Emergence and Reproductive Patterns in the Japanese Beetle, Popillia japonica (Coleoptera: Scarabaeidae)

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Emergence and Reproductive Patterns in the Japanese Beetle, Popillia japonica (Coleoptera: Scarabaeidae)

(TITLE)

BY

Steven J. Van Timmeren

THESIS

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Abstract

Sex-biased emergence patterns are common in insects resulting in either protandry (males emerging before females) or protogyny (females emerging before males). The Japanese beetle, *Popillia japonica* Newman, has been shown to exhibit protandry but shows some characteristics of a protogynous species. For example, females mate and oviposit multiple times and this species displays last male sperm advantage. This study investigated the emergence and egg laying patterns of Japanese beetles in east-central Illinois. Although variation existed among study sites none showed protandry and overall they displayed protogyny. Females did not initiate oviposition until several days after emergence and the number of eggs laid remained relatively constant over time. Thus, emergence patterns and reproductive biology suggest Japanese beetles in this population may be protogynous.
Introduction

In insects, the sexes frequently emerge at different times; males may emerge before females (protandry) or females may emerge before males (protogyny). Protandry is, by far, the most commonly observed of these patterns (e.g. Botterweg 1982, Wiklund and Solbreck 1982, Bulmer 1983a, b, Hastings 1989, Baughman 1991, Kleckner et al. 1995, Sawada et al. 1997, Carvalho et al. 1998). Darwin (1871) proposed that protandry was a result of sexual selection, and this remains the most common explanation for this emergence pattern (Wiklund and Fagerström 1977, Fagerström and Wiklund 1982, Nylin et al. 1993, Simmons et al. 1994 Carvalho et al. 1998, Cueva Del Castillo and Núñez-Farfán 1999.). Early emerging males may have access to more females or may be able to establish territories in the best habitats and better defend territories (Wiklund and Fagerström 1977, Bulmer 1983b, Iwasa et al. 1983, Parker and Courtney 1983). Additionally, protandrous females may be able to increase reproduction by increasing their chance of mating with a superior male, if male longevity is correlated with fitness (Wang et al. 1990). In contrast, protogyny may be selected for if females wait several days before oviposition and there is a last male sperm advantage; later emerging males are thought to have the best chance of reproduction in this situation (Thornhill and Alcock 1983).

Protandry and protogyny are consistently associated with several related, but opposing, life history characteristics of the species (Thornhill and Alcock 1983). For instance, protandry is most often seen in species in which females mate only once soon after reaching sexual maturity (Wiklund and Fagerström 1977, Hastings 1989, Wiklund and Forsberg 1991, Zonneveld 1996, Cueva Del Castillo and Núñez-Farfán 1999).
those protandrous species in which females mate with multiple males, virgin females generally are more valuable to a male (Suzuki 1978, Wiklund and Forsberg 1991, Wedell 1992, Zonneveld 1992). For instance, the majority of the female's eggs may be fertilized with sperm from the first male she mates with, or there may be a substantial period of non-receptivity after mating in which the female lays a number of eggs (Thornhill and Alcock 1983). In addition, females of protandrous species may lay a substantial proportion of their eggs relatively early in life (Milne 1960). Finally, for most protandrous species, males and females are reproductively mature upon emergence (Linsley 1959, Botterweg 1978, Baughman 1991).

Protogynous species, in theory, have opposite characteristics for these same traits (Thornhill and Alcock 1983). For example, protogynous females mate and oviposit multiple times throughout their lives (Cotterell 1920, Nielsen and Nielsen 1953, Thornhill and Alcock 1983); consequently, there is no specific advantage for mating with a virgin female. In addition, protogynous species exhibit last male sperm advantage (Jaycox 1967, Thornhill and Alcock 1983).

