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Physiological and biological patterns of a highland and a coastal population of the European cherry fruit fly during diapause

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ABSTRACT

Adult emergence of univoltine temperate insect species and its synchronization with specific host phenological stages is mainly regulated by obligatory pupal diapause. Although a few studies have investigated the factors affecting diapause intensity, little attention has been paid to the physiological alterations and metabolic regulation that take place during diapause. Here, we describe differences in diapause between a highland and a coastal Greek population of the European cherry fruit fly *Rhagoletis cerasi*, a major pest of sweet and sour cherries in many European countries. Pupae of both populations were exposed to the environmental conditions prevailing in the two areas and diapause termination was observed under laboratory conditions. The regulation of energetic metabolites during the long pupae stage was examined under both field and laboratory conditions. Differences in diapause intensity revealed that the two populations have adapted to the local geographical and climatic conditions and have different requirements for low temperatures to terminate diapause. The coastal population undergoes a shorter diapause and adults emerge more rapidly, especially in the highland area. The highland population failed to terminate diapause (<40% adult emergence) in the coastal area. Both populations draw on their major energetic reserves (lipids and protein) similarly during diapause. Nevertheless, regulation of carbohydrate and glycogen reserves seems to vary between the populations: major peaks of these stored nutrients occur on different dates in the two populations, suggesting a differential regulation. Differences in diapause intensity imply a genetic differentiation between the two populations. The importance of our findings in understanding the physiological patterns during obligatory diapause of a univoltine insect species, as well as the practical implications for the development of specific phenological models for the European cherry fruit fly are discussed.

1. Introduction

Most insects synchronize their life cycles with host availability through the use of one or more seasonal and/or biological adaptations (e.g., diapause, dormancy, quiescence, migration, growth rate) (Kostal, 2006; Tauber and Tauber, 1976). In contrast to processes involved in other types of seasonal adaptations, diapause is an eco-physiological mechanism in which the morphogenesis of an organism is interrupted while, at the same time, basic physiological functions are maintained (Kostal, 2006). During this phase, insects experience a hormonally mediated dynamic state of low metabolic activity (Tauber et al., 1986). Diapause is a programmed inhibition of growth and/or morphogenesis that starts before the onset of environmental adversity and its termination may or may not coincide with the end of the period of adverse conditions. Diapausing organisms sustain their arrestment period (phase) even when environmental conditions may allow development and/or growth and development only resumes after the fulfillment of the scheduled physiological changes (Kostal, 2006). In most cases, diapause induction is triggered by a token stimulus (e.g., photoperiod, pheromones or allelochemicals) during a sensitive stage of the life cycle. However, the induction of obligatory diapause is a fixed component of the insect's ontogenetic program that does not require exogenous-environmental stimuli for its expression (Kostal, 2006).

During periods of adverse environmental conditions, diapause is the main survival mechanism of a variety of herbivorous insects

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that express strict host-specificity (Ragland et al., 2009). Univoltine species use diapause to generate synchrony with their host plants (Dambroski and Feder, 2007). Diapause intensity and within-species diversity in diapause intensity are driven by natural selection pressures and, sometimes, by genetic factors (Demont and Blanckenhorn, 2008; Feder et al., 2003a; Lyons-Sobaski and Berlocher, 2009) as well. Differences in diapause intensity can lead to reproductive isolation due to allochronic adult emergence. For example, *Rhagoletis pomonella* taxa (linked to specific hosts) coexist in natural habitats, but are partially reproductively isolated due to asynchrony in their exhibition of sexual behavior (courtship and copulation on host fruits) and the duration of their diapause phases (temporal divergence of adult emergence and sexual maturity). In many cases, this reproductive isolation is associated with genetic differentiation that may indicate the beginning of sympatric speciation (Feder et al., 2003a,b). Although the above examples refer to life-cycle differentiation due to host-specific adaptation, which is mostly observed among different latitudinal clines, this differentiation can also reflect alterations in the insects' life cycles that occur due to differences in the phenology of their hosts at different altitudes (Smith et al., 2000).

Fruit fly larvae (Diptera: Tephritidae) feed on fruit flesh, accumulating metabolic reserves (e.g., lipids, carbohydrates, proteins, etc.) before their transition into the pupal stage. In insects, the pupal stage is a closed system from an energy point of view, since there is no energy income and some of the energy accumulated during the larval stage is consumed to supply the demands of metamorphosis (Nestel et al., 2003). Many recent studies have focused on the transformation of accumulated energy in several life stages of fruit flies, including the pupal stage (Nestel et al., 2003, 2004, 2005; Warburg and Yuval, 1997). Studies on pupae of medfly (Nestel et al., 2004) and other fruit flies (Dutra et al., 2007) have suggested that energy consumption and metabolic regulation during the pupal stage lead to the fulfillment of a fixed lipid-content requirement for successful adult emergence. This threshold amount is sufficient for the fulfillment of minimal metabolic requirements until the adult fly can find food to replenish its energy reserves. In contrast to what has been observed in multivoltine fruit flies, regulation of energetic metabolites during the pupal stage is expected to be more challenging for univoltine tephritids that undergo obligatory diapause. In these systems, the pupal stage lasts several months and there are physiological alterations due to diapause itself. Thus, it is expected that energy metabolites will be managed according to the demands and dynamics of metamorphosis and diapause. This study focuses on the metabolic regulation of geographically isolated diapausing populations of a univoltine fruit fly, the European cherry fruit fly, *Rhagoletis cerasi* L. (Diptera: Tephritidae), and links metabolic trends with the observed biological patterns of the fly populations.

