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Cost of reproduction in male medflies: The primacy of sexual courting in extreme longevity reduction

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ABSTRACT

In polygynous insect species, male reproductive success is directly related to lifetime mating success. However, the costs for males of sexual activities such as courting, signaling, and mating are largely unknown. We studied the cost of sexual activities in male Mediterranean fruit flies, *Ceratitis capitata* (Tephritidae), a polygynous lekking species, by keeping cohorts of individual male flies under relaxed crowding conditions in the laboratory. We used 5 cohorts among which individuals differed in their opportunities to interact with con-specifics and recorded life span, and in one treatment, mating rate. We found that males kept singly lived more than twice as long as males that interacted intensively with mature virgin females, while male–male interactions caused a smaller reduction in longevity. Because longevity of males that could court but not mate was not significantly different from those that could court and mate, we conclude that courting (not mating) was responsible for the observed longevity reduction. Moreover, we detected high variability in male mating success, when 5 virgin females were offered daily. In contrast to the cohort level, individual males that mated at a high rate lived relatively long, thus indicating heterogeneity in quality or sexual strategy among males.

1. Introduction

Cost of reproduction, defined as reduction in survival or future fecundity as a result of reproduction (Prowse and Partridge, 1997), significantly affects life history evolution including key traits such as survival and age-specific fecundity schedules in animals that reproduce sexually (Maynard Smith, 1958; Partridge and Farquhar, 1983; Partridge et al., 1985). The cost of sexual activities can be partitioned into extrinsic—behavioral and ecological (predation risk, allocation of time and energy to sexual activities; Martin and Hosken, 2004), and intrinsic (physiological; Sakaluk et al., 2004).

There are numerous studies demonstrating cost of reproduction in females (i.e. Bell and Koufopanou, 1986; Chapman et al., 1998; Harshman and Zera, 2006; Langley and Clutton-Brock, 1998; Mangan, 1997; Martin and Hosken, 2004; Molleman et al., 2008; Müller et al., 2002; Sivinski, 1993), but fewer in males, and even fewer attempt to quantify the cost of different male sexual activities (Burton-Chellew et al., 2007; Cordts and Partridge, 1996; Gems and Riddle, 1996; Kotiaho and Simmons, 2003; Martin and Hosken, 2004; Perez-Staples and Aluja, 2006). Theory and empirical evidences suggest that sexual signaling, intensive courting, and ejaculate production all confer costs in polygynous species (Byrne and Rice, 2006, and references there in). Recent lifetime behavioral studies suggest that sexual signaling has no obvious effects on male Mediterranean fruit fly (medfly) lifespan (Papadopoulos et al., 2004; Zhang et al., 2006). However, whether mating or courting are negatively correlated with male longevity is not known. A cost of copulation has been demonstrated recently for males of several insect species (Burton-Chellew et al., 2007; Kotiaho and Simmons, 2003; Martin and Hosken, 2004; McNamara et al., 2008; Simmons and Kotiaho, 2007). For male medflies the relationship between lifetime mating success and life span has not been investigated. However, in another, male lekking, polygynous tephritid the number of copulations was not correlated with life span within individuals (Perez-Staples and Aluja, 2006). The latter study did not include a control cohort that did not interact with females as in our study.

To separate cost of courtship from costs of more general interaction with other individuals we also need to know the costs of non-sexual interactions. It is often assumed that male–male

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antagonistic interactions confer major longevity reductions compared with male–female interactions. Male medflies are believed to defend territories and exhibit intensive aggressive behavior against intruders (Arita and Kaneshiro, 1989; Prokopy and Hendrichs, 1979). An experiment with high densities of medflies in cages suggested that male–male interactions are costly to male medflies, while male–female interactions do not confer additional costs (Gaskin et al., 2002).

Over the last two decades the Mediterranean fruit fly (medfly; *Ceratitis capitata*) has become an important model system in demographic, aging, and genetic research (i.e. Carey and Vaupel, 2003; Loukeris et al., 1995; Malacrida et al., 2007). The sexual behavior of medfly has been studied in great detail (see Aluja and Norrbom, 1999). Reproductive maturity is attained within 5 days for most laboratory strains and considerably longer (7–13 days) in wild flies (Papadopoulos et al., 1998; Liedo et al., 2002). Males are polygynous, and form leks (Yuval and Hendrichs, 1999). It is well documented (in field cages and laboratory studies) that a small proportion of males account for most of the matings (Shelly, 2000). Females are monoandrous or oligoandrous (Bonizzoni et al., 2002, 2006; Kraaijeveld et al., 2005). Within leks, males perform sexual signaling while releasing a sex pheromone to attract virgin, mature females (Prokopy and Hendrichs, 1979). Upon female arrival males initiate a complex courtship behavior, and females select their mates based on male courtship performance. Successful copulation that leads to sperm transfer lasts on average 2–3 h depending on environmental conditions and the medfly biotype (Papadopoulos unpublished data). Nonetheless, most of the data regarding medfly sexual behavior were obtained from studies focusing on relatively young individuals, and the association between sexual signaling and life span has only recently been determined (Papadopoulos et al., 2004; Zhang et al., 2006).

