Original Research Article

Life span changes ofcoffee white stem borers, *Xylotrechusquadripes* (Coleoptera: Cerambycidae) in response to directed homosexual and heterosexual interactions

Abstract

Our study on coffee white stem borers, *Xylotrechusquadripes*investigated the influence of different sex-based clustering factors on beetle survivorship and life spans. Our experiments involved various groups, including individually isolated virgin males, individually isolated virgin females, groups of only males, groups of only females, and mixed-sex groups. First, survivorship reached 100% within the initial 15 days for all groups except for isolated virgin males.In contrast, the survivorship rates of both groups involving only virgin females andonly virgin males ranged from 80% to 90% compared to the maximum survival duration. Notably, in scenarios with males in groups or mixed-gender groups, survival dropped to zero percent after 45 days. Our study also highlighted the comparable costs incurred by males engaging in both homosexual and heterosexual interactions. Males interacting with females and other males exhibited similar survival curves, displaying shorter median life spans compared to isolated virgin males, suggesting that both courting and mounting behaviors, regardless of the recipient's sex, might affect the life span of males.Furthermore, our research revealed that female-female interactions, while less costly than male-male interactions, still reduced survival periods inonly the female group. These findings highlight the cost possibly associated with same-sex interactions and shed light on the dynamics of mating behaviors in this beetle. In conclusion, our findings underscore the significance of studying behavioral and life history traits in different environmental contexts. Social circumstances and ecological factors significantly influence sexual differences in lifespan and highlight the complexity of the interplay between reproductive strategies and survival ratesin *Xylotrechusquadripes*.

Keywords: Coffee white stem borer, survival, coffee, behaviour

Introduction

A fundamental concept within the framework of life cycle theory revolves around the notion of reproductive costs (Stearns 1992; Roff & Fairbairn 2007). Due to its inherent impact on future survival and reproductive potential, reproduction emerges as a costly endeavor. These costs predominantly fallupon females, encompassing expenses related to mating, offspring development, and caregiving. Conversely, the realm of male reproduction involves a spectrum of expenditures, encompassing both material resources and energy investments in gamete production, courtship rituals, competitive encounters, and mating pursuits (Cordts and Partridge 1996; Kotiaho 2001; Martin and Hosken 2004).

Historically, the majority of research concerning the costs of sexual behaviors and interactions has centered on heterosexual encounters. However, in numerous species, both sexes exhibit the potential for aggressive behaviors directed towards individuals of the same sex, including courtship and mounting attempts (Aiken 1981; Serrano *et al.* 1991; Srivastava *et al.* 1991; Andersson 1994; Vasey 1995; Bagemihl 1999; Fang and Clemens 1999; Harari *et al.* 2000; Sommer and Vasey 2006; Vasey *et al.* 2008). These interactions may manifest as elements of intrasexual competition, but distinguishing them from mere perception errors can be challenging within real-world contexts.

Consequently, we have used the term "homosexual relationships" to encompass all interactions between individuals of the same sex. Despite their pivotal significance in models elucidating the evolution and sustenance of sexual behaviors, the costs associated with homosexual relationships have been quantified in only a limited number of species, particularly among females. Engaging in homosexual behaviors can entail substantial costs. In many species, males partake in scramble and/or combat encounters with rivals to gain access to females or territories, often expending considerable time and energy while risking substantial harm (Thornhill and Alcock 1983; Andersson 1994; Kotiaho 2001). Males may also engage in courting and mounting other males, with courtship itself incurring significant expenses (Cordts and Partridge 1996).

Homosexual mounting has been observed across a wide range of taxa, including mammals, birds, reptiles, and insects (Aiken 1981; Thornhill and Alcock 1983; Bagemihl 1999; Harari *et al.* 2000; Switzer *et al.* 2004; Sommer and Vasey 2006). Notably, in insects, homosexual mounting may be more prevalent, accounting for up to 50% of reported mating attempts in some cases (Aiken 1981; Serrano *et al.* 2000). While research on homosexual interactions in mammals has garnered significant attention, it is essential to acknowledge that females exhibit homosexual behaviors in numerous species, even though much of the research has focused on male-to-male relationships (Srivastava *et al.* 1991; Vasey 1995; Fang and Clemens 1999; Harari *et al.* 2000; Sommer and Vasey 2006; Gastal*et al.* 2007).

