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Females Remate for Sperm Replenishment in a Seed Bug: Evidence from Offspring Viability

Qiao Wang[1,2] and Lorraine K. Davis[1]

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Females of many insects mate multiply but why they do so remains controversial. Here we investigated the effects of multiple matings on female reproductive success of a New Zealand seed bug, Nysius huttoni. *We found little evidence for females to gain material (nutritional) benefits through multiple matings because the number of matings did not have significant effect on female fecundity. Females remated to the same males or different males produced similar number of viable offspring, suggesting that females do not obtain genetic benefit from remating in terms of offspring viability. With the increase of the number of matings, however, overall fertility rate significantly increased and daily fertility rate declined significantly slower over time. These results suggest that females remate for the replenishment of sperm. Five matings are sufficient for females to maximize their reproductive success, and additional matings appear to be superfluous. However, the females of this bug mate as many as 68 times if males and females are paired for lifetime. This can be explained by the convenience hypothesis, i.e., females remate superfluously to minimize the costs of harassment by promiscuous males.*

KEY WORDS: Lygaeidae; female remating; sperm replenishment; fecundity; fertility.

INTRODUCTION

Many insects are polyandrous but why females mate multiply still remains controversial. Several hypotheses have been proposed to explain the

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[1] Entomology and IPM Laboratory, Institute of Natural Resources, Massey University, Private Bag 11222, Palmerston North, New Zealand.
[2] To whom correspondence should be addressed. Email: Q.Wang@massey.ac.nz.

benefits of female remating: (1) genetic benefits—females replace sperm of previous mate with sperm from a genetically superior mate, encourage competition between sperm to ensure fertilization by sperm of high quality, ensure genetic diversity, or avoid genetic incompatibility (Madsen *et al.*, 1992; Zeh and Zeh, 1996; Newcomer *et al.*, 1999; Tregenza and Wedell, 2002; Jennions *et al.*, 2004); (2) sperm replenishment-–females may not obtain sufficient sperm to fertilize the full load of their eggs during their life time from only one mating, or sperm they obtain may degrade during long-time storage (Thornhill and Alcock, 1983; Ridley, 1988; Sheldon, 1994); (3) material benefits-–females remate to acquire male derivate nutrients for higher fecundity (Gwynne, 1984; Wiklund *et al.*, 1993; Wang and Millar, 1997; Wilson *et al.*, 1999; Jiménez-Pérez *et al.*, 2003); and (4) convenience-–females remate to minimize the costs of harassment from males (Thornhill and Alcock, 1983; Parker, 1984; Rowe, 1992). However, these hypotheses need not be mutually exclusive (Simmons, 2001).

Nysius huttoni is a polyandrous seed bug from New Zealand, both sexes of which can mate frequently with the same mates or different mates (Eyles, 1965; QW, unpublished data). Mean mating duration is about 2 h and permanently paired bugs in the laboratory can mate an average of 68 times in their lifetime (Wang and Shi, 2004). However, these bugs are not gregarious in the reproduction season in the field (QW, unpublished data). Therefore, the very high number of matings recorded in the laboratory is less likely to occur in the field. Here we carried out a series of mating treatments and monitored female reproductive outputs to determine what benefits *N. huttoni* females might obtain from remating. If female remating is for genetic benefits, then females that mate with different partners are expected to produce more viable offspring than those that remate with the same partners (Tregenza and Wedell, 1998; Jennions and Petrie, 2000). Otherwise, females might remate for other purposes, such as to gain more nutrients and/or sperm.

In the present study, we attempted to determine whether diverse partners or mating frequencies played the major role in maximizing female reproductive success in *N. huttoni*.

MATERIALS AND METHODS

Insects

Insects were collected from twin cress (*Coronopus didymus* (L.) Sm.) on the campus of Massey University, Palmerston North, New Zealand during January (143 adults and 376 nymphs) and mass-reared in a transparent plastic cylinder (25 cm high by 21 cm diameter) with a 15 cm gauze covered hole in the lid for ventilation, at $20 \pm 1^\circ$C, $75 \pm 10\%$ RH, with a photoperiod of 16:8 h (light: dark) (lighting provided with high frequency broad-spectrum biolux tubes, Osram, Germany). Two 6.5 cm medical cotton wool wicks were provided as oviposition sites. *N. huttoni* needs an average of 80 d to complete its lifecycle under these conditions (He *et al.*, 2003). We reared these bugs for 80 d before experiments. Food provided for the colony and following experiments consisted of sunflower seeds and stems of twin cress and was replaced every day.

