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Redefining reproductive dormancy in *Drosophila* as a general stress response to cold temperatures

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ABSTRACT

Organisms regularly encounter unfavorable conditions and the genetic adaptations facilitating survival have been of long-standing interest to evolutionary biologists. Winter is one particularly stressful condition for insects, during which they encounter low temperatures and scarcity of food. Despite dormancy being a well-studied adaptation to facilitate overwintering, there is still considerable controversy about the distribution of dormancy among natural populations and between species in *Drosophila*. The current definition of dormancy as developmental arrest of oogenesis at the previtellogenic stage (stage 7) distinguishes dormancy from general stress related block of oogenesis at early vitellogenic stages (stages 8 – 9). In an attempt to resolve this, we scrutinized reproductive dormancy in *D. melanogaster* and *D. simulans*. We show that dormancy shows the same hallmarks of arrest of oogenesis at stage 9, as described for other stressors and propose a new classification for dormancy. Applying this modified classification, we show that both species express dormancy in cosmopolitan and African populations, further supporting that dormancy uses an ancestral pathway induced by environmental stress. While we found significant differences between individuals and the two *Drosophila* species in their sensitivity to cold temperature stress, we also noted that extreme temperature stress (8 °C) resulted in very strong dormancy incidence, which strongly reduced the differences seen at less extreme temperatures. We conclude that dormancy in *Drosophila* should not be considered a special trait, but is better understood as a generic stress response occurring at low temperatures.

1. Introduction

Organisms are regularly exposed to unfavorable climatic conditions. The impact of such hazards can be reduced through adaptations that provide resistance to these conditions. Flies of the *Drosophila melanogaster* species subgroup are great models to study adaptations. *D. melanogaster* originated in sub-Saharan Africa and subsequently expanded its habitat, possibly in association with humans and agriculture, colonizing temperate habitats in Eurasia and more recently, North America and Australia (David and Capy, 1988; Cogni et al., 2014). Latitudinal and seasonal clines in phenotypes and at the genomic level, spanning temperate to subtropical/tropical regions are usually interpreted as an adaption to spatially variable selection (David et al., 1985; Berry and Kreitman, 1993; Paaby et al., 2010; Fabian et al., 2012; Bergland et al., 2016; Reinhardt et al., 2014; Machado et al., 2015; Behrman et al., 2015; Adrion et al., 2015). The sibling species *D. simulans* probably originated in Madagascar and like *D. melanogaster*, only recently expanded its habitat (Dean and Ballard, 2004). The two members of the melanogaster subgroup that colonized non-African habitats are broadly sympatric (David and Capy, 1988). Nevertheless, the latitudinal differentiation of *D. simulans* is usually much shallower (Capy et al., 1988; Begun et al., 2007; Arthur et al., 2008; Behrman et al., 2015; Zhao et al., 2015a; Machado et al., 2015). Given the abundant molecular, genetic and genomic resources available for *D. melanogaster* (and increasingly for *D. simulans*), these two species provide an excellent opportunity for studying adaptation to novel heterogeneous environments.

One particularly stressful condition for insects is winter, when temperature drops dramatically and feeding resources become scarce. Dormancy is an important adaptation to facilitate overwintering. It is a state of suppressed development, reproduction, metabolic activities and senescence (Denlinger, 2002; Hahn and Denlinger, 2011), which allows the organism to 'escape in time' until the environmental conditions are favorable again (Williams and Sokolowski, 1993; Tatar and Yin, 2001; Zonato et al., 2017). The ability of *D. melanogaster* to overwinter is well studied. Pre-adult life stages do not survive winter conditions, but adults do so by expressing a reproductive dormancy (Izquierdo, 1991; Hoffmann, 2010). Dormant adult female flies have underdeveloped

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ovaries (see below), reduced metabolism, delayed senescence and elevated stress resistance (Tauber et al., 1986; Tatar et al., 2001; Schmidt and Conde, 2006; Kubrak et al., 2014). Nevertheless, dormancy in *D. melanogaster* is considered to be shallow, as transfer to warmer temperatures or topical application of juvenile hormones or ecdysteroids can easily break it (Saunders et al., 1989; Saunders and Gilbert, 1990; Richard et al., 1998; Richard et al., 2001; Tatar et al., 2001; Kubrak et al., 2014). This has generated a debate in literature, regarding whether reproductive dormancy in *D. melanogaster* is expressed in the form of diapause or quiescence (Izquierdo, 1991; Tatar et al., 2001; Schmidt et al., 2005b; Schmidt and Conde, 2006; Lee et al., 2011; Kubrak et al., 2014; Kriesner et al., 2016; Kučerová et al., 2016; Kubrak et al., 2016; see Koštál, 2006 for terminology).