Homosexual mounting and courtship (or courtship-like) behaviors may serve various sexual functions, ranging from establishing dominance over rivals to acting as apractice for heterosexual interactions (Vasey 1995). However, it is worth noting that these actions are often attributed to sex recognition failures or perception errors (Parker 1968; Aiken 1981; Thornhill and Alcock 1983; Serrano *et al.* 1991; Harari *et al.* 2000; Switzer *et al.* 2004). For instance, research on homosexual mounting in the flour beetle *Triboliumcastaneum* suggests the absence of sex recognition in this species, with the frequencies of intra- and intersexual mounting aligning with a random null model (Serrano *et al.* 2000). Although sex recognition exists in many other species, it may be relatively weak. In the water insect *Palmacorixa nana*, males tend to mount larger individuals, likely driven by the size disparity between males and females (Aiken 1981). This preference results in a notable rate of errors (Aiken 1980). Additionally, several studies (Savalli and Fox 1999; Harari *et al.* 2000; Serrano *et al.* 2000; Switzer *et al.* 2004; Van Gossum*et al.* 2005) have provided evidence in support of the perception error hypothesis.

Thornhill and Alcock (1983) propose that homosexual mounting in insects may evolve through male selection if the advantages of increased mating success through rapid, indiscriminate mating attempts outweigh the drawbacks of discrimination. A key prediction stemming from this theory posits that males would tolerate higher costs associated with homosexual mounting because only males stand to benefit from heightened mounting frequency in terms of fitness. Conversely, given that females are less likely to gain from such interactions, selection is expected to act against the evolution of costly female-female interactions (though also Harari *et al.* 2000).

The coffee white stem borer(CWSB),is a severe pest on arabica coffee and farmers are struggling to manage this beetle in coffee plantation and also farmers are shifting from arabica coffee to robusta coffee. While studying this pestwe have noticed male-male mounting and female-female mounting*i.e.* homosexual and heterosexual interactions, this behavior made us hypothesize that theymay incur the cost in survivability of beetles due to their homo and hetreosexual mounting behaviour. Hence, the present study was carried out to know the survivability of beetles in homosexual and heterosexual interactions.

Materials and methods

The experiment is conducted at Bio-control Research Laboratories(BCRL), Bengaluru.*Xylotrechusquadripes*, commonly known as the coffee white stem borer, served as the focal species in a series of experimental investigations to compare the costs associated with homosexual interactions vis-à-vis heterosexual interactions.

This enclosure was specifically designed to house coffee stems infested with the borer, which were traced and uprooted from the farmers fields at Mallenahalli, Chikmagalur, Karnataka and brought to BCRL, Bengaluru for the study. We have created a controlled environment condition, we utilized a nylon net enclosure measuring 3 x 3 x 3 metersand it was situated within the field coordinates of 13.1365° N, 75.6403° E, at an elevation of 970 meters above sea level. The area was kept open yet shaded to mimic natural conditions. Freshly emerged adult beetles were segregated by sex and housed in a laboratory setting, maintaining a controlled temperature of 23°C and relative humidity at 70%. They were given with a cotton wad saturated in a 10% honey.

For our investigation, we exclusively utilized newly emerged virgin males and females. Six distinct experimental groups were established: individually isolatedvirgin males, virgin females, virgin males in group settings, a group of virgin females, mixed-sex groups of males and females, and mixed-sex groups of females and males.