The Japanese beetle (*Popillia japonica* Newman) has been reported to exhibit protandry. Soon after it’s discovery in North America in 1916 (Dickerson and Weiss 1918), researchers noted that males outnumbered females early in the emergence season (Davis 1920, Hadley and Hawley 1934). More recently, Régnière et al. (1981b) found that males emerged earlier than females over the entire emergence period in North Carolina, and that male Japanese beetles took less time to develop into adults from third instar larvae than females (Régnière et al. 1981a). Some aspects of the Japanese beetle's
biology, however, seem to fit more with protogyny than with protandry, and other relevant characteristics have not been studied. First, research with chemosterilants indicates that Japanese beetles exhibit last male sperm advantage (Ladd 1966, 1970a); this result is supported by the extensive post-copulatory mate guarding by the male (Barrows and Gordh 1978). Second, in contrast to most protandrous species, female Japanese beetles mate multiply throughout their lifetime, with relatively short periods of time between matings (Fleming 1972, Barrows and Gordh 1978). Third, although egg laying patterns are thought to be important in the evaluation of skewed emergence patterns, no information currently exists on egg laying patterns in the Japanese beetle.

This study had three goals: (1) To determine emergence patterns for male and female Japanese beetles in Illinois (2) To determine how soon after emergence females start laying eggs, and (3) To investigate whether females lay a majority of eggs early in life.

Methods

Study Area. This study took place in Coles County, Illinois (N 39°25' W 88°05') on the edges of corn and soybean fields in the summer of 1998. Nine study sites were located on average 1.64±0.44 km apart and consisted of grassy areas alongside corn and soybean fields, with bordering habitats ranging from open fields to forest patches. Four of the nine sites contained small creeks or ponds in the immediate vicinity.

Emergence Patterns. To determine the emergence pattern of Japanese beetles I placed 51 emergence traps on the study sites. Each site had four to ten traps that were either placed over grass on the edges of fields (41 traps) or over soybean plants near the edge of the fields (ten traps). Traps consisted of a wooden frame (1.6 m x 0.65 m, inside
area: 0.85 m²) covered with mesh with an aperture smaller than the beetles so they could not escape. The mesh was suspended 0.5 m above the ground, supported in a tent-like fashion by two PVC tubes (1.3 cm diameter) placed in the ground. Emerging adults would move slowly up the sides of the mesh and crawl around near the apex of the trap, where they could be removed alive by lifting up the trap frame. Traps were checked for beetles between 1200 and 1800 hours daily for the entire emergence period (June-August). Male and female beetles were sexed using differences in the tibial spur and first tarsal segment on the front pair of legs (Smith and Hadley 1926).

Previous studies have measured emergence patterns of males and females by either comparing the central tendency of those patterns (Wiklund et al. 1996, Schneider 1997) or by comparing the cumulative frequency (Hastings 1989, Sawada et al. 1997). I used the Kolmogorov-Smirnov two sample test and a Mann-Whitney U test to compare male and female emergence patterns. The Kolmogorov-Smirnov test compares the cumulative frequencies of the male and female emergence patterns (see Sokal and Rohlff 1981) and is often used to test for protandry (Hastings 1989, Sawada et al. 1997, Cuevo Del Castillo and Núñez-Farfán 1999). The Mann-Whitney U test compares the medians of the male and female emergence patterns. All analyses are corrected for ties where appropriate. In all cases means are presented ± se, and an alpha value of 0.05 is used in all hypothesis testing procedures.

