



university of
 groningen

Parental effects on offspring fitness of *Drosophila melanogaster*



Author:

Fábio Manuel Barroso Sousa

S3529096

Supervisors:

Pinar Kohlmeier, PhD student

Prof.dr.Jean-Cristophe Billeter

Parental effects on offspring fitness of *Drosophila melanogaster*

Author: Fábio Manuel Barroso Sousa

Student number: 3529096

Field of study: Biology

Groningen Institute of Life Sciences & Technology

Groningen Institute for evolutionary life sciences

Supervisors: Pinar Kohlmeier, Jean-Christophe Billeter

30-12-2019

Table of Contents

INTRODUCTION	4
MATERIALS AND METHODS.....	6
Model Organism	6
Experimental Evolution	6
Split-brood design	7
Parental generation.....	8
Mating	8
Egg collection and splitting.....	8
Survivability and Developmental Time.....	10
Cold-Shock and Heat-Shock recovery time	10
Statistical Analysis	11
RESULTS	11
<i>Treatment e</i>	11
<i>Treatment c</i>	13
<i>Treatment f2</i>	15
Heat-Shock Recovery.....	17
<i>Treatment e</i>	17
<i>Treatment c</i>	18
<i>Treatment f2</i>	19
Cold Shock Recovery	20
<i>Treatment e</i>	20
<i>Treatment c</i>	21
<i>Treatment f2</i>	22
DISCUSSION	23
Survival and developmental time analysis	24
Heat and Cold-shock analysis	24
Final Remarks	26
CONCLUSION	27
ACKNOWLEDGEMENTS	28
REFERENCES	29

INTRODUCTION

Today's world faces a challenge like never before: climate change. What for some can be an illusion, for others, a devastating phenomenon that can obliterate established species and ecosystems. It is an undeniably present problem that already had severe and irreparable consequences in some environments (Walther *et al.* 2002). Climate change effects have severe impacts both in endotherms as in ectotherms' fitness, particularly the changes in temperature across several regions in the globe. Even the increase of just 1 °C in the mean temperature over time can severely impact some species like tropical insects, as these species have a lower temperature tolerance (Deutsch *et al.* 2008). Such changes in temperature can affect morphology, physiology, behaviour and overall life strategy of organisms. As examples, both endotherm and ectotherm organisms tend to grow larger in cold temperatures and smaller in hot temperatures (Huey *et al.* 1989), additionally, developmental time in insects is negatively correlated with temperature (Dillon *et al.* 2007; Davison & Birch 2008; Chen *et al.* 2014). Ultimately this temperature selection pressure can lead to species extinction when there are no ameliorating factors, as migration and adaptation (Deutsch *et al.* 2008).

Adaptation can be both genetic and non-genetic. Genetic adaptation in organisms is usually a relatively slow process that takes several generations to incur in phenotypical changes and thus relies on the time factor as a key aspect of its success (Orr 2005). In contrast, non-genetic adaptation mechanisms are usually faster processes that can be more advantageous in response to rapid environmental changes (Bonduriansky *et al.* 2012). An important mechanism in non-genetic adaptation is trans-generational plasticity. This mechanism is mediated by non-genetic information, the anticipatory maternal effects (Bonduriansky *et al.* 2012, Bonduriansky & Day 2009). These can be expressed in different ways, usually by conveying information transmitted by the mother to the egg. This information includes RNA molecules, hormones, DNA histones, but also other proteins that convey cues that can be used as maternal information for the offspring to be better prepared for its environment (Jablonka & Raz 2009). This maternal information, when matched with the offspring environmental condition, also mentioned in literature as matched conditions (Uller 2008), can thus prepare offspring for future conditions and may play a huge role in increasing offspring fitness in rapid changing conditions (Badyaev & Uller 2009). For example, on Galloway studies on the monocarpic herb *Campanulastrum americanum*, whose populations

span distinct light environments, either they grow on light gaps or understory, individuals developing in the same light conditions as their mother environment (matched conditions) always had a higher fitness than the populations developing in different conditions from their mother's (mismatched conditions) (Galloway 2005). This proves that maternal information can be of great importance for increasing the offspring fitness when mother-offspring environment matches. However, this maternal information can sometimes be unreliable or misleading. When mothers face unpredictable conditions or different environment from their offspring, this maternal information may turn up as a poor predictor of the offspring environment, which will interfere with offspring plasticity and lead to a mismatch between the offspring's phenotype and environment (Marshall & Uller 2007, Bonduriansky & Day 2009).

There are several mechanisms which lead to transgenerational effects. In vertebrates for example, the transference of antibodies from the mother to the offspring via the placenta or milk, during lactation, may be pivotal for the organism early growth and fitness in early stages of development (Hasselquist & Nilsson 2008) and the way this information is transmitted across generations is variable across species (Mousseau & Dingle 1991). This effects have yet to be clearly showed in experimental studies in animal models and still lack core fundamentals to prove its effectiveness, mainly due to the lack of an appropriate control group in most experiments (Hsu *et al.* 2016).

Having in mind that maternal effects have already been described in insects, regardless of being adaptive or context dependant-effects (Mousseau & Dingle 1991), in this study we aim to understand the existence and significance of this adaptive maternal effects, and whether the change in the environmental conditions predictability over generations affects this transgenerational information, using the model organism *Drosophila melanogaster*. Being an ectotherm species, it relies on its experience through environmental temperature or on maternal information for its fitness, and while being somewhat resilient to temperature changes (Gibert *et al.* 2001), this said effects can play a very important role in offspring fitness of this species (Dillon *et al.* 2007). Therefore, to evaluate maternal effects, we analyse the fitness of individual *Drosophila* with different parental and offspring temperature patterns. Mothers are placed on hot (28°C) and cold (18°C) chambers and are then studied, evaluating some fitness related traits in a 2x2 full factorial design. We evaluate the survivability, developmental time, cold-shock resistance and heat-shock resistance of their offspring.

In this work three treatments with different parental and offspring temperature correlations were analysed and compared. Considering the predictability and reliability of

maternal information, according to each correlation of the treatments studied, we expect to find different interactions of parent-offspring temperatures and different significances of parental information between each group. We expect that treatments with higher correlation values (closer to 1) have more predictable environmental conditions and so in most cases offspring in matching conditions will have higher fitness, and in offspring in mismatching conditions we will observe the inverse pattern. On the other hand, we further expect that treatments with the lowest correlations (zero or negative values), to evidence no impact of the maternal information on offspring, once we think that the predictability of the environmental conditions on these treatments isn't enough to give cues to mothers to convey reliable information to their offspring. This information will be very important to help us understand the adaptive significance of these maternal effects.

MATERIALS AND METHODS

Model Organism

In this study, fruit fly *Drosophila melanogaster* was used as a model organism. The fly population was collected from Groningen in the Netherlands on August 2017. To set up a base population, 249 wild-type female flies were put into the vials individually. The offspring coming from each female were kept for two generations in the lab for identification and then merged into one big population. To keep the contribution of each female equal, 25 female and 25 male offspring per female were used. Afterwards, base population was let to adapt to the lab conditions which are possibly different than nature such as food, light cycle and humidity.

Experimental Evolution

The base population was phenotyped, creating a base population and was then divided into replicates of six different groups (a, b, c, d, e and f) as shown in figure 1. These six groups were created to emulate different environment conditions, using environmental chambers, one with cold temperatures (18°C) and the other with hot temperatures (28°C).

The generational shifts of temperature were created based on temperature correlations between parental and offspring experience over a period of 30 generations.

Treatment *a*, with a correlation of 1 has all generations experiencing cold temperature. Treatment *b*, with a correlation of 1 has all generations experiencing a hot temperature. Treatment *c*, with a correlation of -1 shifts temperatures with every new generation. Treatment *d*, with a correlation of 0 shifts temperature every two generations. Treatment *e*, with a correlation of 1/3 shifts temperature every three generations. Treatment *f* consists of five replicates of different patterns of temperature shifts between generations, all having a correlation of 0. As explained with the previous *f* treatment.

