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Evolution of pre- and post-copulatory traits in female *Drosophila melanogaster* as a correlated response to selection for resistance to cold stress

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ABSTRACT

Exposure to low temperatures reduces gamete viability and fecundity in females of insect species like *Drosophila*. Hence, adaptation to cold stress can in principle involve modifications in reproductive traits in females. Studies on resistance to cold stress have mostly addressed the evolution of adult survivorship post cold shock. Very few studies have addressed the evolution of reproductive traits in females in response to cold stress. We have successfully selected replicate populations of *Drosophila melanogaster* for resistance to cold shock. After 50 generations of selection, we investigated pre- and post-copulatory traits i.e. mating latency, copulation duration, mating frequency and progeny production in female flies exposed to cold shock or control conditions. Post cold shock, females from the selected populations were better at recovery in terms of mating latency, mating success, and progeny production relative to females from the control populations. Performance of the two types of females was not different under control conditions. These findings clearly indicate that adaptation to cold stress involves rapid modification of the reproductive traits.

1. Introduction

Exposure to extreme low temperatures (cold shock) has been shown to have major consequences for the fitness of organisms, especially insects. Apart from its effects on survival at various life stages, cold shock also affects reproductive traits in both males and females of several insect species (Tucic, 1979; Schnebel and Grossfield, 1984; Chen and Walker, 1993; Watson and Hoffmann, 1996; Carriere and Boivin, 1997; Rinehart et al., 2000; Fischer et al., 2003; Anderson et al., 2005; Bubliy and Loeschcke, 2005; Colinet and Hance, 2009; Jakobs, 2014; Singh et al., 2015, 2016).

In species like *Drosophila* where females mate multiple times and store sperm from multiple males, cold shock can affect a range of reproductive traits. Exposure to cold shock can affect the reproductive output of female *Drosophila melanogaster* through its direct effects on the survivorship of stored sperm and eggs as well as its indirect effects on female mating propensity and sperm handling. In *D. melanogaster*, cold shock at sub-zero temperatures leads to the death of stored sperm in mated females (Novitski and Rush, 1949; Lefevre and Jonsson, 1962) and therefore, they fail to produce progeny (Singh et al., 2015) post cold shock. In fact, mated females can be subjected to cold shock to kill the stored sperm so that these females can be used as virgin females (Lefevre and Jonsson, 1962). Post cold shock, females need to remate and gather sperm afresh in order to produce progeny (Singh et al., 2015). Therefore, mating post cold shock is extremely important for female fitness. Multiple studies have documented that female remating frequency is affected by temperature in multiple insects. For example, exposure to low temperature decreases remating frequency in *D. melanogaster* (Best et al., 2012), adzuki bean beetle (*Callosobruchus chinensis*, Katsuki and Miyatake, 2009) and cricket (*Acheta domesticus*, Kindle et al., 2006). Adaptation to lower temperatures increases female mating propensity in *Drosophila pseudoobscura* with females from northern populations mating more frequently than females from the southern populations (Price et al., 2014). Thus, temperature can indirectly affect post-copulatory traits through female remating frequency.

Cold shock can affect reproductive output through direct effects on reproductive tissues (Marshall and Sinclair, 2010). For example, exposure to low temperatures can reduce progeny production in *D. melanogaster* and *Drosophila suzukii* (Marshall and Sinclair, 2010; Jakobs, 2014). Genes related to chorion and egg shell formation are upregulated post cold shock (Zhang et al., 2011), indicating that developing eggs might be damaged during cold shock. Additionally, cold shock could affect reproductive traits indirectly through its effects on behavior. Cold shock is known to perturb ionic balance

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(Kostal et al., 2006; MacMillan and Sinclair, 2011; MacMillan et al., 2015) which leads to loss of neuromuscular activity (MacMillan and Sinclair, 2011). This can affect courtship and mating (Shreve et al., 2004) as well as the post-mating sperm processing by *Drosophila* females that is necessary for laying fertile eggs (reviewed in Wolfner, 1997). Finally, it also possible that exposure to cold can affect resource allocation, moving resources away from reproduction towards repair (Marshall and Sinclair, 2010). Thus cold shock can have major effects on reproductive traits of females.

In the present study we assayed the pre- and post-copulatory traits (such as mating latency, copulation duration, mating success and progeny production) in females from populations of *D. melanogaster* selected for over 50 generations for resistance to cold shock. We found that the females from the selected populations were better at recovery post cold shock in terms of mating latency, mating success and progeny production relative to the control populations.