All experiments were carried out under the above conditions. For the experiments we sexed the 5th instar nymphs according to Eyles (1963a) and reared them individually in glass vials (2.5 cm diameter × 8.0 cm high) with a 1.5 cm diameter mesh-covered hole in lids. The date of emergence was recorded for each insect. Because both sexes became sexually mature about 10 d after emergence (He *et al.*, 2003), 13-d-old adults were used for experiments.

Mating and Reproductive Output

To determine whether the number of times females mated and whether remating with the same and different males affected their reproductive outputs, we set up five female treatments: (1) mated once (M-1), (2) mated twice with the same males (SM-2), (3) mated twice with different males (DM-2), (4) mated five times with the same males (SM-5), and (5) permanently paired with the same males (SM-P). For each of treatments (1) to (3), 45 females were used, and for each of the remaining treatments, 22 females were employed. Each female was reared and mated in a glass vial as mentioned above. First mating was allowed when both sexes were 13-d-old. For treatments (1) to (4), males were removed after mating was completed but for treatment (5) females were paired with the same males until death. In the SM-2 treatment, the once-mated sexes were reared separately for 24 h, after which time females were allowed to mate again with the same males, and then females were reared individually until death. For the DM-2 treatment, once-mated females were maintained for 24 h, after which time they were allowed to mate with 14-d-old males that mated once 24 h ago with other females, and then females were reared individually until death. In the SM-5 treatment, females were allowed to mate with the same males five times at 24-h intervals, and then reared individually until death.

A 2.5 cm medical cotton wood bud was provided as an oviposition substrate for each female 24 h after the first mating, and then replaced daily. Eggs laid on each bud were counted and incubated in a Petri dish (1.2 by

8.8 cm) lined with filter paper. Egg hatch was checked and recorded daily. This allowed us to obtain data of overall and daily reproductive outputs for each female.

Statistical Analysis

Mean number of eggs laid (fecundity) and mean hatch rate (fertility rate) in different treatments were analyzed using an analysis of variance (ANOVA). Means were separated using a Tukey's studentized range test. Mean daily fertility rate was analyzed using an analysis of regression (AOR). The slopes of regression lines were then analyzed using an analysis of covariance (ANCOVA). Means were separated using a least squares means test. Because over 90% of females died 35 d after the first oviposition, daily fertility rate data for the first 34 d of the oviposition period were included in the analysis. Overall and daily fertility rate data were arcsin transformed and normalized prior to ANOVA or ANCOVA. Rejection level was set at $P > 0.05$. All analyses were made using SAS (SAS Institute 1996).

RESULTS

Results show that the total number of eggs laid increased slightly with the number of times *N. huttoni* females had mated (Fig. 1). However, there was no significant difference between treatments (ANOVA: $F = 2.25$; df = 4, 174; $P = 0.066$).

The proportion of full egg load fertilized in the female lifetime (fertility rate) highly depended on the number of matings in this species (ANOVA: $F = 61.01$; df = 4, 174; $P < 0.0001$) (Fig. 2). Once mated (M-1) females had significantly lower fertility rate than females twice-mated to the same males (SM-2) ($P = 0.034$) or to the different males (DM-2) ($P < 0.001$). Although the fertility rate in DM-2 was slightly higher than in SM-2, the difference was not significant between these two treatments ($P = 0.095$). Females mated five times to the same males (SM-5) and paired with the same males for life (SM-P) had significantly higher fertility rate than SM-2 and DM-2 females ($P < 0.0001$). No significant difference in fertility rate was found between SM-5 and SM-P treatments ($P = 0.393$).

In all treatments, daily fertility rates significantly declined over time (Fig. 3A–E). However, the declining rates (slopes) were significantly different between treatments (ANCOVA: $F = 128.68$; df = 3, 164; $P < 0.0001$). Daily fertility rate in M-1 females declined significantly faster than in SM-2 and DM-2 females ($P < 0.0001$) (Fig. 3A–C). There was no signif-

