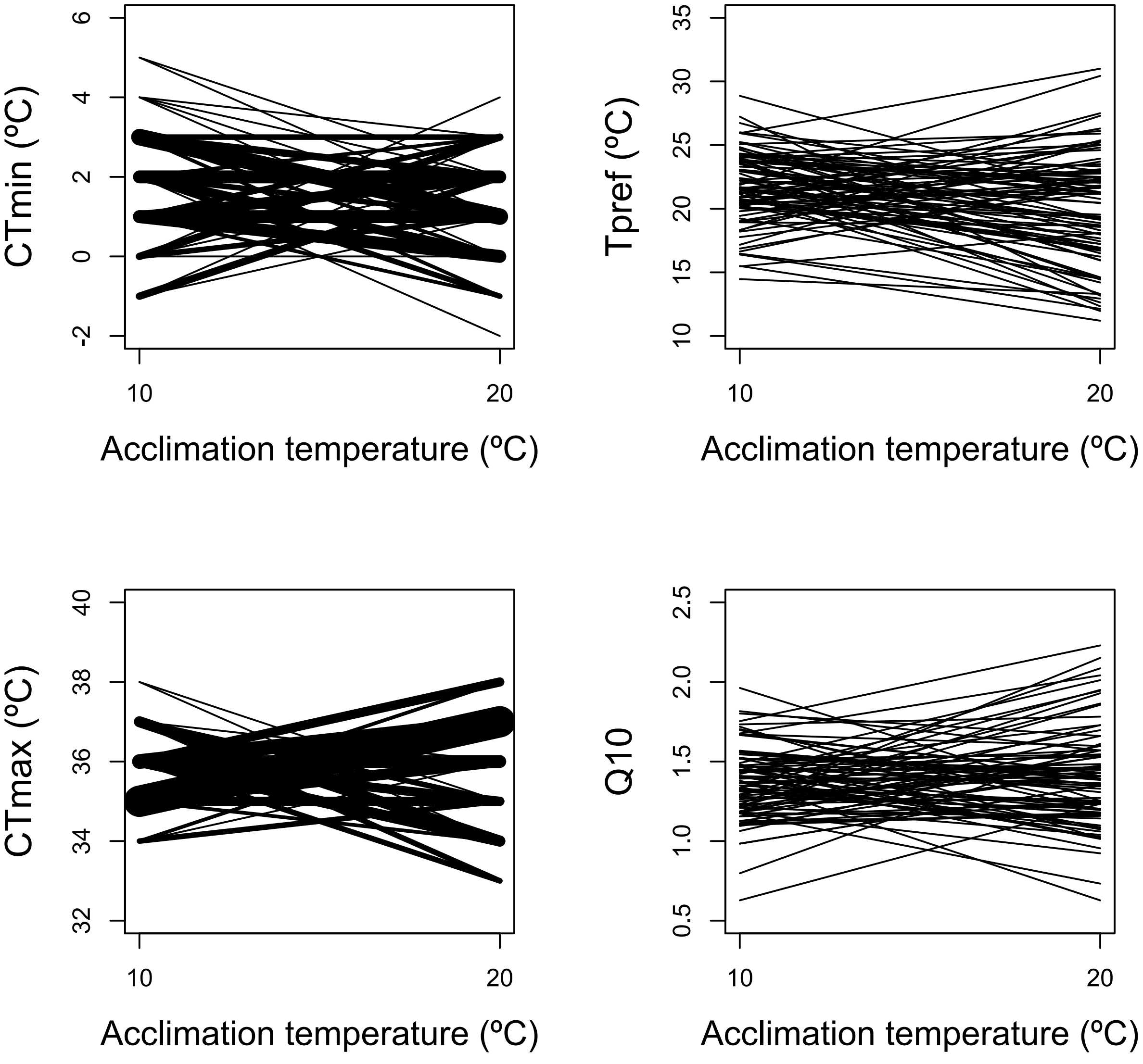
Note: MB was included in all 3 – 27 models