On this study, treatments *e*, *c* and *f2* were studied and are highlighted in figure 1.

Every replicate and treatment were given optimal food and normal laboratory culturing conditions for fruit flies.

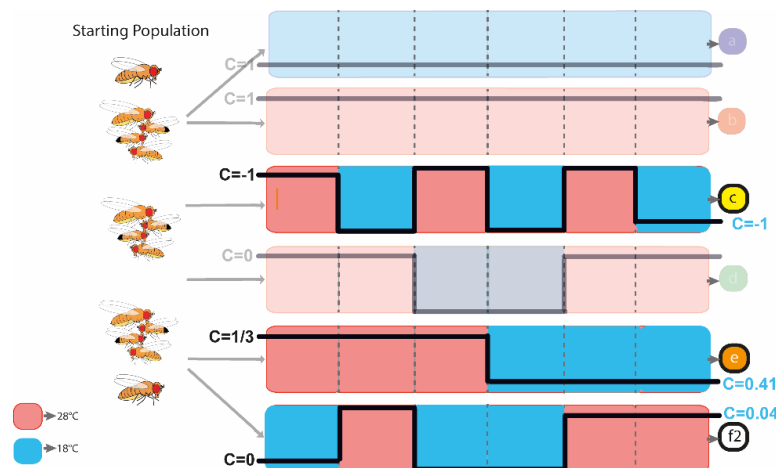


Fig 1. Representation of the six different treatments applied to the starting population. Dashed lines represent a generation switch. Highlighted treatments are the ones analysed in this study. Numbers in black indicate the correlation value over the final period of 30 generations. Numbers in light blue indicate the correlation value at the time this study was made.

Split-brood design

To analyse the impact of both offspring and parental temperatures on offspring phenotype, a match-mismatch split-brood design was used to evaluate the fitness traits

previously mentioned (survivability, developmental time, cold-shock resistance and heat-shock resistance), where we split the eggs of collected females to create four different conditions depending on the mother and offspring environmental temperature (Figure 2). This design was split into chronological events starting with the collection of the parental generation, mating of the females and egg collection and splitting.

Parental generation

Larvae collection from the desired treatment was performed for the split-brood design start. Larvae were collected after egg collection was made on the specific treatment's population. Larvae were collected in the temperature that the parents were developing in that generation.

Mating

Virgin females were transferred to individual vials. Half of them were placed in either a hot (28 °C) or a cold (18 °C) climate chamber. Males were also transferred to vials and placed on the correct temperature (half on cold and half on hot) so mating could occur on the following day. After 24 hours, two males coming from the same temperature as females were added to each individual vial containing a female, in both conditions, for a period of 24 hours, so mating could occur. In the cold room these flies had one more day of acclimation because of slower development due to more adverse conditions.

Egg collection and splitting

After the mating period, males were removed, and each female was given a different identification considering its environmental condition (temperature that they developed in). These females are the parental generation for this design. These females were then placed inside empty vials which in turn were placed on top of a black coloured food batch (obtained by mixing activated carbon with normal food), with a yeast paste dab on top and on a low light environment, usually optimal conditions for egg laying. The food was coloured with activated carbon to make the egg collection process easier, once the eggs are white and therefore harder to look for on normal drosophila food. The food batches were observed every 2 hours to verify the presence of eggs in the hot condition, and every four hours for the cold condition. Within every observation, half of the collected eggs were placed into the cold condition and the other half into the hot condition. This process was repeated by switching vials from one food batch to the other (Figure 3), until around 10 eggs per mother identification

were collected. This parental generation is then mated and again their brood is split into the two different conditions (Figure 2).

This method is used to disentangle the maternal temperature effect on offspring, and also to differentiate the four different studied conditions, two with matched conditions: Hot parents-Hot offspring (HH), Cold parents - Cold offspring (CC); and two with mismatched conditions: Hot parents - Cold offspring (HC) and Cold parents - Hot offspring (CH)). With these four different groups the previously mentioned fitness traits were then analysed. This process was divided into 5 batches, in consecutive days, to create 5 technical replicates of the same treatment.

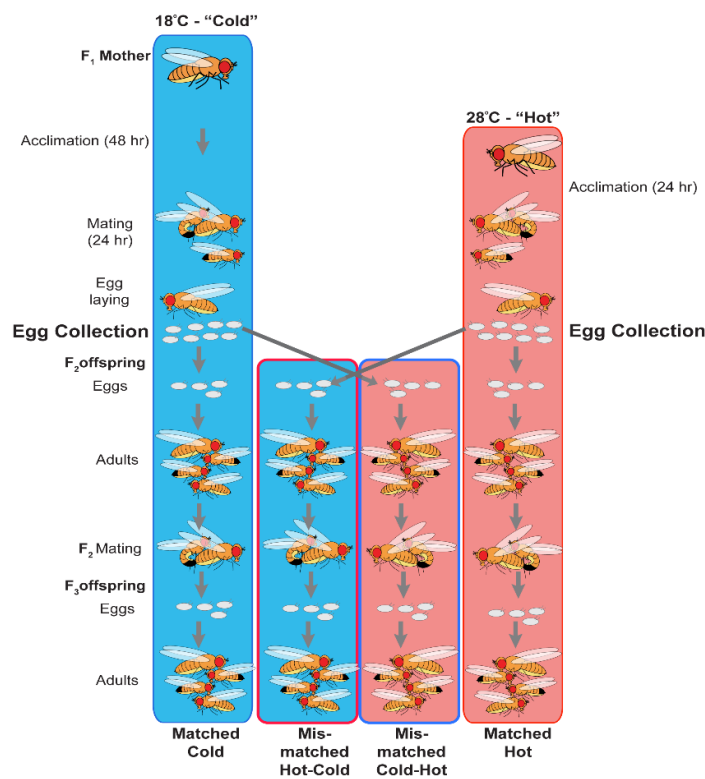


Fig. 2. Schematic illustration of split-brood design used to quantify the effect of maternal and offspring conditions on offspring phenotype.

Egg Collection Process

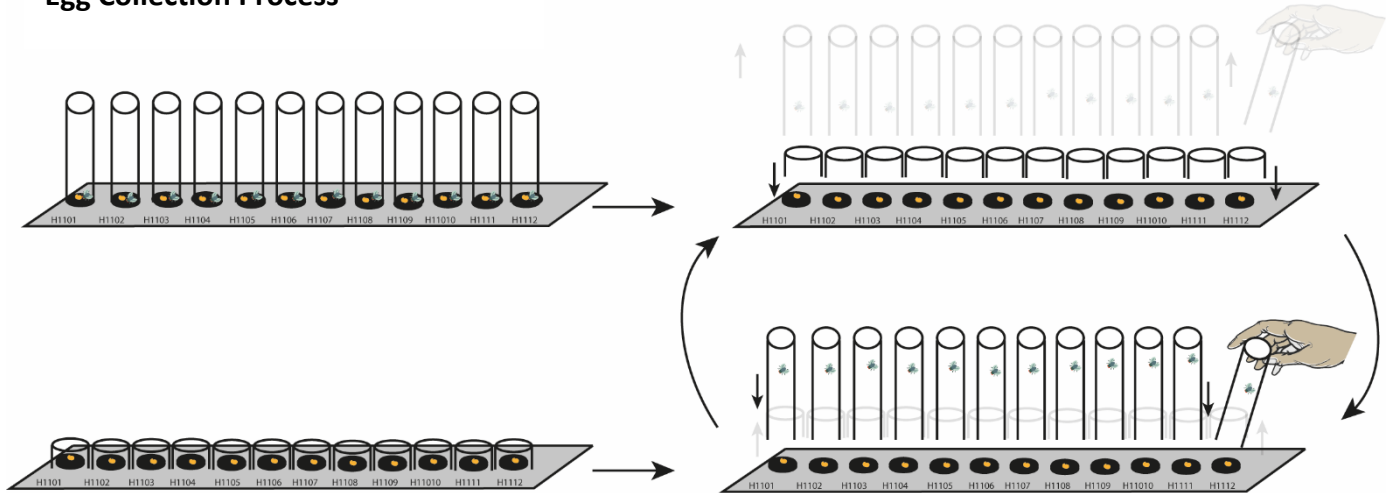


Fig. 3. Schematic representation of the egg collection process highlighted on figure 2.