Drosophila melanogaster adults express reproductive dormancy at low temperatures and/or short photoperiods. Although many studies demonstrate the significance of photoperiod for *D. melanogaster* dormancy (Saunders et al., 1989; Saunders et al., 1990; Saunders and Gilbert, 1990; Saunders, 1990; Williams and Sokolowski, 1993; Pegoraro et al., 2017; Zonato et al., 2017; Nagy et al., 2018), others showed that dormancy is mainly controlled by temperature (Saunders and Gilbert, 1990; Tatar et al., 2001; Emerson et al., 2009; Lee et al., 2011; Anduaga et al., 2018). Schiesari et al. (2011) proposed a more complex scenario where photoperiod controls reproductive dormancy in species-specific temperature ranges. At more extreme temperatures the influence of photoperiod is reduced and temperature becomes the main determinant. For *D. melanogaster*, this range is 10–13 °C. Nevertheless, recent work (Anduaga et al., 2018; Nagy et al., 2018) suggests that the question regarding the relative contribution of temperature and photoperiod to dormancy induction in *D. melanogaster* is not yet fully settled.

Given the Sub-Sahara African origin of *D. melanogaster*, it was assumed that dormancy evolved during the habitat expansion to temperate regions (Eurasia, America and Australia) (Saunders and Gilbert, 1990; Fabian et al., 2015). This hypothesis was supported by the absence of dormancy in sub-Saharan *Drosophila melanogaster* populations (Schmidt et al., 2005b; Schmidt and Conde, 2006; Fabian et al., 2015) and latitudinal clines described for this trait (Schmidt et al., 2005a,b; Schmidt and Conde, 2006; Schmidt and Paaby, 2008; Schmidt et al., 2008; Cogni et al., 2014). However, Zonato et al. (2017) recently found that African populations do express dormancy and proposed that the syndrome has a more ancient history than previously believed. In contrast to *D. melanogaster*, it is generally assumed that *D. simulans* lacks overwintering capability (Boulétreau-Merle and Fouillet, 2002; Boulétreau-Merle et al., 2003; Capy and Gibert, 2004; Machado et al., 2015; Behrman et al., 2015) and reproductive dormancy (Schmidt et al., 2005b; Schmidt and Conde, 2006). Only recently, the possibility of overwintering (Serga et al., 2015) and a dormancy response similar to *D. melanogaster* (Zonato et al., 2017) was described for *D. simulans*.

In the laboratory, dormancy is commonly induced by transferring virgin females, shortly after eclosion under normal conditions (25 °C, LD 12:12) (usually within 2 – 6 h, when they are supposed to have previtellogenic ovaries), into dormancy-inducing conditions (usually 11 – 12 °C, LD 10:14). Ovaries are dissected after 2 – 4 weeks and egg chambers are staged following criteria established by King (1970). Most studies classify flies as dormant if the ovaries have only previtellogenic egg chambers (stages ≤7), while flies with ovaries containing vitellogenic egg chambers (stages 8 – 14, where stage 14 represents the mature egg) are characterized as non-dormant (Saunders et al., 1989; Saunders et al., 1990; Saunders and Gilbert, 1990; Saunders, 1990; Williams and Sokolowski, 1993; Richard et al., 1998; Tatar et al., 2001; Schmidt et al., 2005a,b; Williams et al., 2006; Schmidt and Conde, 2006; Schmidt and Paaby, 2008; Schmidt et al., 2008; Emerson et al., 2009; Lee et al., 2011; Fabian et al., 2015; Zhao et al., 2015b; Schiesari et al., 2016; Zonato et al., 2016; Pegoraro et al., 2017; Zonato et al., 2017). The rationale underlying this classification scheme is that oogenesis should be blocked at previtellogenic egg chambers, before yolk is deposited in oocytes. Avoiding costly investment into oogenesis, the flies save energy and reserves needed to survive adverse winter conditions. This classification has been challenged by Tatar et al. (2001) and Lee et al. (2011), who observed that oogenesis was not arrested at previtellogenic stages, but mostly delayed up to stage 9 of oogenesis. They defined reproductive dormancy as an arrest up to early vitellogenic egg chamber stages (i.e. up to stage 9 rather than stage 7). This difference in dormancy classification has not been resolved so far.

