Parental age at mating affects reproductive attributes of the aphidophagous ladybird beetle, *Coelophora saucia* (Coleoptera: Coccinellidae)

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Key words. Coleoptera, Coccinellidae, *Coelophora saucia*, fecundity, egg viability, paternal age, maternal age

Abstract. Ageing and its affect on life attributes have not been widely explored in ladybirds. The present study investigates the influence of female and male age at mating on the reproductive attributes of the ladybird beetle, *Coelophora saucia* (Mulsant). All reproductive attributes, viz. fecundity, percentage egg hatch, pre-oviposition, oviposition and post-oviposition periods were found to be influenced by the ages of both females and males at mating. This is unlike the results of previous studies on ladybirds, where male age was found to influence only percentage egg hatch. The influence of male age at mating on fecundity and reproductive periods is probably due to age influenced variation in seminal proteins, which influence fecundity and when the eggs are laid.

INTRODUCTION

Senescence can be defined demographically as an age-dependent increase in mortality risk, or functionally as a decline in performance (Medawar, 1952; Williams, 1957; Rose, 1991; Partridge & Gems, 2002), and can be accounted for by several evolutionary and mechanistic theories (see Hughes & Reynolds, 2005). The understanding of the ageing process is based on the relationship between the demographic and functional aspects, which are believed to be tightly linked, as ageing involves a decline in performance of several body functions, leading to increased mortality (Williams, 1999), and is observed in organisms ranging from flies (Grotewiel et al., 2005) to human beings (Burke & Barnes, 2006).

This decline in physiological performance with age is known to be influenced by a number of factors prominent amongst which are dietary restrictions (Piper & Partridge, 2007 and references therein) and reproduction (Rose, 1984; Finch, 1990, 1998; Tatar et al., 1993; Partridge et al., 1999; Finch & Ruvkun, 2001; Robine, 2001). The acceleration of ageing in reproductively active individuals (Arnqvist & Nilsson, 2000; Priest et al., 2002; Mishra & Omkar, 2006), which is reflected in a deterioration in fitness and increase in mortality rate, is reported for a number of insects (Rose, 1984; Van Voorhies, 1992; Simmons & Bradley, 1997; Mishra & Omkar, 2006). The mechanism is explained in terms of reproductive costs (Partridge, 1986; Omkar & Mishra, 2005) and trade offs (Kokko, 1998; Finch & Ruvkun, 2001; Robine, 2001; Mishra & Omkar, 2006).

Ageing is likely to affect mate choice and mortality patterns (Hansen & Price, 1995; Kokko, 1998; Beck & Powell, 2000; Kindlmann et al., 2001; Dixon & Agarwala, 2002; Mishra & Omkar, 2006). Although ageing has considerable implications for individual fitness, even in wild populations (Nesse, 1988; Bonduriansky & Brassil, 2002), studies have typically relied on aggregate statistics from cohorts, while individual and sex specific variation in ageing rates remain poorly understood (Austad, 1996; Service et al., 1998; Partridge & Mangel, 1999; Zens & Peart, 2003; Mishra & Omkar, 2006). Priest et al. (2002) recorded the role of sex-specific ageing on the fitness and survival patterns of future generations; maternal age and to a lesser extent paternal age were found to have a large influence on offspring longevity and their age-specific mortality. This aspect however needs further study.