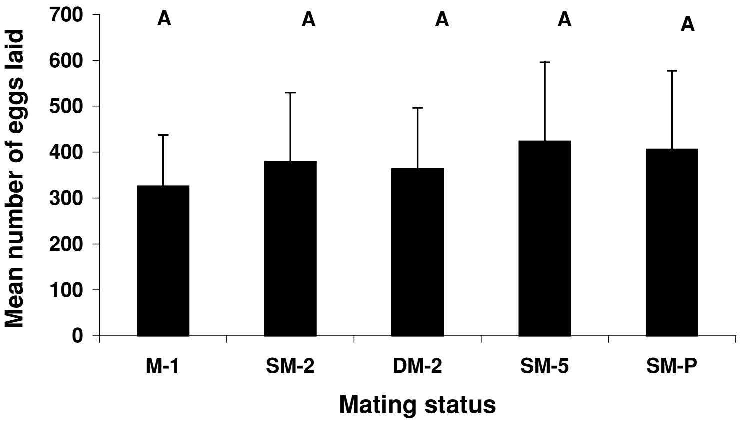


Fig. 1. Mean fecundity of females with different mating status (M-1, females mated once; SM-2, females mated twice to the same males; DM-2, females mated twice to different males; SM-5, females mated five times to the same males; SM-P, females and males paired for life). Bars are SE. Bars with the same letter are not significantly different (ANOVA, $P > 0.05$).

icant difference in regression slopes between SM-2 and DM-2 treatments ($P > 0.05$) (Fig. 3B and C). Furthermore, in twice-mated treatments daily fertility rates declined significantly faster than in SM-5 and SM-P treat-

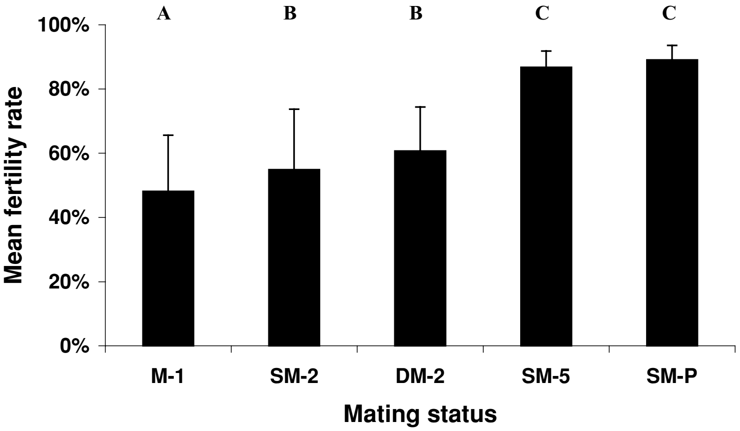


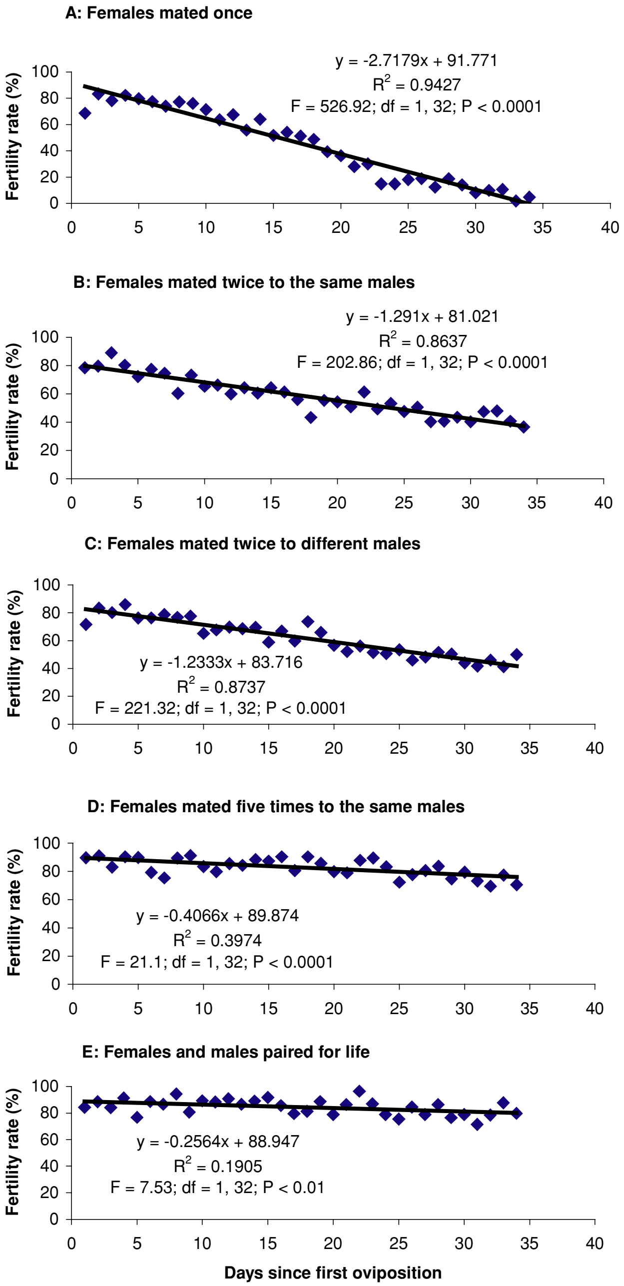
Fig. 2. Mean fertility rate of females with different mating status (M-1, females mated once; SM-2, females mated twice to the same males; DM-2, females mated twice to different males; SM-5, females mated five times to the same males; SM-P, females and males paired for life). Bars are SE. Bars with the same letter are not significantly different (ANOVA, $P > 0.05$).