The European cherry fruit fly is a major pest of sweet (*Prunus avium*) and sour cherries (*P. cerasus*) in many European countries including Greece (Fimiani, 1989). Adults emerge at the end of the spring or the beginning of the summer (depending on the local climate), just before or during the cherries' ripen (when fruit color changes from yellow to reddish). Once they have reached reproductive maturity, females usually oviposit one egg in each ripe or semi-ripe fruit. Soon after hatching, young larvae feed on the fruit mesocarp. Fruits are destroyed by larval activity coupled with secondary fungal or bacterial infections. Mature larvae leave the fruits, fall to the ground and pupate in the soil. Newly formed pupae enter obligatory diapause and remain in this stage until the following spring. Like most univoltine oligophagous species, *R. cerasi* uses obligatory diapause to help it synchronize its life cycle with the phenology of its host.

In Greece and other Mediterranean countries, cherries are produced in highland as well as in coastal areas. There is a temporal divergence of more than a month between the cherry-ripening periods in the highland and the coastal areas. Correspondingly, adult European cherry fruit flies exhibit temporal divergence following the availability of cherries and emerge later at higher altitudes (Kovanci and Kovanci, 2006). For long periods of time, *Rhagoletis cerasi* pupae in the highland and the lowland areas have been exposed to different environmental (mostly temperature) conditions and these conditions have molded diapause termination and post-diapause development. In fact, it is the duration of these two periods that controls adult emergence and allows adult flight to be synchronized with the ripening of the cherries (N.T. Papadopoulos, unpublished data).

Although previous studies have attempted to explain the environmental factors that promote the variability in adult emergence that has been observed among different populations (Danks, 2002; Tauber and Tauber, 1976; Teixeira and Polavarapu, 2005), little is known about the physiological changes that contribute to diapause termination and/or metabolic regulation during diapause. The present study focuses on these issues, taking advantage of the differences in diapause onset and termination observed between highland and lowland populations. We investigated: (a) the diapause intensities of a coastal and a highland population of the European cherry fruit fly, under field conditions; and (b) the trends in energy reserves, specifically protein, lipids, glycogen and carbohydrates, in pupae during the different phases of diapause under both field and constant laboratory conditions. We tested the hypotheses that the pupae of these two *R. cerasi* populations have the same chilling requirements in order to terminate diapause and that pupal energetic metabolites are regulated in a similar manner among different geographic populations, regardless of their diapause and adult emergence patterns.

2. Materials and methods

2.1. Study areas and sources of flies

Flies for this study were collected during 2005–2006 and 2006–2007 in two geographically distinct areas: a highland location (Dafni Kozanis, 1050 m a.s.l., absolute minimum temperature −17.6 °C, absolute maximum temperature 37 °C, northwest mainland Greece, latitude N40°17′8.34″, longitude E21°8′52.56″) and a coastal location (Kala Nera Magnisias, 20 m a.s.l., absolute minimum temperature −6.6 °C, absolute maximum temperature 37.8 °C, coastal area, central Greece, latitude N39°18′53.88″, longitude E23°4′9.96″) (Fig. 1). The climate in the highland area is characterized by cool and dry summers and cold winters with temperatures often falling below 0 °C. The climate in the coastal area is characterized by warm, dry summers and mild, wet winters, with temperatures rarely reaching the freezing point. Climatic data for the two areas (Fig. 2) were obtained from meteorological stations located approx. 20 and 30 km from the experimental areas in the coastal and highland locations, respectively. The elevations of the meteorological stations were similar to those of the sampling locations.

Flies were obtained from infested cherries collected in the two areas. Infested fruits were collected in the coastal area at the beginning of June and in the highland area at the end of June. Cherries were transferred to the laboratory, placed in plastic boxes on a thin layer of dry sand and covered with organdie cloth. Fully grown, mature larvae left the infested cherries and pupated within the thin layer of sand. Pupation was recorded daily and pupae were collected by sieving the sand. Pupae of the two sample populations were randomly divided into batches of 50 (for the diapause experiments) or 30 individuals (for determination of energy reserves). Pupae were placed in plastic Eppendorf tubes and