The aims of this study were better understanding of (a) the cost of reproduction in male medflies, and (b) the division of cost of reproduction into sexual courting and male sexual signaling, male–male interaction (contest and others), and mating per se. We specifically tested the following hypotheses: (1) intensive sexual activities reduce the life span of male medflies; (2) sexual courting and mating are both costly for male medflies; and (3) male–male interactions are more costly than male–female interactions.

2. Methods

2.1. Experimental conditions and insects

The flies were obtained from the Moscamed mass-rearing facility in Metapa, Mexico. Those flies used in our experiments had been reared in the mass-rearing facility for approximately 25 generations. Experiments and observations were conducted under laboratory conditions (25 ± 2 °C, 65% (r.h.) and 12:12 L:D, lights turned on at 06:00) in autumn and winter 2002–2003. Light in the experimental room was provided by day light tubes, and the light intensity in the experimental arena ranged from 1000 to 1500 lx. Adults were sexed upon emergence, maintained in groups of 100 individuals, and offered a diet consisting of sugar and yeast hydrolysate in a ratio of 4:1, respectively.

2.2. Cost of reproduction

Under laboratory conditions cost of reproduction in male medflies may arise because of intrasexual male–male agonistic interaction, sexual signaling, courting, and mating per se. To test whether (and if so which) male reproductive activities incur a life span cost, 1-day-old males were randomly assigned to one of the following five treatments: (a) *no mating*—males engaged in sexual signaling only; individually kept males (one male per cage) having no access to females; (b) *intensive mating*—one male offered five mature virgin females each day until death (one male plus five females per cage). Thus number of matings/day/male could range from 0 to 5; (c) *no mating, courting*—one male was kept with five females that could not mate (one male plus five females per cage); females were mature, mated, and had their ovipositor sealed to preclude any chance of mating; Female ovipositor was cauterized with hot forceps; (d) *no mating, male–male interactions*—males kept in groups of six per cage; (e) *moderate mating*—three males offered three virgin females daily (3 males plus 3 females per cage). Thus number of matings/day/male could range from 0 to 3. There were 60, 60, 60, 15, and 25 cages (replicates) for treatments a, b, c, d, and e, respectively, i.e. 60, 60, 60, 90, and 75 males per treatment, respectively. We used clear plastic, cubic cages of 2 l capacity for all treatments. The density of flies within each cage was rather relaxed (1–6 individuals in 2 l [10 by 20 cm base by 10 cm height] volume) and kept constant by replacing dead flies with similarly treated ones. Replacement flies were marked with a color dot on the thorax to be distinguished from the males of the experimental cohort. A perforated 100 cm^2, mesh-covered window, served for ventilation and access to the cage. Food (yeast hydrolysate and sugar, 1:4, respectively) and water were provided *ad libitum* throughout the experiment. The number of matings was recorded in treatment b (intensive mating). Observers monitored the cages every 15 min, from 08:00 to 17:00, to record mating pairs. To be able to distinguish different matings of the same male within a day (assuming that each female would not mate more than once on a day), virgin females were marked with a within-cage unique color dot on the thorax. Earlier studies have demonstrated that such a marking has no effect on both male and female sexual behavior (McInnis et al., 2002; Shelly and McInnis, 2003). At the end of each day females were removed from cages. New females were placed into experimental cages at 07:00 next day. Within the experimental room there were several males and females held in cages located in close distance to the experimental arena. Neither sexual signaling nor sexual courting was systematically recorded.

2.3. Statistical methods

Statistical analyses included Cox model and Log-rank test to detect differences in survival among the above treatments, time-dependent Cox-proportional hazard model to detect possible associations between number of matings and life span in treatment b intensive mating, and Kaplan Meier estimates of age-specific survival rates (Collett, 2003; Sokal and Rohlf, 1995). Hazard functions for each treatment were obtained by smoothing following the procedure described in Müller et al. (1997) and Wang et al. (1998).

3. Results

Average and record life spans for each of the cohorts tested are given in Table 1. Males maintained individually lived on average of 2.2–2.7 times longer than males exposed to females, and 1.6 times longer than males kept in groups of six. Longevity of males kept in groups of six was 1.7 times longer than that of males exposed daily to five virgin females. The 25% longest lived males that were kept individually (no mating) lived an average of nearly 5 months (i.e. 140 days), while the 25% longest lived males that had the opportunity to mate intensively lived an average of less than 2 months (i.e. 51 days). Average and maximum longevities were positively correlated ($r = 0.95$, $P < 0.05$). Our results clearly demonstrate that sexual activities incur a significant cost in terms of reduced life span for male medflies (Log-rank test, Chi-square = 145.0; df = 4; $P < 0.0001$) (Fig. 1, Table 1). Age-specific mortality rates were low in non-mated males up to day 100 and