Virgin beetles were collected from the infected coffee stems at 24-hour intervals and housed individually in 25ml plastic vials containing cotton wads saturated with 10% honey. In each "isolated" treatment, ten containers were designated for males and ten for females, totaling 10 individuals of each sex. Within the "grouped" treatments, individuals of the same sex were grouped into five containers, each containing ten beetles. To distinguish individual beetles, five individuals in each "grouped" container were marked with white, non-toxic face paint. These marked beetles were reference group to assess mortality among unmarked focal individuals. Whenever a marked beetle perished, it was promptly replaced from the available pool of newly hatched beetles, ensuring that there were consistently five marked beetles in each container at any given time during the experiment. Mortality records were exclusively maintained for the unmarked focal insects, with a total of 25 individuals for each sex.

To establish "mixed-sex groups" for both males and females, we employed similar procedures. However, in this case, instead of using marked beetles of the same sex, we employed marked beetles of the opposite sex (*i.e.*, marked females for the "male mixed-sex" group, and vice versa). For each of the two "mixed-sex" treatments, we have taken five containers, each containing five males and five females, resulting in a total of 25 individuals. In the "male mixed-sex" treatment, only male deaths were recorded, and any deceased females were replaced from the available stock. The order was reversed for the "female mixed-sex" treatment, where only female deaths were recorded, and deceased males were replaced from the stock. Mortality counts were conducted once daily for all treatment types.

To assess potential variations in life expectancy among the different treatments, we conducted survival analysis, utilizing block-mean life duration as the unit of replication for treatments involving groups of animals. However, the results remained qualitatively consistent when individual lifespan served as the unit of replication. We conducted the survival analysis using Excel (Microsoft Corporation, USA).

Results

In our experimental treatments, which included scenarios featuring isolated virgin males, isolated virgin females, males in group settings, females in group settings, and mixed-gender groups, we observed a remarkable trend in beetle survivorship. Specifically, we noted that survivorship reached 100% within the initial 15 days for all groups except for the treatment involving isolated virgin males, where survival extended only up to 50 days.

Furthermore, when examining treatments comprising males in groups, females in groups, males in mixed-gender groups, and females in mixed-gender groups over a 20 to 30-day timeframe, we observed a substantial disparity in survivorship. In these scenarios, beetle survivorship ranged between 40%in mixed groups, compared to a significantly higher 80% to 90% in treatments involving solely virgin females and males.

Notably, in treatments featuring males in groups and males in mixed-gender groups, beetle survival diminished to zero percent over a 45-day duration. Conversely, in the remaining treatments, some beetles displayed remarkable resilience, surviving for up to an impressive 50-day period (Figure 1).

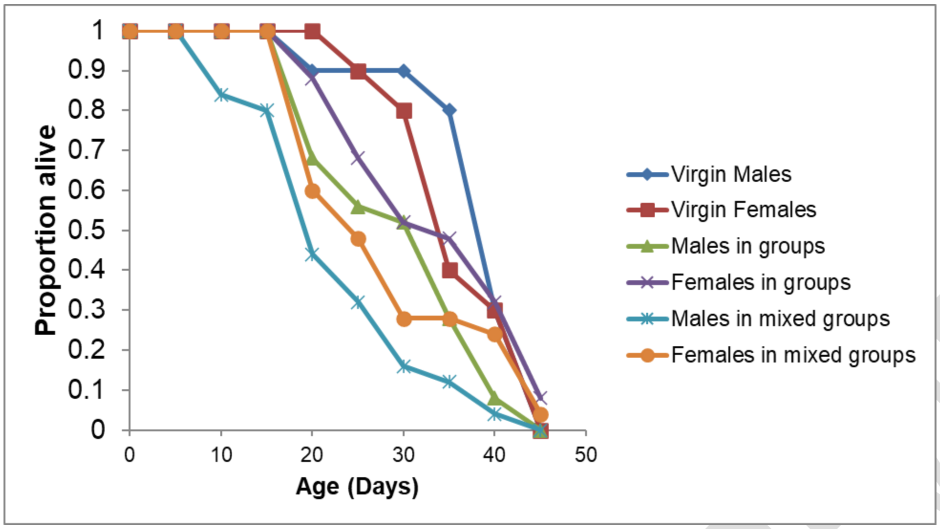

Figure 1. Survival curves of *Xylotrechusquadripes*in different experimental treatments.