As ageing is the outcome of inherent life-history trade offs and the condition of organisms, its effects can be recorded by changes in a number of aspects, such as fecundity, egg hatch and mortality, which have been exhaustively studied in many insects (Williams, 1957; Hamilton, 1966; Kirkwood, 1977; Partridge & Barton, 1993). Recent studies on arthropods have investigated the effect of male or female age on mate choice (Hansen & Price, 1995; Kokko, 1998; Beck & Powell, 2000; Proulx et al., 2002), sperm quality and quantity (Hayashi, 1999; Green, 2003) sperm transfer rates (Jones et al., 2006), last male sperm precedence (LaMunyon, 2001; Schafer & Uhl, 2002; Mack et al., 2003; Radwan et al., 2005), sperm competitive ability (Service & Fales, 1993; Jones et al., 2006), musculature of genital tract (Mack et al., 2002) and sperm storage (Taylor et al., 2001). However, the very basic influence of age on life attributes is yet to be explored in detail in ladybirds, for which there are only a few studies (Dixon & Agarwala, 2002; Pervez et al., 2004; Srivastava & Omkar, 2004; Mishra & Omkar, 2004, 2006; Omkar & Singh, 2005; Omkar et al., 2006; Omkar & Mishra, 2009). While most of these studies have concentrated on the effect of age on various reproductive parameters, the results are dependent on the species studied and require further study. The age at mating is an interesting aspect of age related studies in ladybirds.

*Coelophora saucia* (Mulsant), commonly called the Macular site ladybug and the Tokyo star in China, is an...
aphidophagous ladybird beetle, which is common in Northern India, China, Japan, Taiwan, Thailand, Indonesia, Malaysia, Philippines, Vietnam and Korea (Hirano et al., 1996; Han, 1997; Berg et al., 1997; Omkar et al., 2005; Singh & Tripathi, 2008). It is a relatively little studied ladybird, with only a few studies on its life attributes (Omkar & Pathak, 2006, 2007; Omkar et al., 2005; Pathak, 2008) and none on the effect of parental age at mating. The present study investigates the effect of parental age at mating on reproductive attributes of *C. saucia* and increases the level of knowledge on this beetle and of ladybird physiology in general.

**MATERIAL AND METHODS**

**Stock culture**

Adults of *C. saucia* were collected from colonies of the aphid, *Aphis craccivora* Koch infesting bean (*Dolichos lablab* Linnaeus) in fields around Lucknow and brought to the laboratory (25 ± 2°C, 60 ± 5% R.H and 14L:10D). They were paired and reared in Petri dishes (9.0 × 2.0 cm) on an *ad libitum* supply of *A. craccivora* eggs were collected daily and the larvae reared to the pupal stage in glass beakers (11.0 × 9.0 cm) in which the aphid prey was replenished daily. Beakers containing pupae were observed twice a day and newly emerged (NE) adults were sexed and isolated in Petri dishes and provided with an *ad libitum* supply of *A. craccivora*, which was replaced daily.

**Experimental setup**

(A) Effect of female age

Unmated adults of requisite ages were obtained from the stock culture. Virgin females aged 0 (newly emerged: NE), 1, 2, 4, 6, 8, 10, 20, 30, 40, 50 and 60 days were paired once with 10-day-old males in plastic Petri dishes (9.0 × 2.0 cm). Thereafter, the females were isolated and reared on a daily replenished ad libitum supply of *A. craccivora* for the remainder of their life. Number of eggs laid and percentage egg hatch were recorded once every 24 h. As above there were 10 pairs in each mating group.

**Statistical analysis**

Data were checked for normality and found to be normally distributed. All percentages were arcsine square root transformed prior to statistical analysis. Data on pre-oviposition (time between mating and start of oviposition), oviposition (time spent egg laying) and post-oviposition (time between cessation of egg laying and death of females) periods, fecundity and percentage egg hatch were subjected to one-way ANOVA followed by post hoc Tukey’s honest significance test using statistical software MINITAB (Minitab, 2000) with female or male age as the varying factor. Reproductive rate (fecundity/oviposition period) was also calculated and subjected to ANOVA. Data on NE to 4 day-old males, which did not mate, were not included in the analysis. Fecundity and percentage egg hatch were regressed against both female and male age in order to determine the relationships between these two parameters and age.