**Reproductive Patterns.** I conducted laboratory mating experiments in order to determine the reproductive patterns of female Japanese beetles. Individual trials involved placing beetles in clear plastic cups (top diameter: 10.8 cm, bottom diameter: 8.0 cm, height: 15.2 cm) with 3 cm of moist sandy loam soil in the bottom in a design similar to
that used by Ladd (1987a). Japanese beetles mate and oviposit readily in containers of this size (Ladd 1966). Sandy loam soil was utilized because of its preference by females as an oviposition material (Régnière et al. 1979, Allsopp et al. 1992). I used sassafras leaves (*Sassafras albidum*) as a food source and climbing substrate; sassafras leaves are a preferred food source of the Japanese beetle (Fleming 1972) and maximize female egg production (Ladd 1987a, b). Containers were placed in an environmental chamber 47 cm under two 34 watt cool white fluorescent light bulbs on a 14:10 photoperiod cycle with a mean temperature of 33.5°C, well within the range of activity of the Japanese beetle and consistent with temperatures in the field (Moore and Cole 1921). Each day between 0900 and 1200 the soil and sassafras leaves in each container were replaced. The oviposition substrate from the old container was then sifted for eggs with a size 18 mesh screen.

Mating experiments consisted of a single male and a single female placed in a cup. Each beetle was either a virgin or non-virgin ("experienced"). Beetles obtained from the emergence traps were considered to be virgin because no mating occurred in the emergence traps. I considered beetles to be sexually "experienced" if a male beetle was found mounted on a female beetle; these beetles were caught in the vicinity of emergence traps. Because it is unclear beetles are sexually mature at emergence (Fleming 1972, Vittum 1986), I used "experienced" beetles to ensure a sexually mature mate. Beetles were placed into experiments on the same day as their capture. The number of replicates varied but averaged ten per treatment.

One important characteristic associated with protandry is that most eggs are laid early in life (Thornhill and Alcock 1983). Many protandrous species lay only one clutch of
eggs (Botterweg 1978, Wiklund and Forsberg 1991), and those species that lay more clutches usually only have two to three clutches with the majority of eggs being laid in the first clutch (Milne 1960). For this reason I allowed each replicate in the mating experiments to proceed for at least ten days and thereafter until the female died. This procedure provided the onset of egg laying and the egg laying patterns for at least the first few clutches.

Mating experiments involved four main combinations of virgin and experienced beetles: virgin male: virgin female (VM, VF), virgin male: experienced female (VM, EF), experienced male: virgin female (EM, VF), experienced male: experienced female (EM, EF). Combinations involving virgin females paired with experienced or virgin males allowed for the determination of the onset of egg laying as well as egg laying patterns over time. Combinations involving experienced females provided comparisons for these treatments.

Two additional treatments helped to examine possible confounding variables. To test for possible laboratory effects, some treatments contained virgin females that were isolated in separate containers for five to ten days before being placed into mating experiments. These treatments are referred to as “delayed” treatments (e.g. EM, VF delayed) and they were conducted to determine if laboratory conditions decreased female egg production over time. Additional treatments, referred to as “clutch” treatments (e.g. EM, VF clutch), controlled for any adverse effects on female egg-laying caused by daily changing of oviposition medium. In these treatments the oviposition medium was only replaced when the female was above ground. This also allowed for the calculation of clutch sizes as well as interclutch intervals.
Because egg fertility may change over a female's lifetime, egg production alone may
not give an accurate representation of a female's reproductive patterns (Goonewardene
and Townshend 1975). All eggs laid by females were counted and placed in covered
petri dishes containing filter paper moistened with deionized water. Eggs were kept
under the same temperature and light conditions as the mating experiments and were
allowed to develop until fertility could be ascertained (Ladd 1966).

I compared reproductive patterns using nonparametric tests, and corrected for ties
where appropriate. I tested egg production over time in two ways. First, I compared the
number of eggs a female laid in the first half of her experiment with the number laid in
the last half. Each trial was considered as starting when a female laid her first eggs; this
eliminated any possible biases caused by individual differences in the onset of egg laying.
Second, I looked for correlations between mean egg production and time across females
using Spearman’s rank correlations. Means are presented as ± se.