In our study, we delved into the intriguing realm of the coffee white stem borer, focusing on adult females and males, and examining the nuances of their life spans under various experimental conditions.

We observed that adult female CWSBs exhibited their longest mean life span in treatments where they were housed alone (31±2.34 days), followed by treatments where females were placed in groups (19.68±2.92 days). Their shortest life span was recorded in treatments involving females in mixed-gender groups (16.84±3.25 days). Adult male CWSBs displayed their lengthiest mean life span in treatments where they were isolated, i.e., males alone (24.5±3.7 days), followed by treatments featuring males in mixed-gender groups (14.24±3.23 days), and their shortest life span occurred in treatments with males in group settings (10.8±3.38 days) (Figure 2).

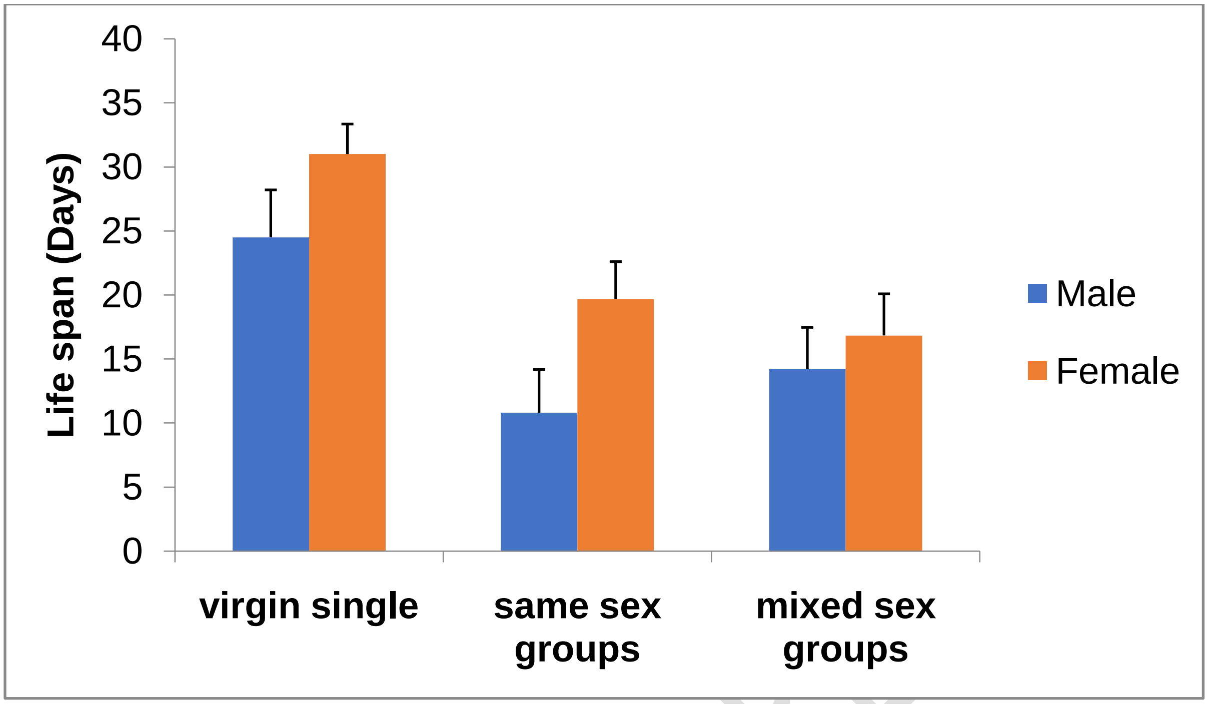

Figure 2. Mean life span ± SE of *Xylotrechusquadripes*in different experimental treatments.