**RESULTS**

(A) Effect of female age

Females in all the age groups mated. Females of *C. saucia* that mated at the age of 20 days laid most eggs (1979.6 ± 197.9; Mean ± SE) and those mated when 60 days old the least (240.0 ± 16.3) (Table 1). Fecundity differed significantly with the age at mating of females (F = 85.09; P < 0.001; d.f. = 11,119; Table 1) and showed a parabolic trend (Y = –35.053X2 + 484.64X – 419.65; r2 = 0.5815; Fig. 1) with a peak at female age of 20 days. Percentage egg hatch was also found to differ with age at mating of females, albeit less significantly (F = 6.23; P < 0.01; d.f. = 11,119; Table 1). It was highest for females

**Table 1. Influence of female age at mating on the reproductive parameters of *C. saucia***

<table>
<thead>
<tr>
<th>Female age at time of mating</th>
<th>Fecundity (no. of eggs)</th>
<th>Percentage egg hatch</th>
<th>Pre-oviposition period (in days)</th>
<th>Oviposition period (in days)</th>
<th>Post oviposition period (in days)</th>
<th>Longevity (in days)</th>
<th>Reproductive rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td>393.1±13.3b</td>
<td>71.9±2.5a</td>
<td>6.5±0.6e</td>
<td>27.8±0.9c</td>
<td>27.2±2.1f</td>
<td>54.0±4.6b</td>
<td>14.1±0.2a</td>
</tr>
<tr>
<td>1</td>
<td>447.6±23.0c</td>
<td>80.7±1.2b</td>
<td>6.2±0.3e</td>
<td>28.2±0.7c</td>
<td>15.4±2.6e</td>
<td>45.3±4.2a</td>
<td>15.9±0.9b</td>
</tr>
<tr>
<td>2</td>
<td>508.3±15.5c</td>
<td>81.2±3.2b</td>
<td>5.3±0.4d</td>
<td>29.5±1.5c</td>
<td>14.9±1.8e</td>
<td>46.9±3.5a</td>
<td>17.6±0.8b</td>
</tr>
<tr>
<td>4</td>
<td>606.5±19.3d</td>
<td>83.4±1.8b</td>
<td>4.2±0.4e</td>
<td>30.7±1.3c</td>
<td>14.2±2.2e</td>
<td>50.5±3.8ab</td>
<td>19.9±0.5b</td>
</tr>
<tr>
<td>6</td>
<td>647.2±34.3d</td>
<td>88.1±1.1</td>
<td>3.9±0.2c</td>
<td>35.3±1.7d</td>
<td>8.3±1.5bc</td>
<td>51.1±3.9ab</td>
<td>18.4±0.7b</td>
</tr>
<tr>
<td>8</td>
<td>1121.1±82.1f</td>
<td>89.6±1.2c</td>
<td>2.9±0.3bc</td>
<td>38.5±2.8d</td>
<td>9.2±2.5c</td>
<td>53.1±4.1ab</td>
<td>30.5±3.1d</td>
</tr>
<tr>
<td>10</td>
<td>1506.4±150.6h</td>
<td>91.5±9.1cd</td>
<td>2.1±0.2bc</td>
<td>49.4±4.9e</td>
<td>5.9±0.6a</td>
<td>63.4±6.3c</td>
<td>27.9±2.8c</td>
</tr>
<tr>
<td>20</td>
<td>1979.6±197.9</td>
<td>93.2±9.3d</td>
<td>1.3±0.1b</td>
<td>56.0±5.6e</td>
<td>7.8±0.8b</td>
<td>65.1±6.5c</td>
<td>35.6±3.6d</td>
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<tr>
<td>30</td>
<td>1219.7±102.9g</td>
<td>89.9±5.4c</td>
<td>2.0±0.2bc</td>
<td>36.9±2.3d</td>
<td>7.4±1.0b</td>
<td>65.5±0.9c</td>
<td>33.9±3.6d</td>
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<td>40</td>
<td>853.8±46.2e</td>
<td>82.7±3.8b</td>
<td>0.3±0.2a</td>
<td>27.7±2.4c</td>
<td>4.2±0.7a</td>
<td>64.9±0.6c</td>
<td>32.5±2.9d</td>
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<tr>
<td>50</td>
<td>458.4±30.2b</td>
<td>76.7±4.3a</td>
<td>0.0±0.0a</td>
<td>20.4±2.3b</td>
<td>12.0±1.1d</td>
<td>73.1±0.04d</td>
<td>24.8±3.1c</td>
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<tr>
<td>60</td>
<td>240.0±16.3a</td>
<td>72.7±3.5a</td>
<td>0.0±0.0a</td>
<td>12.7±1.4a</td>
<td>6.7±0.5b</td>
<td>72.7±3.5d</td>
<td>20.8±2.7b</td>
</tr>
</tbody>
</table>