Survivability and Developmental Time

After the egg collection process in the split-brood design, four different condition groups were created according to the parent-offspring relation: Hot-Hot, Hot-Cold, Cold-Cold and Cold-Hot. For the survival analysis, the number of eggs placed in each vial was registered so we could calculate the percentage of eggs that reached adult stage.

For the developmental time analysis, each day, the vials containing the eggs coming from the split-brood design were checked for adults, in three set checkpoints during the day: 9:30, 14:30 and 18:00. These time points were used to calculate the developmental time from egg to adult per individual.

Cold-Shock and Heat-Shock recovery time

For the cold-shock recovery time experiment, sex-separated flies were placed on ice for a period of 5 hours and 17 minutes at 0°C. After the set period, flies were placed at room temperature on mini petri dishes (35*15mm) and each individual's recovery time (time until the fly stands back on its legs) was recorded.

The heat-shock recovery time experiment consisted of placing 2-3 males and 2-3 females per mother ID in small glass vials that were inserted on a floating platform. The

floating platform was placed in a heat bath set at 41°C for a period of 7 minutes and 20 seconds. After this period, they were placed into an observation platform at room temperature and the flies' recovery time was registered with the help of the android application: Multi Timer version 2.5.5.

Collection of flies for cold and heat-shock recovery time experiments was performed during at the same time as the aforementioned developmental time checks, each day, in three set times during the day. A total of ~ 10 parental female IDs per population replicate and per experimental batch were collected. Each parental female ID had 2-3 females and 2-3 males, creating a total of ~ 150 adult flies per batch, used for cold and heat-shock analysis. The collected parental female IDs were separated in two groups of equal number, divided for cold-shock and heat-shock studies.

Statistical Analysis

Survivability, developmental time and heat and cold-shock resistance were analysed for treatments *e*, *c* and *f2*. The effects of maternal and offspring temperature as well as their interaction were tested and correlated with our generation's correlation numbers, to check for the changes when compared between treatments. The effects were tested using a two-way ANOVA and subsequent pairwise comparisons. Importantly, the residuals plot showed a clear normality pattern.

RESULTS

Treatment e

Survivability and developmental time

On the survivability analysis for *treatment e*, the impact of both the mother and offspring condition on offspring fitness was highly significant ($P < 0,001$), while the interaction mother:offspring condition was less significant ($P = 0,021$) (Table 1).

The overall survivability means of all groups (HH, HC, CH and CC, first letter being mother

condition, second letter being offspring condition) was 78,8%. The groups with the highest survivability percentage were the groups that had parents living in Hot conditions, with 86,7% of survivability for the HH group, followed by group HC with 82,3%. Groups of offspring that had mothers living in cold conditions overall had a lower survival rate with group CH showing a 79,8% survival rate and group CC having the lowest percentage of survivability at 59,2% (Figure 4).

Developmental time values were very different between offspring developing in hot conditions or in cold conditions for every treatment. Offspring developing in hot conditions had a lower average developmental time then flies developing in cold conditions with 188,38 and 433,25 hours respectively. The flies in group HH took in average 186,1 hours to develop, while flies in the CH group averaged 192,3 hours of developmental. Flies in cold conditions reached adult stage more than two times slower than flies developing in hot conditions, in average showing 433,25 hours to fully develop, 428,40 hours for the HC group and 444,40 hours for the CC group.

Table 1. Survivability statistical significance of treatment's e Mother and Offspring conditions and their interaction.

Condition	Chisq	P(>Chisq)
Mother condition	201,33	<0,001
Offspring condition	1525,04	<0,001
Mother:Offspring interaction	5,33	0.021

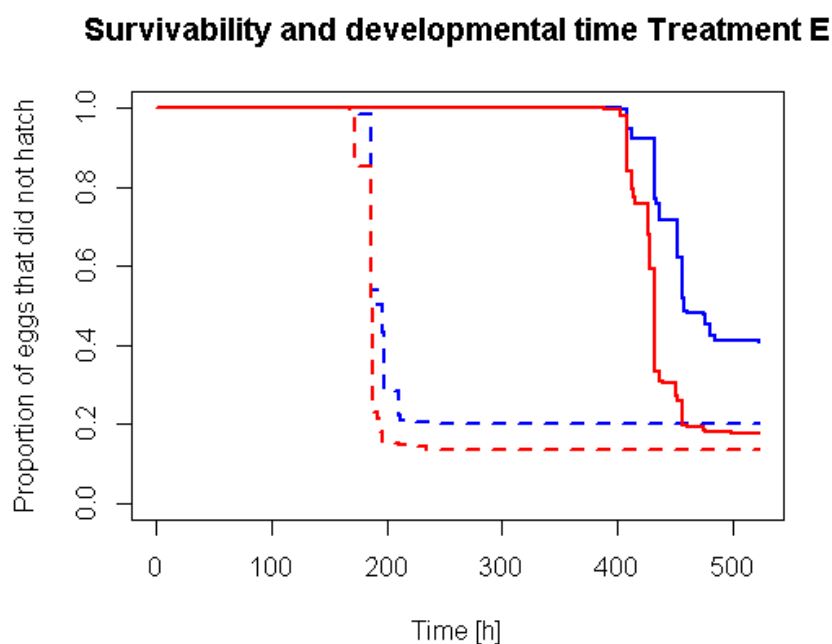


Figure 4. Survivability and developmental time graph for treatment e.

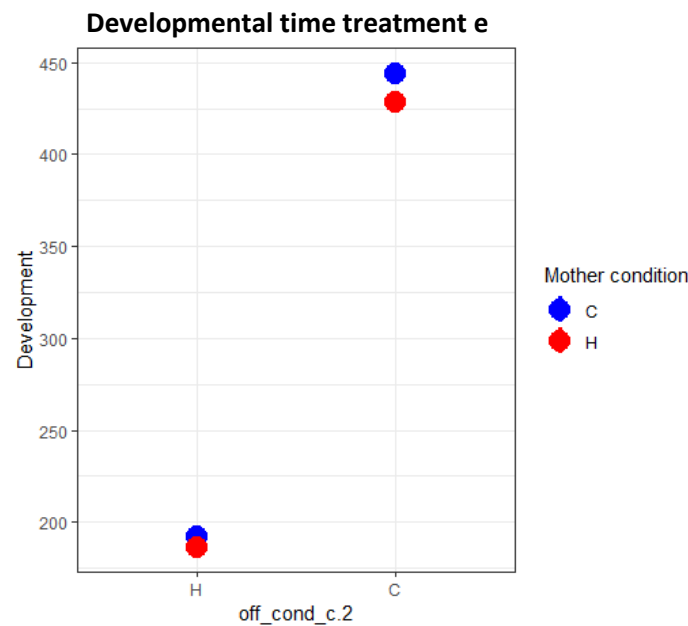


Figure 5. Developmental time graph for treatment e.

Treatment c

Survivability and developmental time

On the survivability analysis for *treatment c*, the impact of both the offspring condition and the interaction mother:offspring condition on offspring fitness was highly significant ($P < 0,001$), while the mother condition was less significant ($P = 0,016$) (Table 2).

The overall survivability means of all groups was 85,9%. The groups with the highest survivability rates were those that had offspring developing in Hot conditions, with 87,3% for the group HH, and 86,3% for group CH. The groups with offspring developing in cold conditions had an overall lower survivability with group HC showing a percentage of survival of 85,6% and group CC with the lowest survivability at 83,7%.

