

# Group Living Accelerates Bed Bug (Hemiptera: Cimicidae) Development

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**ABSTRACT** For many insect species, group living provides physiological and behavioral benefits, including faster development. Bed bugs (*Cimex lectularius* L.) live in aggregations composed of eggs, nymphs, and adults of various ages. Our aim was to determine whether bed bug nymphs reared in groups develop faster than solitary nymphs. We reared first instars either in isolation or in groups from hatching to adult emergence and recorded their development time. In addition, we investigated the effects of group housing on same-age nymphs versus nymphs reared with adults. Nymphal development was 2.2 d faster in grouped nymphs than in solitary-housed nymphs, representing 7.3% faster overall development. However, this grouping effect did not appear to be influenced by group composition. Thus, similar to other gregarious insect species, nymph development in bed bugs is faster in aggregations than in isolation.

**KEY WORDS** aggregation, bed bug, development, social interaction, Cimicidae

Bed bugs live in aggregations in cracks and crevices in proximity to their hosts. These aggregations are composed of individuals of different sexes, life stages, and feeding and mating status (Usinger 1966, Reinhardt and Siva-Jothy 2007).

Group living may provide benefits, including a more favorable microenvironment (e.g., higher humidity and group thermoregulation), access to symbiotic microbes, lower risk of predation, and more encounters with mates (Krause and Ruxton 2002). Group living with or without social interactions can also result in social facilitation or “group effect,” a form of phenotypic plasticity resulting in morphological, behavioral, and physiological changes that may benefit individuals within the aggregation. An example of group effect includes faster development in cockroaches (Wharton et al. 1968, Izutsu et al. 1970), crickets (McFarlane 1962), and desert locusts (Norris 1954), where juveniles reared in groups grow faster and reach the adult stage sooner than those insects reared in isolation. In cockroaches, adult females and males show faster sexual maturation in aggregations (Gadot et al. 1989; Holbrook et al. 2000; Uzsák and Schal 2012, 2013). Experimental evidence for adaptive benefits of group living in bed bugs is lacking.

We investigated the effects of grouping and isolation on the development rate of bed bug nymphs. We hypothesized that development time in *Cimex lectularius* L. nymphs would be affected by social interac-

tions, with grouped nymphs developing faster than isolated nymphs.

## Materials and Methods

**Insects.** We used bed bugs from a *C. lectularius* colony that originated from an infested apartment in Jersey City, NJ, and was in culture in the laboratory for 3 yr. Insects were maintained in an incubator at  $27 \pm 0.5^\circ\text{C}$ ,  $50 \pm 10\%$  RH, and a photoperiod of 12:12 (L:D) h. Mated adult females were fed, separated, and allowed to oviposit. Newly hatched first instars were immediately removed and used in experiments.

**In Vitro Feeding System.** Insects were fed using an artificial membrane feeding system, which consisted of custom-built water-jacketed glass feeders connected to a thermal circulator water bath (B. Braun Biotech, Inc., Allentown, PA) that was maintained at  $37^\circ\text{C}$ . Each feeder could hold up to 4 ml of blood, which was retained by a membrane stretched across the bottom of the feeder (NESCOFILM, Karlan, Cottonwood, AZ). Bed bugs were fed defibrinated rabbit blood (Quad Five, Ryegate, MT). Although bed bugs do not require frequent feeding, we offered them blood three times per week throughout the experiments to ensure that they had ready access to a blood meal.

**Grouping and Isolation of *C. lectularius* Nymphs.** One or five newly hatched first instars were placed in 1-ml polypropylene cages, which were constructed by cutting 5-ml polypropylene low temperature freezer vials (VWR International, Radnor, PA) to 1-ml volume (17.5 mm in height by 13.0 mm in diameter), and by heat-sealing both ends with plankton netting fabric (0.3-mm mesh opening, 0.2-mm fabric thickness; Bio-

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Quip Products, Inc., Compton, CA) through which bed bugs could feed. A piece of twice-folded manila folder cardboard (17 by 12 mm) was inserted into each cage so bed bugs could crawl up to the top of the cage and feed. Total nymph development time by sex was recorded when nymphs reached the adult stage.

**Effects of Group Composition.** Newly hatched first instars were reared either in a group of 50 or in a group of 50 first instars with 10 additional adult males. We used adult males rather than females to avoid the confounding effects of eggs (and ultimately more nymphs) oviposited by the females. Bed bugs were placed in plastic cages that were constructed from 5-by 5-cm clear polystyrene wide-mouth threaded round jars (Consolidated Plastics Company, Inc., Stow, OH). The base of the jar was removed and replaced with plankton netting. Two pieces of quadruple-folded manila cardboard (6.6 by 4.4 cm) were inserted in the cage. We recorded development time by sex when the nymphs reached the adult stage.

**Statistical Analyses.** Data were analyzed with an unpaired Student *t*-test by using SAS 9.1.3 software (SAS Institute Inc. 2002–2003, Cary, NC). Significance level for rejecting the null hypothesis was set at  $\alpha = 0.05$ . Variation around the mean is represented by the standard error of the mean (SEM).

## Results

**Effects of Grouping and Isolation on Development Time of *C. lectularius* Nymphs.** Grouped bed bugs reached the adult stage significantly faster ( $27.5 \pm 0.33$  d;  $P = 0.0002$ ) than bed bugs reared in isolation ( $29.6 \pm 0.45$  d; Fig. 1A). No significant differences in development time were observed between males and females in the two treatments. We observed lower mortality in grouped nymphs (6%) than in solitary nymphs (18%), but this difference was not significant ( $P = 0.06$ ).