ments ($P < 0.0001$) but the latter treatments were not significantly different ($P > 0.05$) (Fig. 3B–E).

DISCUSSION

Matings are costly, such as the energy costs of sexual behavior and the risks of predation, disease transmission and injury inflicted by the male (Daly, 1978). Therefore, the benefits of multiple matings must outweigh the costs to justify rematings. A number of studies have shown a positive relationship between the number of matings and fecundity in females, suggesting that females acquire nutrients from the male by remating (Kon *et al.*, 1993; Pardo *et al.*, 1995; Wang and Millar, 1997; Wilson *et al.*, 1999; Jiménez-Pérez *et al.*, 2003). However, the present study does not support the material benefit hypothesis because neither the number of matings nor the number of partners females had significantly affected female fecundity in *N. huttoni* (Fig. 1). Similar results were also reported for other insects (Kraan and Straten, 1988; Svärd and Wiklund, 1988; Ono *et al.*, 1995; Rodríguez, 1998; Kawagoe *et al.*, 2001). In addition, males of this seed bug do not provide food for females to obtain mating. It is suggested that males of these insects do not invest in nutritional substances as a reward for females during mating.

Cases of polyandry for genetic benefits are common in the recent literature (Zeh and Zeh, 1996; Newcomer *et al.*, 1999; Tregenza and Wedell, 2002; Jennions *et al.*, 2004; Neff and Pitcher, 2005). Jennions and Petrie (2000) even suggested that multiple matings by females for purely non-genetic benefits is unlikely as rematings invariably lead to the possibility of genetic benefits. In a previous study, Yang and Wang (2004) found that male genital traits rather than non-sexual traits such as body size and weight were highly selected by *N. huttoni* females in premating sexual selection. This preference of males with certain genital traits by females implies that to some extent females may obtain genetic benefits such as good genes from remating in this bug. Among various kinds of genetic benefits from female multiple matings with more than one male, offspring viability or hatching success rate is the most obvious (Madsen *et al.*, 1992; Olsson *et al.*, 1994; Tregenza and Wedell, 1998, 2002; Jennions and Petrie, 2000). However, the present study demonstrates that *N. huttoni* females remated to the same males or to the different males produced similar numbers of viable offspring. This result suggests that females do not obtain genetic benefit from remating in terms of offspring viability. Because how these viable offspring perform in their later life remains unknown, we cannot exclude the possibility that *N. hut-*



A: Females mated once

B: Females mated twice to the same males

C: Females mated twice to different males

D: Females mated five times to the same males

E: Females and males paired for life

Fig. 3. Daily fertility rate decline over time.

toni females may obtain other genetic benefits from multiple matings, such as genetic diversity and future reproductive advantage of these offspring.

A previous study showed that one mating could provide enough sperm to fertilize eggs throughout a female's lifespan in *N. huttoni* although noted that the fertility declined over time (Eyles, 1963b). Our results clearly demonstrate that one mating by females was not sufficient to fertilize the full egg load in *N. huttoni*. Furthermore, with the increase of the number of matings, whether this be with the same males or different males, the overall fertility rate significantly increased and the daily fertility rate declined significantly slower. It is thus strongly suggested that the replenishment of sperm is a major benefit females obtain from multiple matings in this bug. In his review of the relationship between mating frequency and fertility, Ridley (1988) found that the females of over half of 48 species for which there were data ran out of sperm if not allowed to remate. Thornhill and Alcock (1983), Hunter *et al.* (1993) and Sheldon (1994) also provided the value of evidence for sperm replenishment by female multiple matings. Our studies indicate that around 10% of eggs laid by *N. huttoni* females mating multiply failed to hatch. This cannot be attributed to the depletion of sperm. It is thus possible that a proportion of eggs and/or sperm are naturally sterile or unviable in this bug.