Results

Emergence Patterns. Pooled results from emergence trap data suggest that Japanese
beetles in this population may be protogynous. With all sites pooled, females emerged
significantly earlier than males, although this may be due to a single site with a very large
sample size that was significantly protogynous (Fig. 1; Kolmogorov-Smirnov; D=0.153;
P<0.04). Of the five individual sites with sample sizes of at least 20 beetles, one showed
a significant trend toward protogyny and four showed relatively synchronous patterns of
emergence. Both median and cumulative frequency comparisons yielded similar results
(Table 1).
**Egg Laying Patterns.** Females in the mating experiments waited between one and 19 days before the first oviposition, on average waiting generally a little less than one week ($VM, VF$: $6.6\pm1.1$, $N=16$; $VM, EF$: $5.3\pm0.9$, $N=8$; $EM, VF$: $6.3\pm1.3$, $N=10$; $EM, EF$: $5.6\pm1.5$, $N=9$). There was no significant difference in the onset of egg laying among treatments (Kruskal-Wallis; $H=0.52$, $P=0.913$), suggesting both virgin and experienced females were waiting the same amount of time. A comparison between the days till first eggs laid and days till first fertile eggs laid indicated that the fertility of the eggs was not a factor affecting the onset of egg laying (Kruskal-Wallis; $H=1.934$, $P=0.963$). Thus, females could lay eggs as early as one day after the experiment started, but most waited almost a week.

In order to test whether females lay the majority of their eggs early in adult life, I compared the reproductive output of virgin females in the first and last half of their trials with the analysis starting on the day that the first eggs were laid. Egg production in these trials showed no significant trend toward either the first or last half (Wilcoxon; $VM, VF$: 1st half, $15.7\pm2.9$; 2nd half, $14.0\pm3.1$; $T=54.5$, $N=15$, $P=0.755$; $EM, VF$: 1st half, $9.7\pm1.8$; 2nd half, $10.1\pm4.5$; $T=12.5$, $N=10$, $P=0.163$; $EM, VF\text{ clutch}$: 1st half, $12.7\pm2.5$; 2nd half, $9.9\pm2.0$; $T=15$, $N=10$, $P=0.240$). Delayed treatments show the same pattern of egg laying as normal trials, indicating laboratory conditions likely did not adversely affect egg laying (Wilcoxon; $VM, VF, delayed$: 1st half, $12.8\pm3.5$; 2nd half, $14.2\pm2.8$; $T=4.0$, $N=5$, $P=0.715$; $EM, VF, delayed$: 1st half, $17.5\pm3.3$; 2nd half, $12.4\pm2.2$; $T=12$, $N=9$, $P=0.313$). In no case was there any significant indication the beetles were laying more eggs earlier in life. This same lack of trend was present when only considering the fertile eggs as well, indicating egg fertility did not significantly drop during the treatment
Correlational analysis also suggests that females are laying eggs consistently over time. With all females pooled, no significant trend existed toward early egg production (Spearman's Rank Correlation; Day versus Eggs; VM, VF: $r_s=0.030$, $N=15$, $P=0.873$; EM, VF: $r_s=0.125$, $N=9$, $P=0.623$; EM, VF clutch: $r_s=0.021$, $N=10$, $P=0.944$).

Since the time between clutches may affect emergence patterns, I analyzed the interclutch interval for the female Japanese beetles. Female Japanese beetles are waiting, on average, $5.1\pm0.8$ days between clutches. I also determined the mean clutch size for females in the experiments. Females laid, on average, $7.59\pm0.98$ eggs per clutch.

**Discussion**

In contrast to Régnière et al. (1981a, b), Japanese beetles in this study were not protandrous. Overall, the emergence pattern was protogynous, although only one individual site showed a significant trend in that direction. Laboratory mating experiments indicated that females waited approximately one week before onset of oviposition, after which time they started to lay steady numbers of eggs at regular intervals. These results are consistent with either a protogynous or synchronous pattern of emergence.

