

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

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**ABSTRACT:** Sex-biased emergence patterns are common in insects, resulting in either protandry (males emerging before females) or more rarely protogyny (females emerging before males). Previous studies have suggested that the Japanese beetle, *Popillia japonica* Newman, was protandrous; interestingly, however, they exhibit some characteristics of a protogynous species. For example, Japanese beetles exhibit a last male sperm advantage and females mate and oviposit multiple times. This study investigated the emergence and egg laying patterns of Japanese beetles in east-central Illinois. For both sexes, the size of emerging individuals tended to decline as the season progressed. Overall, female emergence was skewed significantly earlier than that of males. Females did not initiate oviposition until several days after emergence, and the number of eggs laid remained relatively constant over time. Thus, in this population, emergence patterns and reproductive biology suggest that Japanese beetles are not protandrous and may be protogynous.

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 two 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, selection may favor protogyny 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). In those protandrous species in which females mate with multiple males, virgin females generally have a higher reproductive value to males (Suzuki, 1978; Wiklund and Forsberg, 1991; Wedell, 1992; Zonneveld, 1992). For example, 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 during which the female lays a number of eggs (Thornhill and Alcock, 1983). In addition,

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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 its 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, field studies have found that males emerged earlier than females over the entire emergence period in North Carolina; laboratory studies have demonstrated that male Japanese beetles take less time to develop into adults from larvae than females (Goonewardene et al., 1973; Régnière et al., 1981a, b). 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 multiple times 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, little information currently exists about egg laying patterns in the Japanese beetle.

This study had three goals: (1) To determine emergence patterns for male and female Japanese beetles in east-central 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.

#### Materials and Methods

**STUDY AREA FOR EMERGENCE PATTERNS:** This study took place in Southeastern Coles County, in east-central Illinois (39°25'N, 88°05'W) in the summer of 1998. To determine the emergence pattern of Japanese beetles we placed 51 emergence traps over grass at the edges of fields (41 traps) or over soybean plants near the edge of the fields (10 traps). Bordering habitats consisted of corn fields, soybean fields, pasture or forest patches; some areas had small creeks or ponds in the vicinity.

Traps consisted of a wooden frame (1.6 m × 0.65 m, inside area: 0.85 m<sup>2</sup>) covered with identical mesh with an aperture size smaller than the beetles so they could not escape. The total area of ground covered by our emergence traps exceeded that of previous studies on the species (cf. Morrill and Dobson, 1978; Régnière et al., 1981a). 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). Beetle gender was determined by 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 either by 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). We used the Kolmogorov-Smirnov two sample test (Sokal and Rohlf, 1981) and the Mann-Whitney *U*-test (Zar, 1999) to compare male and female emergence patterns. The Kolmogorov-Smirnov test compares the cumulative frequencies of the male and female emergence patterns 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.

Using a dissecting scope with an ocular micrometer, we recorded the size of the emerged beetles as the maximum body width. We had previously determined that maximum body width was positively and highly correlated with 6 other morphological measures for both males and females ( $n = 50$  of each sex; dry mass, face width, elytra length, middle left tarsus, pronotum width, and ventral spur; all  $r > 0.58$  with a mean  $\pm$  SE =  $0.74 \pm 0.03$ ; all Fisher's  $r$  to  $z$   $P < 0.0001$ ).

Analyses were corrected for ties where appropriate. Means are presented  $\pm$  SE, and an alpha value of 0.05 was used in all hypothesis testing procedures.

**REPRODUCTIVE PATTERNS:** We 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). We used sassafras leaves (*Sassafras albidum* (Nutt.)) 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; two lines of evidence strongly suggest that mating did not occur in the emergence traps. First, on 209/244 (86%) of the occasions that a trap caught an emerging beetle, either only a single beetle was captured or multiple beetles were of the same sex. Second, for those traps that simultaneously captured individuals of different sexes, mating was still extremely unlikely. Although enough time may have elapsed between emergence and collection for copulation to take place, we never observed males copulating or mate guarding in the

traps, even though males mate guard for many hours after copulation (Barrows and Gordh, 1978; Kruse and Switzer, unpubl. data). Lack of mating may have been at least partially due to the interior conditions of the traps (e.g., relatively dark) were not conducive to normal activity of Japanese beetles (e.g., Moore and Cole, 1921). We considered beetles to be sexually "experienced" if a male beetle was found mounted on a female; these individuals were caught in the vicinity of emergence traps. Because it's unclear whether beetles are sexually mature at emergence (Fleming, 1972; Vittum, 1986), we used experienced beetles to try 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 we allowed each replicate in the mating experiments to proceed for at least ten days and thereafter until the female died. This procedure provided information on 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: (1) virgin male: virgin female (VM, VF), (2) virgin male: experienced female (VM, EF), (3) experienced male: virgin female (EM, VF), and (4) 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. Clutch treatments also allowed for the calculation of clutch sizes as well as inter-clutch 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).