Finally, the results indicate that five matings were sufficient for *N. huttoni* females to maximize their reproductive success (Figs. 2 and 3D and E). Additional matings appear to be superfluous in terms of fertilization. However, the females of this bug could mate as many as 68 times if a male and a female were paired for their lifetime in the laboratory (Wang and Shi, 2004). In a longicorn beetle *Nadezhdiella cantori*, females could mate on average 46 times per day (Wang and Zeng, 2004) and in some other animal species, females could mate hundreds of times per each clutch produce(see review by Hunter *et al.*, 1993). Such a large number of matings are obviously not necessary for the fertilization of the eggs. Parker (1984) proposed the convenience hypothesis to explain this phenomenon, which suggests that females may mate multiply simply because the costs of repeated harassment from males outweigh the costs of additional matings. *N. huttoni* males are almost always promiscuous and can mate every day (QW, unpublished data). This property may result in superfluous matings for 'convenience' by females of this bug in the laboratory. However, this phenomenon may be less likely to occur in the field as mentioned earlier in the paper.

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REFERNCES

Daly, M. (1978). The cost of mating. *Am. Nat.* **112:** 771–774.

Eyles, A. C. (1963a). Fecundity and oviposition rhythms in *Nysius huttoni White (Heteroptera: Lygaeidae)*. *NZ J. Sci.* **6:** 186–207.

Eyles, A. C. (1963b). Incubation period and nymphal development in *Nysius huttoni White (Heteroptera: Lygaeidae: Orisillinae)*. *NZ J. Sci.* **6:** 446–461.

Eyles, A. C. (1965). Notes on the ecology of *Nysius huttoni* White (Heteroptera: Lygaeidae). *NZ J. Sci.* **8:** 494–502.

Gwynne, D. T. (1984). Courtship feeding increases female reproductive success in bushcrickets. *Nature* **307:** 361–363.

He, X. Z., Wang, Q., and Carpenter, A. (2003). Thermal requirements for the development and reproduction of *Nysius huttoni* White (Heteroptera: Lygaeidae). *J. Econ. Entomol.* **96:** 1119–1125.

Hunter, F. M., Petrie, M., Otronen, M., Birkhead, T., and Møller, A. P. (1993). Why do females copulate repeatedly with one male? *TREE* **8:** 21–26.

Jennions, M. D., Hunt, J., Graham, R., and Brooks, R. (2004). No evidence for inbreeding avoidance through postcopulatory mechanisms in the black field cricket, *Teleogryllus commodus*. *Evol.* **58:** 2472–2477.

Jennions, M. D., and Petrie, M. (2000). Why do females mate multiply? A review of the genetic benefits. *Biol. Rev.* **75:** 21–64.

Jiménez-Pérez, A., Wang, Q., and Markwick, N. (2003). Remating behavior of *Cnephasia jactatana* Walker females (Lepidoptera: Tortricidae). *J. Insect Behav.* **16:** 797–809.

Kawagoe, T., Suzuki, N. and Matsumoto, K. (2001). Multiple mating reduces longevity of females of the windmill butterfly *Atrophaneura Alcinous*. *Ecol. Entomol.* **26:** 258–262.

Kon, M., Oe, A., and Numata, H. (1993). Intra- and interspecific copulations in two congeneric green stink bugs, *Nezara antennata* and *N. viridula* (Heteroptera: Pentatomidae), with reference to postcopulatory changes in the spermatheca. *J. Ethol.* **11:** 83–89.

Kraan, C., and Straten, M. (1988). Effects of mating rate and delayed mating on the fecundity of *Adoxophyes orana*. *Entomol. Exp. Appl.* **48:** 15–23.

Madsen, T., Shine, R., Loman, J., and Håkansson, T. (1992). Why do female adders copulate so frequently? *Nature* **355:** 440–441.

Neff, B. D., and Pitcher, T. E. (2005) Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Mol. Ecol.* **14:** 19–38.