Values are Mean ± S.E. * and ** indicate F-values significant at P < 0.01 and P < 0.001, respectively. Values in a column followed by different letters are significantly different.
mated when 20 days old (93.2 ± 9.3%) and lowest for females mated at 60 days of age (72.7 ± 3.5%) (Y = –0.6211X² + 8.1866X + 63.917; r² = 0.9118; Fig. 1). The reproductive periods, viz. pre-oviposition (F = 60.08; P < 0.001; d.f. = 11,119), oviposition (F = 43.55; P < 0.001; d.f. = 11,119) and post-oviposition (F = 13.22; P < 0.001; d.f. = 11,119) also differed significantly for the females mated at different ages. Pre-oviposition period decreased with increase in female age at mating up to 20 days and then increased, with a reverse trend in the oviposition period. Post-oviposition period on the other hand decreased with increase in female age at mating up to 40 days and then increased. The reproductive rate showed a parabolic relationship with female age at mating (F = 13.84; P < 0.001; d.f. = 11,119; Table 1) with the peak at 20 days. Longevity increased with female age at mating. Thus, female age at mating significantly affected all the reproductive parameters, viz. pre-oviposition, oviposition and post-oviposition periods, fecundity and percentage egg hatch.

(B) Effect of male age

Recently emerged to 4 day old males did not mate and thus no reproductive parameters were recorded for them. In all the other treatments the males mated with females. The pre-oviposition period of female C. saucia mated with males of different ages differed significantly (F = 7.27; P < 0.001; d.f. = 7,79; Table 2), as did the oviposition (F = 35.73; P < 0.001; d.f. = 7,79; Table 2) and post-oviposition periods (F = 7.11; P < 0.001; d.f. = 7,79; Table 2). Fecundity also differed significantly with male age at mating (F = 49.82; P < 0.001; d.f. = 7,79; Table 2) with those females mated to males aged 30 days having the highest fecundity. However, there was no significant difference in the fecundity of females that were mated with 10-, 20- or 30-day old males. Fecundity in C. saucia increased with the age of the males that were mated up to the age of 30 days, after which it declined (Y = –95.077X² + 844.81X – 361.54 r² = 0.7808; Fig. 2). Percentage egg hatch was also influenced by paternal age at mating (F = 4.17; P < 0.01; d.f. = 7,79; Table 2) and was highest when males were 30 days old (Y = –0.8698X² + 13.84**