We compared reproductive patterns using nonparametric tests (Zar, 1999), and corrected for ties where appropriate. We tested egg production over time in two ways. First, we compared the number of eggs a female laid in the first half of her trial 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 differ-

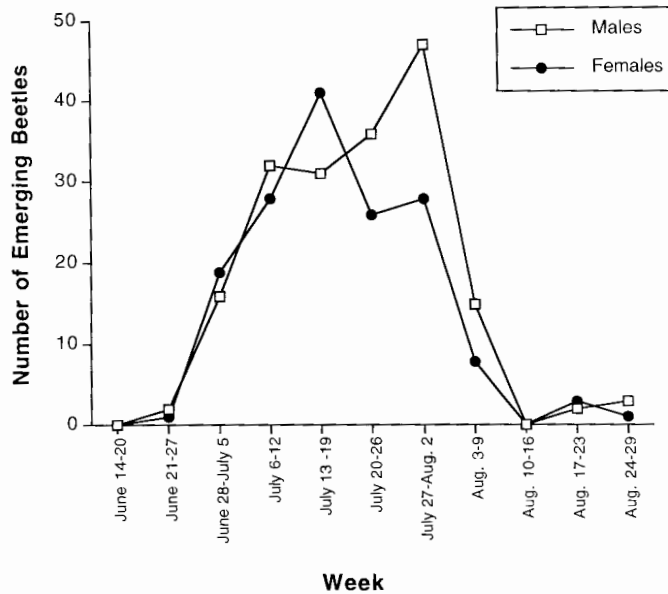


Fig. 1. The number of male and female Japanese beetles caught in emergence traps during each week of the emergence season.

ences in the onset of egg laying. Second, we looked for correlations between mean egg production and time using Spearman's rank correlations. Means are presented as  $\pm$  SE.

### Results

**EMERGENCE PATTERNS:** We captured a total of 339 emerging beetles (184 males, 155 females). Although the number of males and females emerging was similar for the first few weeks of emergence (Fig. 1), when considering the entire emergence period, the median emergence day (i.e., days after the first beetle emerged) for females (day 22) was earlier than that of males (day 27). This skew towards earlier female emergence was significant when comparing both the cumulative frequency (Kolmogorov-Smirnov,  $D = 0.153$ ,  $P = 0.039$ ) and the median (Mann-Whitney,  $U = 16,056.5$ ;  $P = 0.045$ ) of the distribution. Thus, the Japanese beetles were protogynous during this study.

Male beetles were significantly smaller than females (Males:  $5.62 \pm 0.02$  mm,  $n = 178$ ; Females:  $6.09 \pm 0.02$  mm,  $n = 152$ ; Mann-Whitney Test,  $U = 2920$ ,  $P < 0.0001$ ). Size of both male and female beetles decreased over the emergence period (Fig. 2), although the trend was only significant for males (Spearman; Males:  $r_s = -0.26$ ,  $P = 0.0007$ ; Females:  $r_s = -0.13$ ;  $P = 0.10$ ).

**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:  $6.6 \pm 1.1$  days,  $N = 16$ ; VM, EF:  $5.3 \pm 0.9$ ,  $N = 8$ ; EM, VF:  $6.3 \pm 1.3$ ,  $N = 10$ ; EM, EF:  $5.6 \pm 1.5$ ,  $N = 9$ ). 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