2. Methods and materials

2.1. Stock populations

2.1.1. Blue Ridge Base line population

Details of the maintenance of the baseline populations have been explained in our previous study (Singh et al., 2015). Briefly, 19 isofemales lines were set up from wild inseminated females of *D. melanogaster* that were captured from the Blue Ridge Mountains, Georgia, USA. These isofemales lines were maintained in Prof. Daniel Promislow's laboratory. Prof. Promislow very generously provided us with these lines in 2010. They were maintained for 6 generations on standard banana-yeast-jaggery food (hereafter referred to as "food") at standard laboratory conditions (25°C temperature, 50–60% relative humidity, 12 h:12 h light/dark cycle, on a 14-day discrete generation cycle). Following this, in 2011, a population was established by mixing equal number of males and females from each of the 19 isofemales lines. This population was labeled as Blue Ridge Base line (BRB). This population was further maintained at standard laboratory culture conditions as mentioned above with adult numbers of ~2800 for 10 generations. Subsequently, this population was further split into five replicate populations referred to as BRB 1–5. These populations are maintained at standard laboratory culture conditions as described above.

2.1.2. Derivation and maintenance of the selected (FSB) and the control (FCB) populations

A detailed account of the origin and maintenance of the selected (FSB; Cold shock Selected population derived from BRB population) and their control (FCB; Cold shock Control derived from BRB population) populations has been given in our previous study (Singh et al., 2015). In short, after 35 generations of laboratory maintenance of BRB 1–5 populations, we established one FSB and one FCB population from each of the ancestral BRB 1–5 populations. For instance FSB 1 and FCB 1 populations were created from BRB 1, FSB 2 and FCB 2 populations were created from BRB 2 and so on. Thus we had 10 populations: five FSB and five FCB populations. Populations having the same numerical label, by virtue of being derived from the same BRB population, were more closely related to each other than to any other population and hence form a statistical block. For example, FSB 1 is more closely related to FCB 1 by ancestry than to FSB 2 or FCB 2 or any other population. Thus FSB 1 and FCB 1 have been included in the 'Block 1' in our analyses.

FSB populations were maintained on a 13-day discrete generation cycle at standard laboratory culture conditions as mentioned earlier. In this population, peak of eclosion is on the 10th day post egg collection and by the 12th day post egg collection almost all flies eclose. Thus by the 12th day post egg collection, flies are roughly 2–3 days old as adults and would have already mated. On the 12th day post egg collection flies were moved from food vials to clean, empty, dry glass vials (25 mm diameter × 90 mm height) and the cotton plugs were inserted deep into the vials to make sure that the flies were constrained to the bottom 1/3 of the vials. After that, the vials containing flies were subjected to cold shock for one hour in water-ice-salt slurry maintained at −5°C. Soon after the cold shock the flies were transferred to a Plexiglas cage (25 cm length × 20 cm width × 15 cm height) and provided food. To collect eggs to start the next generation, twenty-four hours after the cold shock, Petri plates containing fresh food were introduced into the respective cages for a period of 18 h to allow flies to oviposit. Eggs were collected at a density of about 100 eggs per vial containing 6 ml of food (since the egg viability post cold shock was around 70%, close to 70 larvae hatched out of the eggs in each vial). For each of the FSB 1–5 populations twenty such vials were established.

FCB 1–5 populations were maintained in a similar manner except that these populations were subjected to 25°C for one hour in a water-bath instead of cold shock (−5°C). Both the FSB and the FCB populations were maintained with large population size (N of ~1400).

2.2. Experimental protocol

2.2.1. Standardization of flies

In order to equalize non-genetic parental effects (Rose, 1984), each of the FSB and the FCB populations were raised in common laboratory environment for one generation. This process is known as standardization and the flies are called standardized flies. In order to standardize flies, twenty vials containing eggs at a density of 70 per vial were collected from each of the five FSB and FCB populations and incubated at standard laboratory culture conditions (25°C temperature, 50–60% relative humidity, 12 h:12 h light/dark cycle). On the 12^{th} day after egg collection flies from each of the populations were separately transferred into Plexiglas cages. Experimental flies were generated by collecting eggs laid by these standardized flies.

2.2.2. Generation of experimental flies

Experiments 1 and 2 were performed after 49 and 50 generations of selection respectively. Eggs were collected from standardized selected (FSB 1–5) and control (FCB 1–5) populations. To generate experimental flies, eggs were collected at a density of 70 per vial and incubated at standard laboratory culture conditions (25°C temperature, 50–60% relative humidity, 12 h:12 h light/dark cycle). For each of the FSB (1–5) and FCB (1–5) populations, sixteen such vials were set up. On 9–10^{th} day post egg collection, virgin females were collected during the peak of their eclosion using mild CO_2 anesthesia. These flies were held in vials containing 2 ml of food at a density of 10 flies per vial until the 12^{th} day after egg collection on which day the flies were subjected to cold shock or no shock treatment. Thus the females used in the experiments were about 2–3 days old as adults. In the selection regime, flies are exposed to cold shock on the 12^{th} day post egg collection by which time flies are roughly 2–3 days old as adults. Hence, we used 2–3 days old adults for experiments. Additionally, exposure of flies to CO_2 can affect their cold shock resistance negatively (Nilson et al., 2006). Hence the two day gap also helped to ameliorate the negative effects of CO_2 anesthesia.

2.2.3. Generation of common males from ancestral populations (BRB)

In our experiments, FSB and FCB females were exposed to common ancestral (BRB) males. Thus, differences, if any, in the pre- and post-copulatory traits could be ascribed to differences between the