<table>
<thead>
<tr>
<th>Male age at time of mating</th>
<th>Fecundity (no. of eggs)</th>
<th>Percentage egg hatch</th>
<th>Pre-oviposition period (in days)</th>
<th>Oviposition period (in days)</th>
<th>Post-oviposition period (in days)</th>
<th>Longevity (in days)</th>
<th>Reproductive rate</th>
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<tr>
<td>NE</td>
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</tr>
<tr>
<td>6</td>
<td>433.6±43.4a</td>
<td>84.4±8.4a</td>
<td>4.3±0.4c</td>
<td>20.7±2.1a</td>
<td>17.3±1.7d</td>
<td>51.1±5.1a</td>
<td>21.7±2.2a</td>
</tr>
<tr>
<td>8</td>
<td>673.0±67.3b</td>
<td>88.1±8.8a</td>
<td>3.8±0.4</td>
<td>31.0±3.1b</td>
<td>13.6±1.4c</td>
<td>47.3±4.7a</td>
<td>21.7±2.2a</td>
</tr>
<tr>
<td>10</td>
<td>1506.4±150.7d</td>
<td>91.5±9.2ab</td>
<td>2.1±0.2a</td>
<td>49.4±4.9d</td>
<td>5.9±0.6b</td>
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<td>27.9±2.8b</td>
</tr>
<tr>
<td>20</td>
<td>1653.3±165.3d</td>
<td>92.8±9.3b</td>
<td>2.8±0.3ab</td>
<td>38.4±3.8c</td>
<td>1.5±1.0a</td>
<td>55.0±5.5a</td>
<td>43.7±4.4c</td>
</tr>
<tr>
<td>30</td>
<td>1724.0±172.4d</td>
<td>94.7±9.5b</td>
<td>2.2±0.2a</td>
<td>39.2±3.9c</td>
<td>11.2±1.1c</td>
<td>60.5±6.1a</td>
<td>45.4±4.5c</td>
</tr>
<tr>
<td>40</td>
<td>904.0±90.4c</td>
<td>90.3±9.0ab</td>
<td>3.0±0.3b</td>
<td>32.9±3.3b</td>
<td>17.4±1.7d</td>
<td>63.7±6.4a</td>
<td>27.9±2.8b</td>
</tr>
<tr>
<td>50</td>
<td>696.5±69.6b</td>
<td>87.7±8.8a</td>
<td>2.3±0.2a</td>
<td>30.2±3.0b</td>
<td>17.4±1.7d</td>
<td>60.6±6.1a</td>
<td>23.9±2.4a</td>
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<tr>
<td>60</td>
<td>534.3±53.4a</td>
<td>81.4±8.1a</td>
<td>3.2±0.3ab</td>
<td>24.4±2.0a</td>
<td>20.4±2.0e</td>
<td>56.5±53.4a</td>
<td>22.1±2.2a</td>
</tr>
</tbody>
</table>

Values are Mean ± S.E. * and ** indicate F-values significant at P < 0.01 and P < 0.001, respectively. Values in a column followed by different letters are significantly different.

Fig. 1. The relationships between female age at mating, fecundity and egg hatch in C. saucia. Bold line indicates the trend in fecundity and the dashed line the trend in percentage egg hatch.

Fig. 2. The relationships between male age at mating, fecundity and egg hatch in C. saucia. Bold line indicates the trend in fecundity and the dashed line the trend in percentage egg hatch.
7.5392X + 77.123; \( r^2 = 0.964 \); Fig. 2). The reproductive rate was also significantly dependent on male age at mating (\( F = 13.84; P < 0.001 \); d.f. = 7,79; Table 2). It was highest when the females were mated with 30 day old males, but not statistically different from when mated with 20 day old males.

**DISCUSSION**

The results reveal that the ages of both the female and male at mating affect reproductive performance in *C. saucia*. All reproductive parameters, viz. pre-oviposition, oviposition and post-oviposition periods, fecundity, percentage egg hatch and reproductive rate, were influenced by the ages of both the female and male at mating. An effect of the age of both sexes at mating on all reproductive parameters has not been reported before in ladybirds. Previous studies on ladybirds, viz. *Propylea dissecta* (Mulsant), *Cheilomenes sexmaculata* (Fabricius), and *Coccinella septempunctata* Linnaeus revealed the effect of male age is limited to percentage egg hatch and had no effect on fecundity and the length of the reproductive period (Pervez et al., 2004; Srivastava & Omkar, 2004; Mishra & Omkar, 2004; Omkar et al., 2006).