Mirroring the results shown on *treatment e*, flies with offspring developing in hot conditions had a striking difference in developmental time when compared to offspring developing in cold conditions. Developmental time values in hot conditions averaged 185,30 hours while in the cold room, flies averaged 433,19 hours to reach adult stage. In the hot room, flies averaged 183,43 hours of developmental time for group HH and 188,83 hours for group CH. Flies in cold conditions averaged 437,66 hours of developmental time for the HC group and 433,19 hours for group CC.

Table 2. Survivability statistical significance of treatment's c Mother and Offspring conditions and their interaction.

Condition	Chisq	P(>Chisq)
Mother condition	5,76	0,016
Offspring condition	1694,99	<0,001
Mother:Offspring interaction	63,97	<0,001

Survivability and developmental time Treatment C

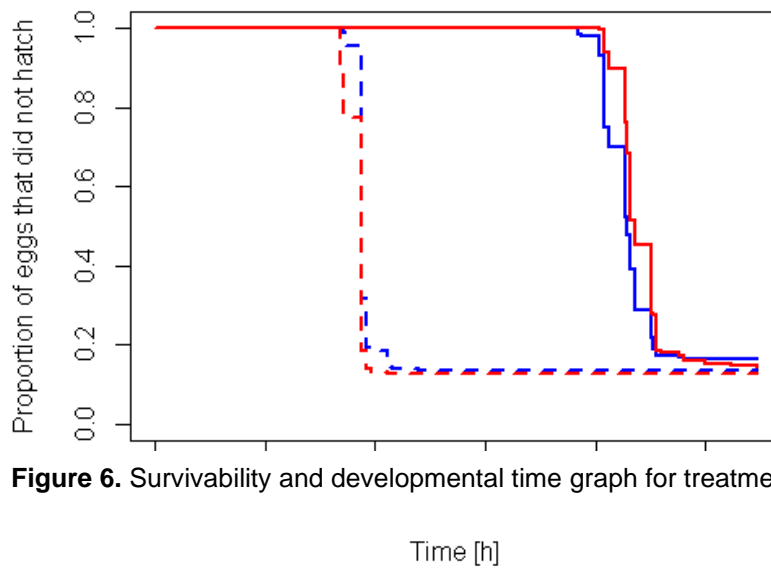


Figure 6. Survivability and developmental time graph for treatment c.

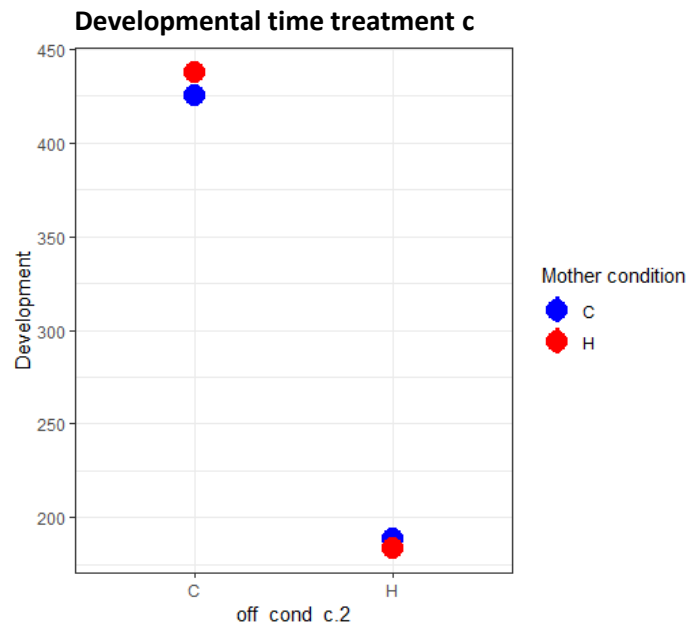


Figure 7. Developmental time graph for treatment c.

Treatment f2

Survivability and developmental time

On the survivability analysis for *treatment f2*, the impact of both the offspring condition and the interaction mother:offspring condition on offspring fitness was highly significant ($P < 0,001$), while the mother condition had no significant impact (Table 3).

On the survivability analysis for *treatment f2* (Figure 6), the overall survivability mean of all groups was 82,3%. The groups with the highest survivability percentages were the ones that had offspring developing in Hot conditions, with a value of 89,6% for the CH group, and 86,2% for the HH group. In contrast, groups with offspring developing in Cold conditions had a lower percentage of survivability with group CC evidencing a survival rate of 83,3% and the group HC with the lowest survivability rate at 74,4%.

As described in previous treatments, the same gap between the developmental time of offspring developing in hot and cold conditions was observed. Developmental time values in hot conditions averaged 184,49 hours while on cold conditions they averaged 418,25 hours. For offspring developing in hot conditions, for group HH, the developmental time averaged 181,87 hours and 189,86 hours for group CH. Flies in cold conditions reached adult stage around the 421,46 hours for group HC and 412,75 hours for the group CC.

Table 3. Survivability statistical significance of treatment's f2 Mother and Offspring conditions and their interaction.

Condition	Chisq	P(>Chisq)
Mother condition	1,58	0,209
Offspring condition	366,04	<0,001
Mother:Offspring interaction	15,62	<0,001

Survivability and developmental time Treatment f2

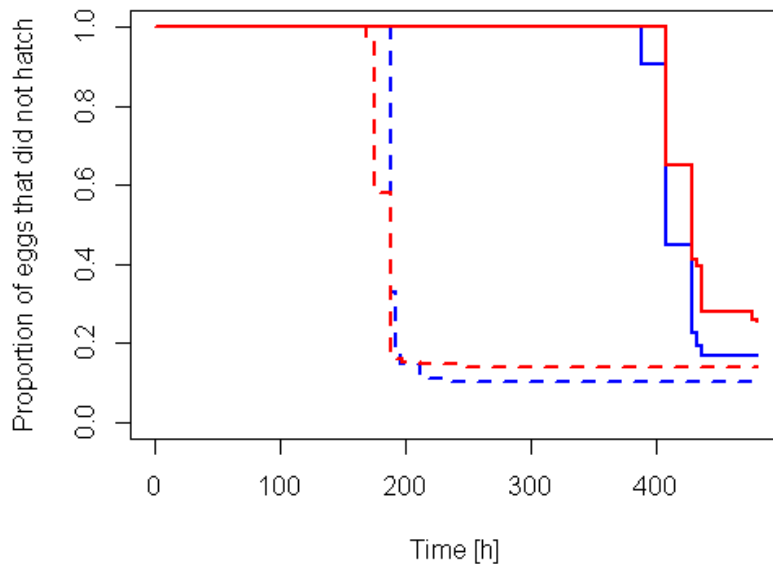


Figure 8. Survivability and developmental time graph for treatment f2.

Developmental time treatment f2

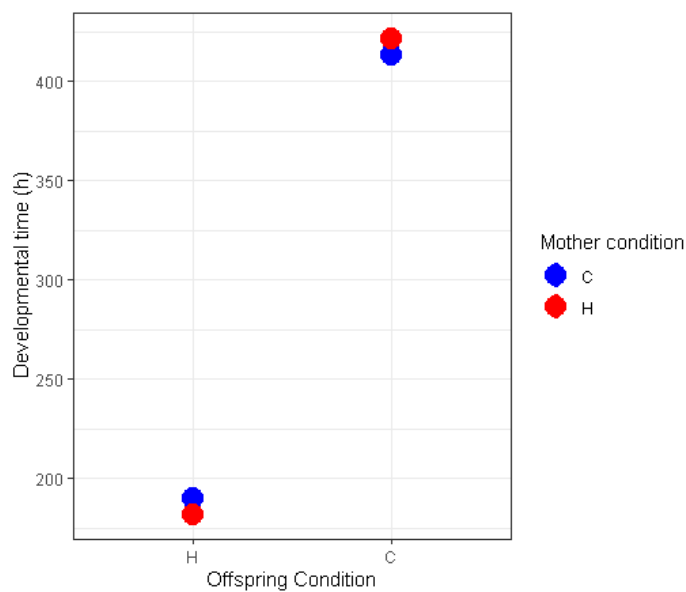


Figure 9. Developmental time graph for treatment e.