Newcomer, S. D., Zeh, J. A., and Zeh, D. W. (1999). Genetic benefits enhance the reproductive success of polyandrous females. *Proc. Natl. Acad. Sci. USA* **96:** 10236–10241.

Olsson, M., Gullberg, A., Tegelström, H., Madsen, T., and Shine, R. (1994). Can female adders multiply? *Nature* **369:** 528.

Ono, T., Hayakawa, F., Matsuura, Y., Shiraishi, M., Yasui, H., Nakamura, T., and Arakawa, M. (1995). Reproductive biology and function of multiple mating in the mating system of a tree cricket, *Truljalia hibinonis* (Orthoptera, Podoscritinae). *J. Insect. Behav*. **8:** 813–824.

Pardo, M. C., López-León, M. D., Hewitt, G. M., and Camacho, J. P. M. (1995). Female fitness is increased by frequent mating in grasshoppers. *Heredity* **74:** 654–660.

Parker, G. A. (1984). Sperm competition and the evolution of animal mating strategies. In Smith, R. L. (ed.), *Sperm Competition and the Evolution of Animal Mating Systems*, Academic Press, London, pp 2–60.

Ridley, M. (1988). Mating frequency and fecundity in insects. *Biol. Rev.* **63:** 509–549.

Rodríguez, R. L. (1998). Possible female choice during mating in *Ozophora baranowskii* (Heteroptera: Lygaeidae): female behavior, multiple matings, and sperm transfer. *J. Insect. Behav*. **11:** 725–741.

Rowe, L. (1992). Convenience polyandry in a water strider: foraging conflicts and female control of copulation frequency and guarding duration. *Anim. Behav.* **44:** 189–202.

SAS Institute (1996). *Users manual*. Version 6.12. ed. SAS Institute, Cary, NC.

Sheldon, B. C. (1994). Male phenotype, fertility, and the pursuir of extra-pair copulations by female birds. *Proc. Roy.Soc. Lond. B* **257:** 25–30.

Simmons, L. W. (2001) *Sperm Competition and Its Evolutionary Consequences in the Insects*. Princeton University Press, Princeton, NJ.

Svärd, L., and Wiklund, C. (1988). Fecundity, egg weight and longevity in relation to multiple matings in females of the monarch butterfly. *Behav. Ecol. Sociobiol.* **23:** 39–43.

Thornhill, R., and Alcock, J. (1983). *The Evolution of Insect Mating System*. Harvard University Press, Cambridge, MA.

Tregenza, T., and Wedell, N. (1998). Benefits of multiple mates in the cricket *Gryllus bimaculatus*. *Evol.* **52:** 1726–1730.

Tregenza, T., and Wedell, N. (2002). Polyandrous females avoid costs of inbreeding. *Nature* **415:** 71–73.

Wang, Q., and Millar, J. G. (1997). Reproductive behavior of *Thyanta pallidovirens* (Heteroptera: Pentatomidae). *Ann. Entomol. Soc. Am.* **90:** 380–388.

Wang, Q., and Shi, G. L. (2004). Mating frequency, duration, and circadian mating rhythm of New Zealand wheat bug *Nysius huttoni* White (Heteroptera: Lygaeidae). *NZ Entomol.* **27:** 113–117.

Wang, Q., and Zeng, W. Y. (2004). Sexual selection and male aggression of *Nadezhdiella cantori* (Hope) (Coleoptera: Cerambycidae: Cerambycinae) in relation to body size. *Environ. Entomol*. **33:** 657–661.

Wiklund, C., Kaitala, A., Lindfors, V., and Abenius, J. (1993). Polyandry and its effects on female reproduction in the green-veined white betterfly (*Pieris napi* L.). *Behav. Evol. Sociobiol.* **33:** 25–33.

Wilson, N., Tufton, T. J., and Eady, P. E. (1999). The effect of single, double, and triple matings on the lifetime fecundity of *Callosobruchus analis* and *Callosobruchus maculatus* (Coleoptera: Bruchidae). *J. Insect Behav.* **12:** 295–306.

Yang, L. H. & Wang, Q. (2004). Precopulation sexual selection in *Nysius huttoni* White (Heteroptera: Lygaeidae) in relation to morphometric traits. *J. Insect Behav.* **17:** 695–707.

Zeh, J. A., and Zeh, D. W. (1996). The evolution of polyandry I: intragenomic conflict and genetic incompatibility. *Proc. Roy. Soc. Lond. B* **263:** 1711–1717.