**Effect of Group Composition on Nymph Development Time.** Nymphs reared in a group of 50 similarly-aged bed bugs did not develop faster ( $29.1 \pm 0.29$  d;  $P = 0.51$ ) than 50 nymphs grouped with 10 adult males ( $28.8 \pm 0.42$  d; Fig. 1B). As in the previous experiment, we did not observe significant differences in development time between males and females in either treatment. Mortality was higher in nymphs grouped with adults (20%) than in nymphs grouped with similarly-aged nymphs (12%), but this difference was not significant ( $P = 0.51$ ). In both experiments, most of the mortality was observed within the first 2 wk of the experiment during early instar development.

## Discussion

This is the first study to document the effects of aggregation on bed bug development. Our results showed that grouped nymphs developed 2.2 d faster than solitary nymphs, a significant 7.3% difference (Fig. 1A). This socially mediated difference in development rate is comparable with differences observed in other species. For example, McFarlane (1962)

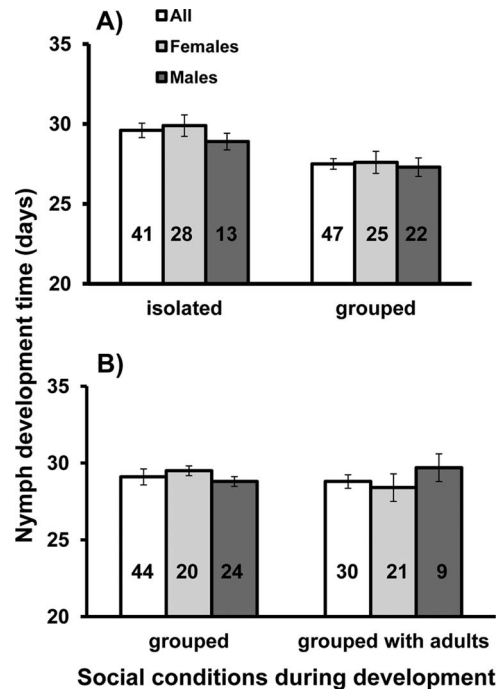


Fig. 1. (A) Effects of isolation and grouping on nymph development time. Mean ( $\pm$ SE) nymph development time for isolated and grouped bed bugs. The difference between the two treatments was significant ( $P = 0.0002$ ; *t*-test). (B) Effects of grouping on development time of 50 same-age grouped nymphs and 50 nymphs grouped with 10 adult males. The difference between these two treatments was not significant ( $P = 0.51$ ; *t*-test). Numbers of individuals per treatment are shown inside the bars.

found that grouped male nymphs of the house cricket [*Acheta domesticus* (L.)] developed 11.1% (4.4 d) faster than isolated males, and grouped female nymphs developed 8.0% (3.0 d) faster than females reared in isolation. Similarly, grouped nymphs of the German cockroach (*Blattella germanica* L.) developed 11.9% (7.8 d) faster than isolated nymphs (Izutsu et al. 1970).

The presence of adult males did not affect the development of grouped bed bug nymphs. These results suggest two important features of bed bug aggregations: First, it appears that the effects of grouping are the same regardless of the age of the individuals in the group, as shown previously for German cockroach nymphs grouped with either same age or older nymphs (Izutsu et al. 1970). Second, our results suggest that newly hatched bed bugs do not require interaction with older, fed bed bugs to achieve maximal developmental rates, implying that development might be independent of horizontally transmitted symbiotic microbes.

Gregariousness may have evolved in bed bugs for protection from natural enemies, facilitating mate-finding, horizontal transfer of symbionts, nutrients, or both, and for protection from unfavorable environmental conditions such as low humidity (Benoit et al. 2007, Pinto et al. 2007). The general absence of pred-

ators in the indoor environment, coupled with obvious advantages to cursorial predators (e.g., spiders) when encountering prey aggregations, make the predator avoidance argument unlikely. The value of aggregations for mate-finding is an attractive hypothesis because bed bugs are wingless and do not appear to use long-range sexual communication. However, the presence of nymphs in bed bug aggregations makes a purely sexual function unlikely. Aggregation, however, seems to be important for the maintenance of a moist microclimate in harborages because all bed bug life stages are sensitive to desiccation, especially during molting (Usinger 1966, Benoit et al. 2007).

Here, we report that a second important benefit of grouping in bed bugs is significantly faster development. As hypothesized for the German cockroach (Uzsák and Schal 2012), this life history trait might be particularly adaptive for a colonizer species with obligatory sexual reproduction. In aggregations, nymphs should maximize their development rate to rapidly reach the reproductive adult stage. Recent evidence suggests that infestations start from small, genetically depauperate propagules (Booth et al. 2012, Saenz et al. 2012). When a solitary male or female nymph is the sole colonizer, it would be maladaptive to rapidly reach the adult stage without the prospect of successful reproduction. It is plausible, therefore, that social facilitation of development evolved in certain insect species—particularly in pest species—in response to periodic population bottlenecks and frequent colonization events in a highly fragmented environment.

Many aspects of this fascinating system remain to be investigated, including whether there are particular stages in nymph development that are affected more or less by grouping, whether aggregation influences reproduction and survivorship of adult bed bugs, the sensory cues that trigger faster development in grouped bed bugs and their coupling to neuroendocrine mechanisms that accelerate development, and other fitness advantages conferred in aggregations. It is worth noting, in particular, that the sensory cues that facilitate faster development are not known. If volatile pheromones are involved, we might have underestimated the effect of grouping because both solitary and grouped nymphs were maintained in the same incubator.

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