Heat-Shock Recovery

Treatment e

On this treatment only the Mother condition had a highly significant impact on offspring fitness ($P < 0,001$), while both offspring condition and the mother:offspring condition interaction had no significant impact (Table 4).

Flies from *treatment e* submitted to the heat shock treatment (Figure 7), across all conditions (HH, HC, CH, CC), took in average 155,84 seconds to recover. Groups with offspring developing in Hot conditions had the lowest average recovery times at 128,48 seconds for group HH followed by group CH recovering in average after 148,9 seconds. Groups with offspring developing in cold conditions showed a higher average recovery time, with 169,89 seconds for group HC and 174,53 seconds for group CC.

Table 4. Heat-Shock recovery statistical significance of treatment's e Mother and Offspring conditions and their interaction.

Condition	F value	P(>F)
Mother condition	16,38	<0,001
Offspring condition	1,70	~0,196
Mother:Offspring interaction	0,93	~0,336

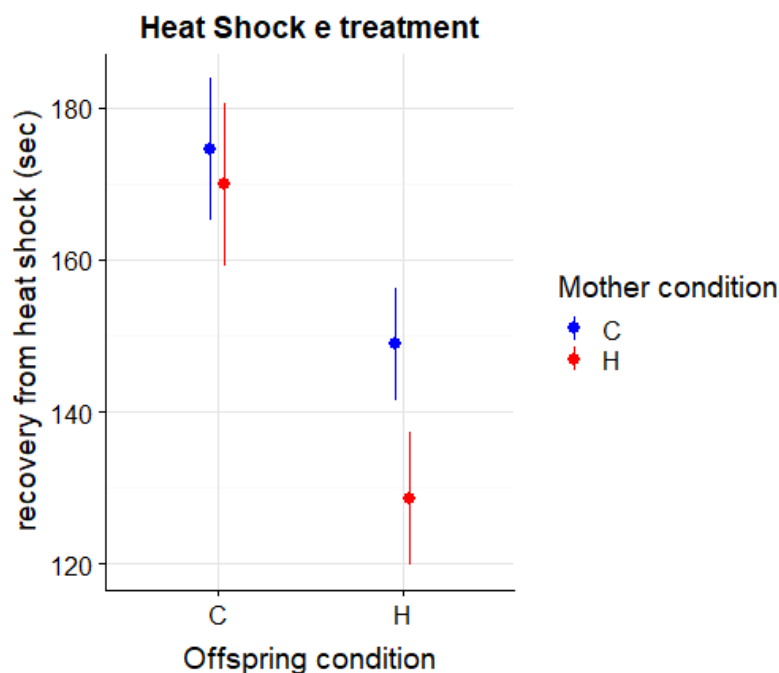


Figure 10. Heat Shock recovery time graph for treatment e.

Treatment c

On this treatment, all the conditions (offspring, mother and mother:offspring interaction) had a significant impact on offspring fitness ($P < 0,05$) with the mother condition being the most impactful condition of all ($P < 0,001$) (Table 5).

Flies from treatment c submitted to the heat shock treatment (Figure 8), across all conditions (HH, HC, CH, CC), took in average 157,62 seconds to recover. The same trend evidenced on treatment e flies was observed on this treatment, where groups where the offspring developed in hot conditions in average showed a lower recovery time then groups of flies with offspring developing in cold conditions, averaging 125,38 seconds of recovery time for group HH, followed by group CH recovering in average after 155,68 seconds. Groups with offspring developing in cold conditions recovered, in average, after 180,53 seconds for group HC and 184,58 seconds for group CC.

Table 5. Heat-Shock recovery statistical significance of treatment's c Mother and Offspring conditions and their interaction.

Condition	F value	P(>F)
Mother condition	49,14	<0,001
Offspring condition	5,88	~0,017
Mother:Offspring interaction	5,58	~0,018

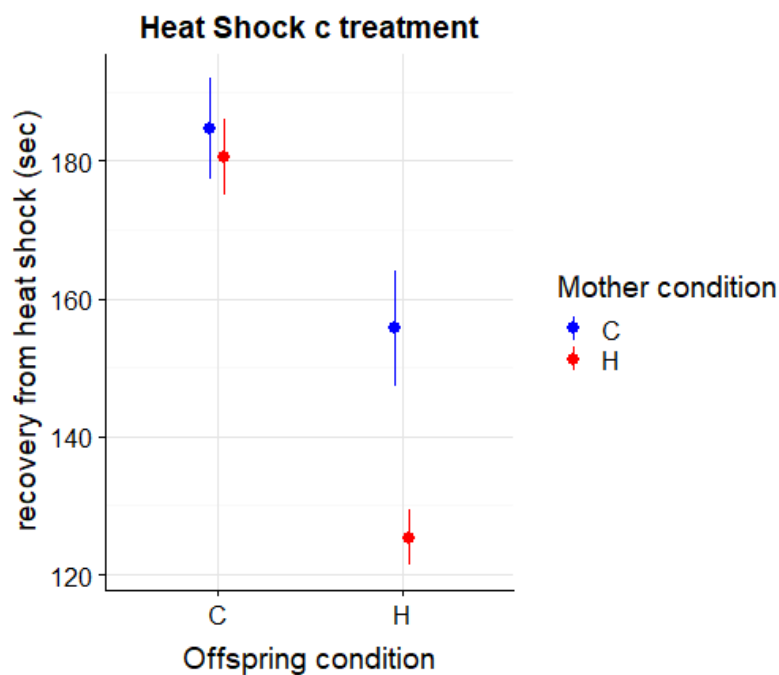


Figure 11. Heat Shock recovery time graph for treatment c.

Treatment f2

The mother condition had a very significant impact on offspring fitness ($P < 0,001$), while Offspring still had a significant but lower impact ($P = 0,013$). The mother:offspring interaction had no significant impact on their fitness (Table 6).

Flies submitted to the heat shock treatment (Figure 9), across all conditions (HH, HC, CH, CC), took in average 211,59 seconds to recover. Following the trend described in the previous treatments, groups with offspring developing in hot conditions recovered faster than groups with offspring developing in cold conditions. HH group was the group with the lowest recovery time, in average recovering after 165,68 seconds. CH group followed, recovering in average after 219,13 seconds. HC and CC groups had the highest recovery times averaging 221,82 and 297,17 seconds respectively.

Table 6. Heat-Shock recovery statistical significance of treatment's *f2* Mother and Offspring conditions and their interaction.

Condition	F value	P(>F)
Mother condition	15,15	<0,001
Offspring condition	11,45	~0,013
Mother:Offspring interaction	0,35	~0,554

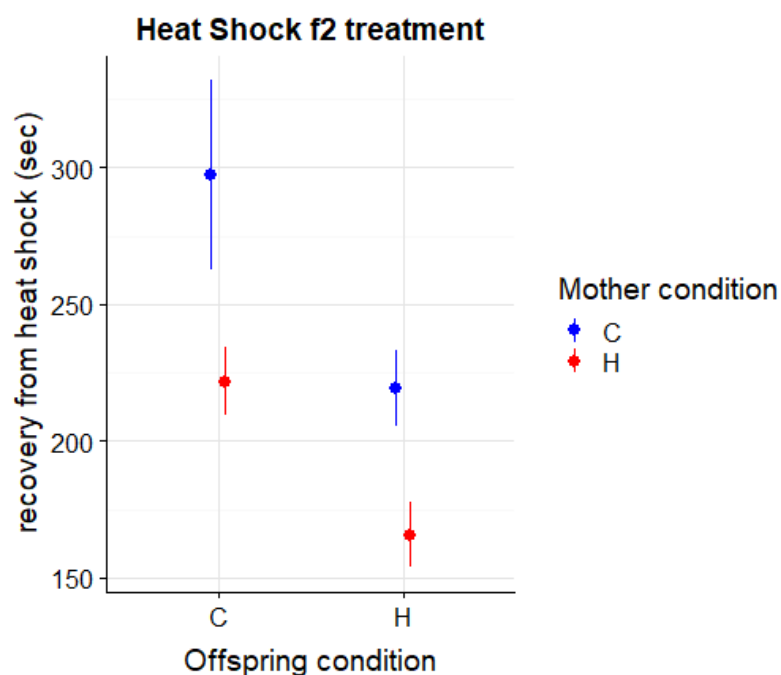


Figure 12. Heat Shock recovery time graph for treatment f2.