The delay in onset of oviposition by females, along with the fact that adult beetles are reproductively mature upon emergence and exhibit last male sperm advantage, suggests there is no advantage for males to emerge before females (Ladd 1966, 1970a, Fleming 1972, Régnière et al. 1979). Emerging just before females begin ovipositing would increase a male's chance of being the last male to mate with a female. An important
factor that still needs to be investigated here is the female behavior in the pre-oviposition period and whether they are available to males for mating. In addition, there seems to be no advantage for males to mate with virgin females, since females laid constant numbers of eggs over time and since the Japanese beetle's lifespan is 30 to 40 days (Davis 1920, Fleming 1972). The interclutch interval of around five days appears too short to be a factor that would influence the evolution of protandry. However, because the daily survival rates of individual females have never been investigated in the Japanese beetle, it cannot be ascertained whether the expected future reproduction for the female is small enough to favor protandry. If female daily survival rates are low enough males may preferentially favor virgin females as mates. Determining female survival in the field may be especially difficult because of evidence suggesting extensive dispersal behavior (Régnière et al. 1983).

Interestingly, certain aspects of the Japanese beetle's behavior support a protandrous emergence pattern. Virgin females emit a sex pheromone that is highly attractive to males (Fleming 1972); this results in intense competition among males to mate with virgin females (Smith and Hadley 1926). After a female has mated for the first time she stops producing this sex pheromone, making her less attractive to males (Ladd 1970b, Klein 1981). The presence of such highly sought after virgin females suggests there may be some advantage for males that emerge earlier.

This behavioral biology along with previously documented patterns of emergence conflict with the results of this study. Variations in emergence patterns could be caused by differences in environmental conditions, and may be either short term or long term. Short term differences (e.g. year to year) could be the result of fluctuations in male life
expectancy or duration of the female emergence period (Hastings 1989). Long term variations in emergence patterns may be due to more consistent differences among localities. For example, different geographic locations may have fluctuating levels of larval risk factors (e.g. milky disease; Beard 1945, Sharpe and Detroy 1979) which may vary the relative costs and benefits associated with male and female emergence times (Thornhill and Alcock 1983). Additional research is needed to sort out these observed variations in the biology and emergence patterns. Especially important are investigations on female behavior prior to laying her first clutch of eggs, daily female survival rates, and additional studies of the emergence patterns of males and females in different locations. Such research is needed to fully understand the behavioral ecology of the Japanese beetle, and may lead to insights concerning the control of this resilient pest in the future.
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Table 1. Kolomogorov-Smirnov, Mann-Whitney U, and median emergence day values for Japanese beetles emerging on nine sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>No. of Males</th>
<th>No. of Females</th>
<th>Kolmogorov-Smirnov D-Value</th>
<th>P-Value</th>
<th>Mann-Whitney U-Value</th>
<th>P-Value</th>
<th>Median Emergence Day</th>
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<tr>
<td>1</td>
<td>26</td>
<td>15</td>
<td>0.113</td>
<td>0.999</td>
<td>204.5</td>
<td>0.797</td>
<td>13</td>
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<tr>
<td>2</td>
<td>13</td>
<td>7</td>
<td>0.319</td>
<td>0.794</td>
<td>56.5</td>
<td>0.374</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
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</tr>
<tr>
<td>4</td>
<td>16</td>
<td>9</td>
<td>0.188</td>
<td>0.999</td>
<td>73.0</td>
<td>0.955</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
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<tr>
<td>7</td>
<td>99</td>
<td>82</td>
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<td>0.0538</td>
<td>4894.0</td>
<td>0.0172</td>
<td>29</td>
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<td>0.227</td>
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<td>0.211</td>
<td>16</td>
</tr>
<tr>
<td>9</td>
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<td>10</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
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<td>155</td>
<td>0.153</td>
<td>0.0390</td>
<td>16056.5</td>
<td>0.0455</td>
<td>27</td>
</tr>
</tbody>
</table>

- Sample sizes too small to achieve significance at alpha = 0.05.

- Emergence day values indicated by days after first recorded beetle emergence
Fig. 1. The number of male and female Japanese beetles caught in emergence traps on all sites during each week of the emergence season.