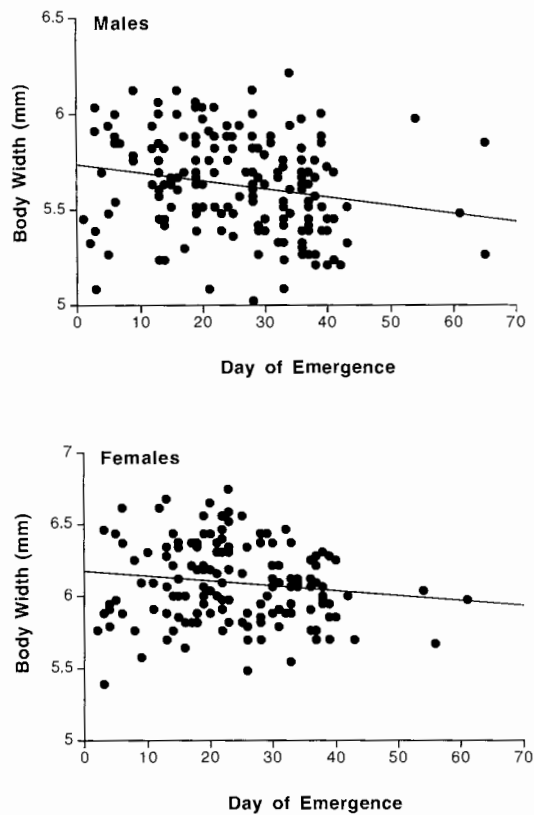


Fig. 2. Sizes of male ( $N = 178$ ) and female ( $N = 152$ ) beetles over the emergence period. Day 1 of the emergence period was June 15. Lines represent simple linear fits to the data and are for illustrating the general trend.

time. A similar analysis of onset of oviposition involving only those eggs that were fertile yielded a similar lack of difference among treatments (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, we 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,  $15.7 \pm 2.9$ ; 2nd half,  $14.0 \pm 3.1$ ;  $T = 54.5$ ,  $n = 15$ ,  $P = 0.755$ ; EM, VF: 1st half,  $9.7 \pm 1.8$ ; 2nd half,  $10.1 \pm 4.5$ ;  $T = 12.5$ ,  $n = 10$ ,  $P = 0.163$ ; EM, VF clutch: 1st half,  $12.7 \pm 2.5$ ; 2nd half,  $9.9 \pm 2.0$ ;  $T = 15$ ,  $n = 10$ ,  $P = 0.240$ ). 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,  $12.8 \pm 3.5$ ; 2nd half,  $14.2 \pm 2.8$ ;  $T = 4.0$ ,  $n = 5$ ,  $P = 0.715$ ; EM, VF delayed: 1st half,  $17.5 \pm 3.3$ ; 2nd half,  $12.4 \pm 2.2$ ;  $T = 12$ ,  $n = 9$ ,  $P = 0.313$ ). 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 period.

Correlational analysis also suggests that females are laying eggs consistently over time. No significant trend existed toward early egg production (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:  $r_s = 0.021$ ,  $n = 10$ ,  $P = 0.944$ ).

Because the time between clutches may affect emergence patterns, we analyzed the interclutch interval for the females. Females are waiting, on average,  $5.1 \pm 0.8$  days between clutches with an average clutch size of  $7.59 \pm 0.98$  eggs ( $n = 12$ ).

### Discussion

Surprisingly, in comparison to previous studies, the Japanese beetles in this study were not protandrous; in fact, the overall emergence pattern was significantly protogynous in the year of this study. Laboratory mating experiments indicated that females waited approximately one week before the onset of oviposition, after which time they started to lay varying number of eggs at an average interval of approximately 5 days. Females did not lay more eggs early in life. These results are more consistent with protogyny or at least synchrony rather than protandry.

Female Japanese beetles lay on average 40–60 eggs over a one month adult lifespan (Davis, 1920; Smith and Hadley, 1926; Hadley and Hawley, 1934). In this study, we found that this egg production was not concentrated early in a female's life, as is normally associated with a protandrous emergence pattern (Thornhill and Alcock, 1983). Furthermore, females began to lay eggs approximately 6 days after emergence. Thus, males emerging after females might be at an evolutionary advantage by emerging when females are ready to oviposit, especially because Japanese beetles have last male advantage (Ladd, 1966, 1970a; Thornhill and Alcock, 1983).

This argument for protogyny partially rests on our observation that the onset of oviposition is delayed. However, our trials involving experienced females, who presumably were reproductively mature, also exhibited a delay in oviposition (although this delay was slightly less than that shown by virgin females). These results may indicate that laboratory conditions are responsible for these delays in oviposition. Alternatively, because we do not know the reproductive histories of our experienced females, these females either may have been recently emerged or have recently oviposited. In support of this latter possibility, we found that the interclutch interval was just over 5 days, which is very similar to the initial delay that we observed in experienced females. In any case, even if females did not naturally delay oviposition, our results would support a synchronous emergence pattern, rather than protandry.