Cold Shock Recovery

Treatment e

For this treatment both the mother and offspring conditions had a very significant impact on offspring fitness ($P < 0,001$), while the mother:offspring interaction had no impact (Table 7).

Flies from treatment e submitted to the cold shock treatment (Figure 10), across all conditions (HH, HC, CH, CC), took in average 1371,48 seconds to recover. Overall, groups with offspring developing in cold conditions, in average, recovered faster than groups where offspring developed in hot conditions. CC group was the group with the lowest recovery time, in average recovering after 1157,58 seconds followed by group HC recovering in average after 1310,99 seconds. CH and HH groups had the highest recovery times averaging 1363,67 and 1655,27 seconds respectively.

Table 7. Cold-Shock recovery statistical significance of treatment's e Mother and Offspring conditions and their interaction.

Condition	F value	P(>F)
Mother condition	40,60	<0,001
Offspring condition	20,13	<0,001
Mother:Offspring interaction	2,55	0,1111

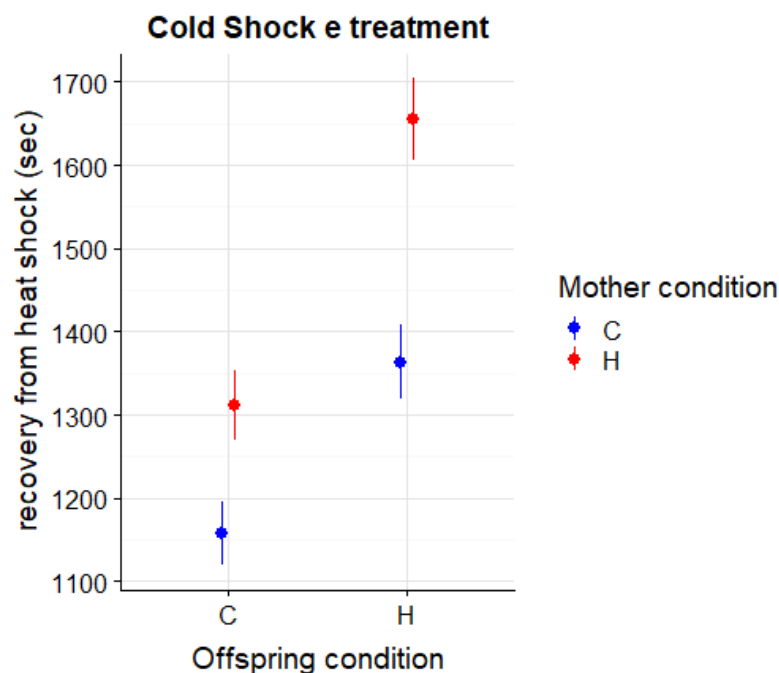


Figure 13. Cold Shock recovery time graph for treatment e.

Treatment c

On this treatment all the conditions had a highly significant impact on the offspring fitness ($P < 0,001$) (Table 8).

Flies from treatment c submitted to the cold shock treatment (Figure 11), across all conditions (HH, HC, CH, CC), took in average 1295,04 seconds to recover. In accordance with the results shown for cold shock recovery on *treatment e*, recovery times were lower for groups with offspring developing in cold conditions. HC group was the group with the lowest recovery time, in average recovering after 1126,97 seconds followed by group CC recovering in average after 1195,05 seconds. Groups with offspring developing on hot conditions had the highest recovery times with group HH recovering, in average, after 1295,30 seconds and group CH averaging 1591,95 seconds of recovery time.

Table 11. Cold-Shock recovery statistical significance of treatment's c Mother and Offspring conditions and their interaction.

Condition	F value	P(>F)
Mother condition	105,83	<0,001
Offspring condition	18,34	<0,001
Mother:Offspring interaction	17,02	<0,001

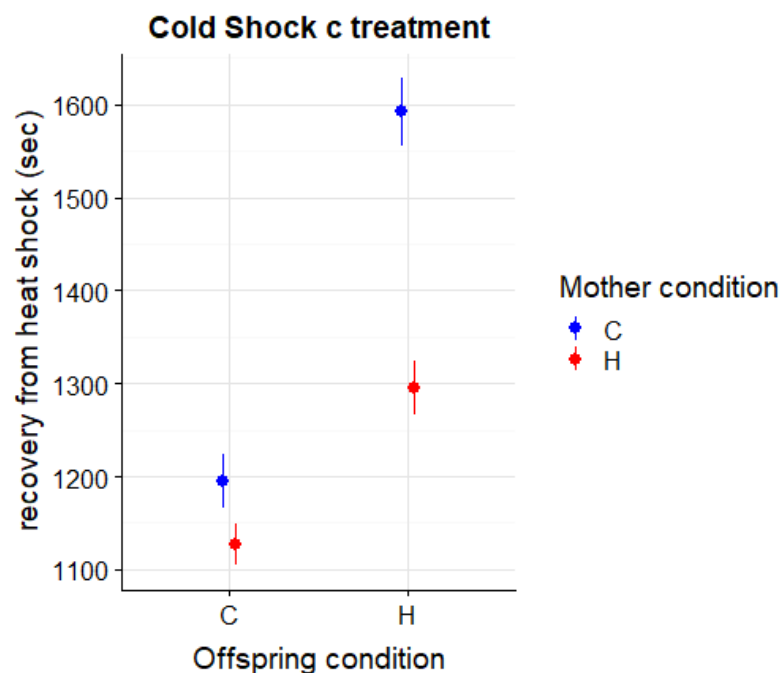


Figure 14. Cold Shock recovery time graph for treatment c.

Treatment f2

No significance of the conditions (mother offspring and mother:offspring interaction) was found in this treatment (Table 12).

Flies from *treatment f2* submitted to the cold shock treatment (Figure 12), across all conditions (HH, HC, CH, CC), took in average 1272,99 seconds to recover. The same trend evidenced in previous treatments was observed, where groups with offspring developing in cold conditions recovered faster than groups with offspring developing in hot conditions. HC group was the group with the lowest recovery time, in average recovering after 1191,03 seconds. CC group followed, recovering in average after 1249 seconds. CH and HH groups had the highest recovery times averaging 1320,08 and 1346,09 seconds, respectively.

Table 12. Cold-Shock recovery statistical significance of treatment's f2 Mother and Offspring conditions and their interaction.