This difference in classification reflects important biological differences. The actual yolk-demanding oogenesis stage is stage 10. Large amounts of yolk are deposited in stage 10 egg chambers, causing substantial increase in size, compared to stage 9 egg chambers (He et al., 2011). Most yolk is synthesized by the fat body, released in the haemolymph and sequestered in the egg chambers from stage 10 onwards. In contrast, the yolk deposited in egg chambers of stage 8 and 9 is synthesized by the chambers themselves (Brennan et al., 1982). Dormant flies do synthesize yolk proteins in the fat body and accumulate them in the haemolymph, without sequestering them in the oocytes (Saunders et al., 1990; Richard et al., 2001). Thus, the proposed block of vitellogenesis under dormancy-inducing conditions seems to be related to the deposition of yolk in egg chambers at stage 10, rather than stage 8.

The difference in classification becomes also relevant in the light of generic stress response in *Drosophila*. The block of oogenesis at early vitellogenic egg chamber stages under dormancy-inducing conditions may reflect a well described general mechanism of stress response: the degeneration of early vitellogenic egg chambers (or mid-oogenesis checkpoint). Upon transfer to a yeast-free diet (protein-poor medium) (Drummond-Barbosa and Spradling, 2001; Terashima and Bownes, 2004; McCall, 2004; Terashima et al., 2005; Terashima and Bownes, 2006; Bass et al., 2009; Uryu et al., 2015), after heat stress (Gruntenko et al., 2003; Gruntenko and Rauschenbach, 2008), in the absence of males (Soller et al., 1997; Soller et al., 1999), when flies are mechanically unable to oviposit, in the absence of suitable oviposition sites, due to adult crowding (Wilson, 1985) and in old flies (Klepsatel et al., 2013), egg chambers of stage 8 and 9 undergo degeneration to arrest oogenesis before reaching the yolk-demanding stage 10 (McCall, 2004). This mid-oogenesis checkpoint is controlled by ecdysteroids (Pétavy, 1990; Buszczak et al., 1999; Buszczak and Cooley, 2000; Carney and Bender, 2000; McCall, 2004; Terashima et al., 2005; Terashima and Bownes, 2006; Schwedes et al., 2011) and juvenile hormones (Wilson, 1982; He et al., 2011), just like dormancy (Richard et al., 1998; Richard et al., 2001; Tatar et al., 2001; Flatt et al., 2005; Allen, 2007) (also see Pritchett et al. (2009) for other molecular components of dormancy that are implicated in cell death in egg chambers). It is an intriguing question whether the same (degeneration) mechanism also underpins dormancy.

Given the ongoing research on the relative contribution of temperature and photoperiod to dormancy incidence in *Drosophila* in other labs (Anduaga et al., 2018; Nagy et al., 2018), our study focuses on the physiological basis of dormancy and the evolution of the trait in *Drosophila*. We performed a series of assays using *D. melanogaster* and *D. simulans* to a) distinguish between a block of vitellogenesis and early vitellogenic egg chambers degeneration during dormancy; b) examine whether other stressors also affect dormancy expression. Based on these results and evidence from the literature, we propose a modified dormancy phenotyping scheme and evaluate the proposed theory of the evolution of this syndrome.

2. Materials and methods

The first set of experiments used only dormancy-inducing conditions, while in the second set of experiments we combined dormancy-inducing conditions with additional stress factors. The strains used are detailed in Table S1. In every assay, freshly eclosed virgin flies were collected within a two-hour window post eclosion under normal