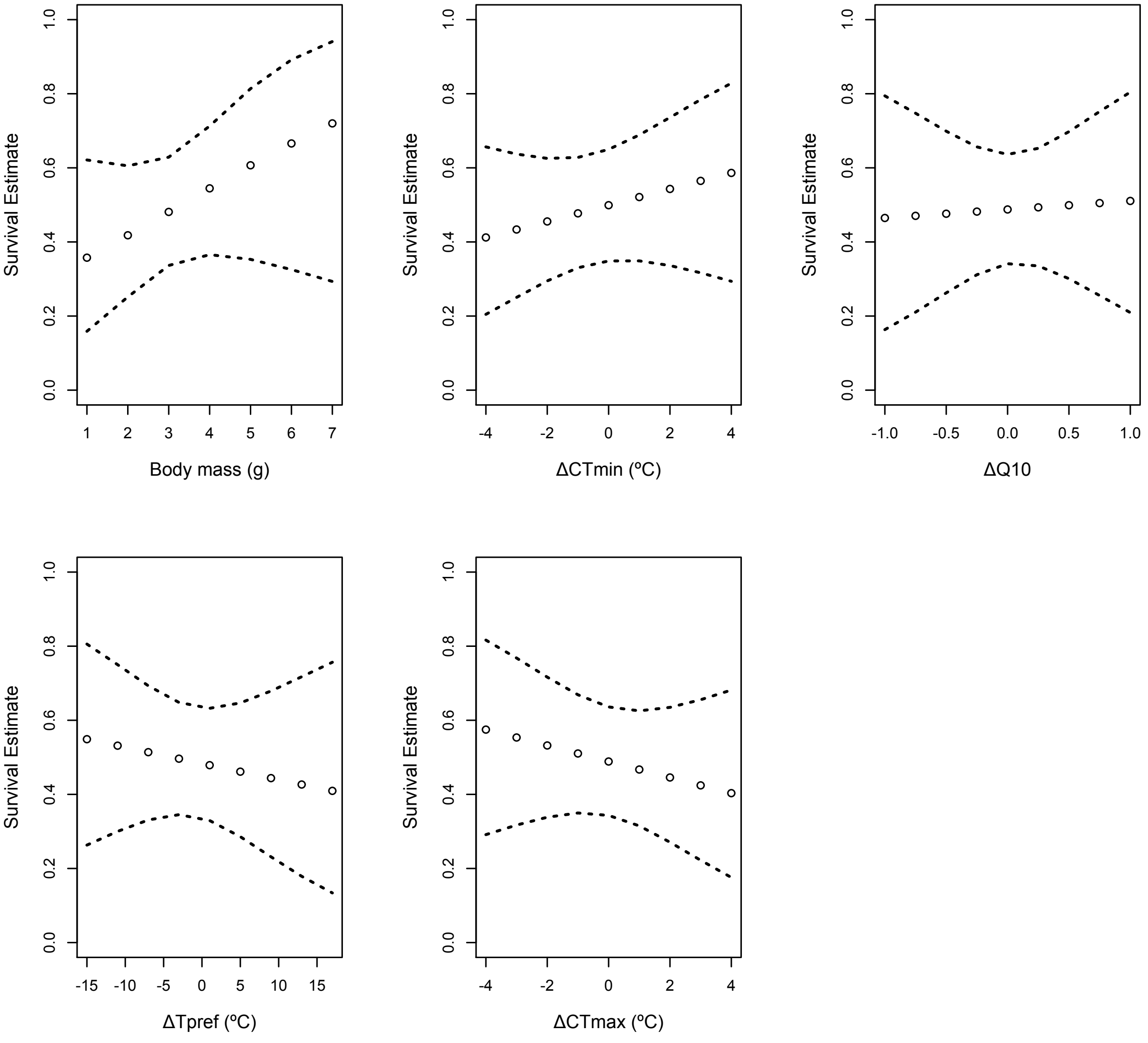
K = number of parameters.
AICc: AIC values corrected for small sample sizes.
w_i: Akaike weights.



Acclimation 10ºC

Acclimation 20ºC


CTmin (ºC)
6
4
2
0
-2
10
20
Acclimation temperature (ºC)
Tpref (ºC)
35
30
25
20
15
10
10
20
Acclimation temperature (ºC)
CTmax (ºC)
40
38
36
34
32
10
20
Acclimation temperature (ºC)
Q10
2.5
2.0
1.5
1.0
0.5
10
20
Acclimation temperature (ºC)



Survival Estimate
0.0
0.2
0.4
0.6
0.8
1.0
1
2
3
4
5
6
7
Body mass (g)
Survival Estimate
0.0
0.2
0.4
0.6
0.8
1.0
-4
-2
0
2
4
ΔCTmin (ºC)
Survival Estimate
0.0
0.2
0.4
0.6
0.8
1.0
-1.0
-0.5
0.0
0.5
1.0
ΔQ10
Survival Estimate
0.0
0.2
0.4
0.6
0.8
1.0
-15
-10
-5
0
5
10
15
ΔTpref (ºC)
Survival Estimate
0.0
0.2
0.4
0.6
0.8
1.0
-4
-2
0
2
4
ΔCTmax (ºC)