Condition	F value	P(>F)
Mother condition	3,55	0,062
Offspring condition	0,031	0,863
Mother:Offspring interaction	0,311	0,578

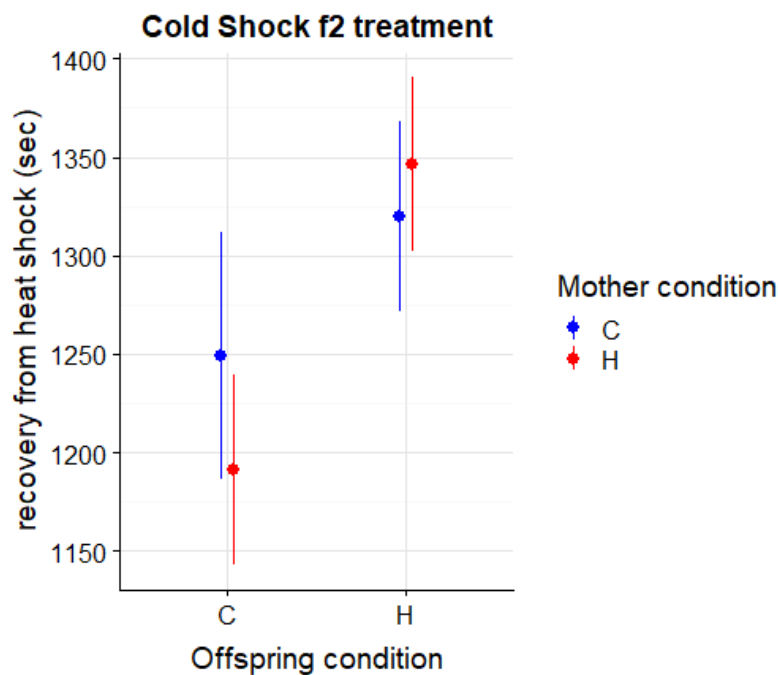


Figure 15. Cold Shock recovery time graph for treatment f2.

DISCUSSION

In order to evaluate the adaptive value of maternal effects, some conditions must be met. For the maternal effects to have adaptive value there must be a change in the environment conditions so that a response is triggered on the offspring. Also, another factor that is pivotal for the adaptive value of the maternal information, is its reliability. For maternal information to be reliable, environmental conditions must be predictable (Marshall & Uller 2007). On this study, we want to discuss the parental temperature effect on offspring fitness traits and understand whether this information may have an adaptive significance.

Individuals were divided into six treatments, two treatments with constant environmental temperature, either constant Hot (28°C): *treatment b*; or constant Cold (18°C): *treatment a* and four other groups (c, d, e and f) with different temperature shifts across generations. In this study we analysed treatments at a midway point, around the 10th generation, from the planned 30 generations analysis, to evaluate whether the different correlations from the correlations calculated for the 30 generations period, have an impact on how maternal information is conveyed to the offspring. We analysed treatments e, c and f2 which have a correlation value of 0,41, -1 and 0,039 respectively where the values calculated for 30 generations, are 1/3, -1 and 0. This difference in correlation values, compared to the ones planned over the 30 generations might have some impact on the end results, once they can change the predictability and reliability of the maternal information and thus may explain why some results immensely deviate from the values we predicted.

In accordance with previous literature, regardless of how reliable the maternal information is, flies have an increased fitness on hot temperatures (Cavicchi *et al.* 1989). Flies that developed in the hot room (28°C), and thus closer to its optimal fitness temperature (25°C)(Cohet 1975), scored better in terms of the fitness traits evaluated. On the other hand, flies developing in cold conditions (18°C) showed a lower survivability, took more time to develop and recovered slower from heat shock. Flies coming from the cold room only scored better on fitness traits when compared to the hot room flies, when it comes to the cold shock recovery time. This can be explained by the offspring condition prior to the cold shock test, once that even though a temperature of 28°C isn't optimal for the flies, a temperature of 18°C is much more harmful and has an increased influence on the flies' fitness (Kingsolver & Huey 2008). These temperature effects on offspring fitness, can mask the adaptive value of the

maternal information. To understand the importance of the maternal effects, we then analysed more fitness traits, in order to have an overall picture of these effects on offspring fitness.

When it comes to the survivability analysis, there was a striking difference between offspring developing in hot and in cold conditions. Flies developing in cold conditions had much lower rates of survivability than flies developing in hot conditions. This big difference in survival rate may be, as mentioned before, attributed to the fact that the hot temperature used in this study (28°C), even though not being optimal for this species development, is still not as harmful as the cold temperature used (18°C). The latter deviates further from the optimal range of temperature preference of this organism (Hamada *et al.* 2008).

Survival and developmental time analysis

All three treatments studied showed a statistical significance of the Mother:Offspring condition interaction when it comes to the survivability analysis ($p < 0,001$), which might mean that maternal information is of extreme importance for early time development and survival, as reported in other species (Wood *et al.* 1980; Dettling *et al.* 2002; Butts & Litvak 2007).

Interestingly on *treatment f2*, the Mother condition evidenced no significance on the survival analysis, as opposed to treatments *e* and *c*. This can be due to several factors. It may be connected to the unpredictability of the environmental conditions on this treatment, once the correlation value is close to zero, thus providing mothers with cues that lead to unreliable information for the offspring. Or even to the lower sample size present for this treatment, when compared to the other treatments studied.

Similar to the survival analysis, there was also a very significant impact of the Maternal:Offspring interaction ($p < 0,001$) on the developmental time for all treatments studied. As mentioned before, this may be largely attributed to the fundamental value of maternal information in early life stages.

Heat and Cold-shock analysis

Both heat and cold shocks showed no statistical relevance of the Mother:Offspring condition's interaction for treatments *e* and *f2*, these being the two treatment with the highest

correlation value, while in treatment *c* this interaction was clearly significant. These results highly deviate from our predictions. In accordance with the conditions required for maternal information to be valuable (Marshall & Uller 2007), we predicted treatment *e* to show the highest impact of the Mother:Offspring interaction, once this treatment changes temperature every three generations and, consequently, at the 10th generation shows a correlation value of 0,41, the highest correlation value apart from the two treatments with constant temperatures. Thus, when looking at this correlation value we hypothesised that based on the correlation alone, this treatment gathered the perfect conditions of both predictability of environmental conditions and reliability of maternal information which are necessary to improve the Offspring fitness values the most. Contrarily, the results showed no significance for this interaction on both Cold and Heat shocks for this treatment. On the other hand, as treatment *f2* has a random pattern (assigned with a correlation value of zero), we conjectured that treatment *f2* did not meet the conditions for the maternal information to be reliable and thus we expected the Mother:Offspring interaction to show no significance. These predictions were verified by the results, with this interaction showing no statistical significance on this treatment. Similarly, analysing the predictability of the environmental conditions for treatment *c* and considering this treatment shows the lowest correlation value of all the treatments, we predicted that the reliability of the maternal information would be very diminished, and, as so, we expected the mother:offspring interaction to show no significance on this treatment. Interestingly, this treatment was the only one that showed statistical significance for the Mother:Offspring interaction. This results highly deviate from our predictions and raise question on how to hypothesize about the predictability of the environmental conditions of the treatments. Treatment *c*, switches temperature every generation. This constant generational shift made us theorize that the predictability of the environmental conditions for flies in this treatment would be very low. These results on the other hand show us that the conditions of predictability and reliability for this maternal information to be adaptive are ideal. This could be explained by the fact that, even though offspring in this treatment always live in opposite conditions of their parents, when we look at the pattern of generational shifts, it is a constant pattern over the 30 generations. So, when analysing this correlation value, considering the modular value for all treatments could better explain the predictability and yield better analysis of posterior results.

As said before, Treatment *c* was the only treatment where maternal effects were present, and the Mother:Offspring interaction was highly significant ($P < 0,001$). Even though

the Mother:Offspring interaction was significant, the way these two variables interacted was still unpredictable. Offspring that developed in cold temperatures with cold mothers (matched conditions) recovered slower from heat shock than the ones with mothers in unmatched conditions, even though these differences were not significant. This non significance of the maternal condition could be explained by their low fitness at adverse conditions, even in the presence of maternal information (Cohet 1975). In contrast, offspring that developed in the hot condition had a clear influence of maternal information. When these flies developed in matching conditions, with mothers coming from the hot condition, they recovered significantly faster from heat stress ($P < 0,0007$) than the ones with mothers coming from the cold condition.