Fecundity in *C. saucia* was greatest when the females were first mated at the age of 20 days and for 10 day old females mated with 30 day old males. In another ladybeetle, *Cryptolaemus montrouzieri* Mulsant, 5–15 day old females laid the highest number of eggs (Jalali et al., 1993a, b; Fox et al., 1995a; Savalli & Fox, 1999a, b; Savalli et al., 2000). That this may occur in *C. saucia* is indicated by the increase in the longevity of females that mate many times compared with those that only mated once or a few times, as is recorded in another publication on this beetle (Omkar et al., 2010). Nutritional inputs provided by males are reported to take the form of (a) gifts of food (Arnvist & Nilsson, 2000) and/or (b) by varying the quantity and quality of the ejaculates (Arnvist & Nilsson, 2000). The former practice has not been observed in *C. saucia*, so the latter is likely to play a role in the change in reproductive parameters of females mated with males of different ages. A study by Fox et al. (1995b) on *C. maculatus* revealed a change in the quantity and quality of the ejaculate with age. Gillot (2003) reviewed the effect of male age on the quality and concentration of proteins in semen that are known to influence female fecundity and time of oviposition. Change in the frequency with which females mate along with male age might also determine the change in reproductive parameters (Fox et al., 1995b). A reduction in the quantity of sperm transferred with increase in male age may also account for the reduced fecundity of females.

The influence of paternal age on percentage egg hatch is reported for ladybirds (Pervez et al., 2004; Srivastava & Omkar, 2004). This is most likely a consequence of age related changes in the physiological state of males. This might manifest itself in four ways (a) sexual maturity of male (Pitnick et al., 1995; Baker et al., 2003), (b) change in quality or quantity of sperm with age (Hayashi, 1999; Taylor et al., 2001; Green, 2003), (c) change in the ability to transfer sperm (Jones et al., 2006), and/or (d) change in sperm precedence and competitive ability
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ACHIEVING GREATER FERTILIZATION SUCCESS WHEN COMPETING WITH YOUNG OR OLD MALES (JONES ET AL., 2006).

OLDER MALES ARE MORE LIKELY TO TRANSFER SPERM SUCCESSFULLY AND A GREATER QUANTITY OF SPERM THAN YOUNG MALES. SPERM COMPETITIVE ABILITY VARIES WITH MIDDLE AGED MALES ACHIEVING GREATER FERTILIZATION SUCCESS WHEN COMPETING WITH YOUNG OR OLD MALES (JONES ET AL., 2006).

THE RESULTS OF THE PRESENT STUDY MAY ALSO INDICATE MATE CHOICE IN C. SAUCIA. MATE CHOICE MODELS FOR INSECTS REVEAL THAT IN CERTAIN CASES YOUNG MALES HAVE A SELECTIVE ADVANTAGE OVER OLDER MALES (HANSEN & PRICE, 1995). THE POSSIBLE REASON FOR THIS IS THEIR BETTER GENETIC CONSTITUTION (FEWER MUTATIONS AND LOWER PLIEOTROPIC ACTIVITY OF LATE ACTING GENES; PARTRIDGE & BARTON, 1993; PLETCHER & CURSTINGER, 1998). THE HIGH REPRODUCTIVE OUTPUT OF THE FEMALES MATED WITH 10 TO 30-DAY-OLD MALES MAY INDICATE Males IN THAT AGE RANGE ARE LIKELY TO BE THE PREFERRED MATES. THIS NEEDS TO BE TESTED EXPERIMENTALLY BY AGE-BASED STUDIES ON MATE CHOICE IN WHICH THE FITNESS CONSEQUENCES ARE RECORDED.

Thus, the present study reveals that both male and female age at mating altered the reproductive parameters, with the 20-DAY-OLD FEMALES MATED WITH 10 TO 30-DAY-OLD MALES BEING THE MOST PRODUCTIVE.

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