Interestingly, despite all the evidence for a protogynous or synchronous emergence pattern, certain aspects of the Japanese beetle's behavior do seem to support protandry. For instance, virgin females emit a sex pheromone after emergence that is highly attractive to males and that results in intense male-male competition (Smith and Hadley, 1926; Fleming, 1972). After a female has mated, she stops producing this pheromone, making her less attractive to males (Ladd, 1970b; Klein, 1981). This pattern of pheromone production and male preference suggests that males may gain some advantage from mating with a virgin female, as would be predicted by protandry (Thornhill and Alcock, 1983). Conversely, because females can fertilize multiple clutches after mating with only one male (Ladd, 1987a), perhaps this be-

havior is driven by advantages to the female and has nothing to do with the evolution of emergence patterns.

Thus, the results of our study (i.e., no protandry) are inconsistent with both previously documented patterns of temporal differences in presence or emergence between the sexes (Davis, 1920; Hadley and Hawley, 1934; Goonewardene et al., 1973; Régnière et al., 1981a, b; Vittum, 1986) and possibly some aspects of Japanese beetle behavior. Some of these previous studies (e.g., Davis, 1920; Hadley and Hawley, 1934; Vittum, 1986) captured free-living adults, and in some cases used pheromone traps to do so. The fact that these studies were not capturing adults at emergence may have obscured the true emergence pattern. Laboratory studies of development time (e.g., Goonewardene et al., 1973; Régnière et al., 1981b) and studies capturing emerging beetles in the field (e.g., Régnière et al., 1981a) are more difficult to dismiss, but several possible explanations exist for the inconsistencies between those studies and our results. First, our sample size of emerging beetles may have been too small. However, our number of emerging beetles was similar to that of other studies on Japanese beetle emergence (e.g., Régnière et al., 1981a) and the difference we found between female and male emergence was statistically, if not biologically, significant. Second, the inconsistencies could be caused by variation in environmental conditions and may reflect either short or long term differences within and between localities. For example, male life expectancy or the duration of the female emergence period may vary from year to year due to factors such as temperature or precipitation (Hastings, 1989). In support of this hypothesis, evidence suggests that temperature and precipitation may affect the emergence of Japanese beetles (Morrill and Dobson, 1978; Régnière et al., 1979, 1981b). For instance, in their study of Japanese beetle development times, Régnière et al. (1981b) found that the magnitude of the difference between males and females depended upon temperature. Multi-year studies in the same location could test for this possibility. Additionally, specific geographic locations may have different levels of larval risk factors (e.g., milky spore 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). To test this hypothesis one would need a large scale study that samples a number of sites both for emerging beetles and for potential larval pathogens and predators.

An additional pattern in our results was that early emerging males tended to be larger than males emerging later; this same trend was present in females but was not significant. This seasonal pattern in size has been found in a wide variety of insects, including other Coleoptera as well as Diptera, Ephemeroptera, Hymenoptera, and Odonata (e.g., Packer and Corbet, 1989; Ohgushi, 1996; Alcock, 1997; Thompson, 1997; Watanabe et al., 1998). The usual explanations for this size pattern involve relationships between decreasing food availability, temperature differences, developmental rate, and/or the timing of diapause (e.g., Kleckner et al., 1995; Ohgushi, 1996; Thompson, 1997). To the extent that size governs competitive ability or fecundity, this seasonal trend may lead to differences in reproductive success among individuals emerging at different times (Packer and Corbet, 1989; Alcock, 1997; Thompson, 1997). How these factors may relate to the seasonal decrease in size in Japanese beetles is unknown. However, because temperature does affect the rate of larval development and the size of adult Japanese beetles (Ludwig, 1928, 1932), and because individuals may initiate diapause in different instars (Fleming, 1972), temperature and



the timing of diapause, at least, may play a role in the seasonal trend. Overall, whatever the explanation for this trend, date of emergence did not explain much of the variance in size.

Clearly, the available evidence from this and other studies on the emergence patterns of Japanese beetles is mixed. We demonstrated that Japanese beetles do not fit the typical protandry model. In fact, we found that emergence in this population this year was significantly protogynous, and additional aspects of their biology support either protogyny or synchrony. Additional research is needed to more fully understand the observed relationships between Japanese beetle biology and emergence patterns. Especially important will be investigations on female behavior prior to laying the first clutch of eggs and studies relating environmental conditions to emergence patterns both in the same location and among geographically separate locations. Such information on the behavioral ecology of the Japanese beetle should lead to insights concerning the control of this resilient insect pest.

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