On the cold-shock analysis, maternal effects were again, only evidenced on treatment *c* where the Maternal:Offspring condition interaction was significant. On this treatment, offspring that developed on the cold condition unpredictably recovered faster when in unmatched conditions (hot mothers), but this difference between matched and unmatched conditions was not significant. In contrast, offspring that developed in the hot condition that are in matched conditions (hot mothers) recovered much faster from cold stress ($p < 0,001$) than the ones with mothers coming from the cold condition.

Final Remarks

Overall, we can assume that maternal effects are adaptive and consequently of great importance when we look at the survivability and developmental time analysis. For almost every treatment, flies have higher fitness if they come from matched conditions. For the heat and cold shocks, values were surprising for treatment *e* and *c*, which may indicate that further analysis and interpretation of the results must be done in order to have a broader understanding of the final study, when all treatments are analysed together (treatments *a*, *b*, *c*, *d*, *e* and *f*).

Even though the mother:offspring interaction was significant on treatment *c*, for both cold and heat shocks, its adaptive value is still unclear, once that the mother condition was only significant when flies developed in hot conditions. As mentioned before, this could be related to the conditions that flies are developing in. Even though both temperatures used in this study are non-ideal, the cold temperature is reported much more harmful to flies than the hot temperature. These conditions alone, might have masked the value of the maternal

information yielded in the results and raise questions on whether temperature should be the main variable in this study.

It is also noteworthy to mention that, while this study was being made, eggs were collected for fecundity tests, over a collection period of 5 days. This data will later be analysed, and fecundity scores will be compared between treatments. This analysis should complement this study and should give a broader and more reliable interpretation of the overall final data.

Even though an analysis of the maternal effects adaptive value was made at the 9th or 10th generations in this study, one more analysis will be done, with the final analysis being at the 30th generation. Values may shift and be more concise once the final analysis is made, combining all three of the generation's phenotyping studies. Although this study is a preliminary analysis of this 30 generations study, positive results suggest that further generations will increase the statistical values of every treatment evaluated.

For future improvement of the phenotype analysis of these treatments, and specifically for treatment f, to attenuate the effect of the low number of flies, more batches could be set up in a longer time span to increase the number of replicates and mother IDs. Also, using other variables to analyse fitness effects, instead of the temperature, could eliminate the presence of unwanted effects, and the masking effects that temperature incurs.

CONCLUSION

The adaptive value of maternal effects, depending on the model organism, requires a devoted and time-consuming analysis over a period of several generations. Even though hard to prove, their importance is unarguable. With this study, we suggest that maternal effects can be adaptive and have a huge value on the of *Drosophila melanogaster* offspring fitness, depending on how their environment changes and on how predictable their environmental conditions are. This study also sheds a light on how predictability can be open to interpretation and should not be assumed as a linear variable.

ACKNOWLEDGEMENTS

I would like to thank everyone that helped this study come to a fruitful conclusion, in particular to Bart van Schaik for all the help and guidance given throughout this study, Gerrard Overkamp, cooking the food and preparing the vials for all our flies, Jean-Cristophe Billeter for the help with writing the report, and with all the motivation given. Special thanks to Mário Mira, for helping with the flies' collection and help with the R scripts. Lastly, I would like to thank Pinar Kohlmeier for all effort and help given to me in the supervision of this study and all the help writing the report.

REFERENCES

- Badyaev, A. V & Uller, T. (2009). Parental effects in ecology and evolution: mechanisms, processes and implications. *Philos. Trans. R. Soc.*, 1169–1177.
- Bonduriansky, R., Crean, A.J. & Day, T. (2012). The implications of nongenetic inheritance for evolution in changing environments. *Evol. Appl.*, 5, 192–201.
- Bonduriansky, R. & Day, T. (2009). Nongenetic Inheritance and Its Evolutionary Implications. *Annual review of ecology evolution and systematics.*, 40, 103-125.
- Butts, I.A.E. & Litvak, M.K. (2007). Stock and parental effects on embryonic and early larval development of winter flounder *Pseudopleuronectes americanus* (Walbaum). *J. Fish Biol.*, 70, 1070–1087.
- Cavicchi, S., Guerra, D., Natali, V., Pezzoli, C. & Giorgi, G. (1989). Temperature-related divergence in experimental populations of *Drosophila melanogaster*. II. Correlation between fitness and body dimensions. *J. Evol. Biol.*, 2, 235–251.
- Chen, S.Y., Leask, K.P., MacKinnon, S.W., Ramanaden, Y.J. & Yoon, J.H. (2014). The effects of temperature on the time to maturation of *Drosophila melanogaster*. *Expedition*, 3.
- Cohet, Y. (1975). *Drosophila: existence of an optimal growth temperature for adult longevity*. Pergamon Press.
- Davison, K.K. & Birch, L.L. (2008). Review: Thermal preference in *Drosophila*, 64, 2391–2404.
- Dettling, A.C., Feldon, J. & Pryce, C.R. (2002). Repeated Parental Deprivation in the Infant Common Marmoset (*Callithrix jacchus*, Primates) and Analysis of its Effects on Early Development. *Biol Psychiatry.*, 52, 11, 1037-1046.
- Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., et al. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *National academy of sciences.*, 105, 18, 6668-6672
- Dillon, M.E., Cahn, L.R.Y. & Huey, R.B. (2007). Life history consequences of temperature transients in *Drosophila melanogaster*. *J. Exp. Biol.*, 210, 2897–2904.
- Galloway, L.F. (2005). Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytol.*, 166, 93–100.
- Gibert, P., Huey, R.B. & Gilchrist, G.W. (2001). Locomotor performance of *drosophila melanogaster*: interactions among developmental and adult temperatures, age, and geography. *205 Br. Commun. Evol.*
- Hamada, F.N., Rosenzweig, M., Kang, K., Pulver, S., Ghezzi, A., Jegla, T.J., et al. (2008). An internal thermal sensor controlling temperature preference in *Drosophila*. *Nature*, 454, 217–220.

- Hasselquist, D. & Nilsson, J.-A. & K. (2008). Maternal transfer of antibodies in vertebrates: trans-generational effects on offspring immunity. *Philosophical Trans. R. Soc.*, 51–60.
- Hsu, B.Y., Dijkstra, C., Darras, V.M., de Vries, B. & Groothuis, T.G.G. (2016). Maternal adjustment or constraint: Differential effects of food availability on maternal deposition of macro-nutrients, steroids and thyroid hormones in rock pigeon eggs. *Ecol. Evol.*, 6, 397–411.
- Huey, R., Kingsolver, J., Huey, R.B. & Kingsolver, J.G. (1989). Evolution of Thermal Sensitivity of Ectotherm Performance. *Evolutionary Ecology Research.*, 10, 2, 251-268
- Jablonka, E. & Raz, G. (2009). Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Quarterly Review of Biology.*, 84, 2, 131-176
- Kingsolver, J.G. & Huey, R.B. (2008). Size, temperature, and fitness: three rules. *Evolutionary Ecology Research.*, 10, 2, 251-268
- Marshall, D.J. & Uller, T. (2007). When is a maternal effect adaptive? *Oikos*, 116, 1957–1963.
- Mousseau, T. & Dingle, H. (1991). Maternal Effects In Insect Life Histories. *Annu. Rev. Entomol.*, 36, 511–534.
- Orr, H.A. (2005). The genetic theory of adaptation: a brief history., *Nature Reviews Genetics.*, 6, 2, 119-127
- Uller, T. (2008). Developmental plasticity and the evolution of parental effects. *Trends Ecol. Evol.*, 23, 432–438.
- Walther, G.-R., Post², E., Convey³, P., Menzel, A., Parmesan, C., Beebee, T.J.C., et al. (2002). Ecological responses to recent climate change., *Nature.*, 416, 6879, 389-395
- Wood, W.B., Hecht, R., Carr, S., Vanderslice, R., Wolf, N. & Hirsh, D. (1980). Parental Effects and Phenotypic Characterization of Mutations that Affect Early Development in *Caenorhabditis elegans*. *Developmental Biology.*, 74, 2, 446-469