We observed that males interacting with females and those interacting with other males exhibited analogous survival curves. Both scenarios displayed shorter median life spans when contrasted with virgin males kept individually isolated (Figures 1 and 2). This underscores the substantial expenses associated not only with courting but also mounting among same-sex conspecifics. It's worth emphasizing that these same-sex courtship and mounting behaviors are likely - effective on both the initiator and the recipient.

Notably, male CWSBs exhibit vigorous pursuit and mounting of other males (Mangalgikar, P., personal observations), which is believed to demand considerable energy and hydration resources. Furthermore, during such interactions, there's a potential risk of injuries or even rapid mortality if one male's genitalia becomes entangled beneath their elytra (Mangalgikar, P., personal observations). It's worth noting that CWSB males do not engage in physical combat(Mangalgikar, P., personal observations).

Our principal discovery hinges on the observation that males experience both homosexual and heterosexual interactions at roughly equivalent costs. In contrast, this parity is not observed among females. Additionally, our study revealed that beetles maintained in groups of ten individuals, with no separation and prompt replacement of deceased individuals, experienced a notable reduction in the life expectancy of females in group by 11.2% compared to solitary females (Figure 2). This reduction suggests potential costs associated with female-female interactions, even though they do not involve overt agonistic behaviors such as fighting. In CWSB, females do indeed exhibit mounting behaviors toward other females (Mangalgikar, P., personal observations). In contrast, our analysis indicated that female survival in CWSB was considerably less affected by homosexual interactions when compared to their male counterparts (Figure 2).

Discussion

Our research findings underscore the paramount significance of investigating behavioral and life history traits within the context of their developmental environments. Our study suggests that the costs associated with sexual behaviors such as courtship and mounting, often meticulously quantified within heterosexual interactions, exhibit substantial variation contingent upon social and ecological factors, including the adult sex ratio. This variability arises from a significant portion of the net cost attributed to behaviors directed towards individuals of the same sex. While certain behaviors aimed at conspecifics of the same sex may play a role in thesexual competition, others, exemplified by gay mounting in *Callosobruchus maculatus*, appear to manifest as nonadaptive consequences of highly indiscriminate male responses. Consequently, the relative prevalence of such behaviors can markedly influence their net loss and benefits.

Furthermore, our findingscontribute to unveil intriguing implications for understanding the emergence of sex-based disparities in life expectancy. The prevalent phenomenon of sexual dimorphism in lifespan is often attributed to differential reproductive costs between the sexes (Trivers 1972; Liker and Szekely 2005). Given that sexual dimorphism in lifespan is a recurring pattern in *C. maculatus* (Fox *et al.* 2003, 2004; Maklakov *et al.* 2007), we purposefully manipulated reproductive opportunities in our study. Remarkably, when individuals were housed in same-sex cohorts, the contrast in lifespan between virgin males and virgin females in *C. maculatus* surged to 42%. In contrast, when males and females were allowed to mate (*i.e.*, maintained in mixed-sex environments), we detected no discernible sexual differences in lifespan.

Therefore, it becomes evident that social circumstances exert a profound influence on sexual dimorphism in lifespan: in isolation, virgin females outlived males significantly. However, as soon as individuals of both sexes were provided opportunities for interaction and reproduction, sexual dimorphism in lifespan virtually dissipated. This observation indicates that despite their intrinsic potential for a longer lifespan, females actually exhibit mortality rates on par with those of males under conventional reproductive conditions, thereby implying that selection acts comparably on survival (and conceivably, the rate of aging) in both sexes. Consequently, sex-based disparities in various other life history traits (e.g., resource acquisition) and sexual behaviors, which are deeply rooted in sex-specific reproductive strategies, may potentially emanate as indirect by-products of the sexual dimorphism in lifespan frequently documented in studies involving virgin cohorts (Bilde *et al.* 2009).

Furthermore, it is noteworthy that male homosexual interactions substantially diminish survival, to an extent comparable to the overall costs of reproduction, as our findings elucidate. This highlights the importance of quantifying the costs associated with homosexual interactions, a facet of animal behavior that has often been presumed to be modest despite its long-standing interest in the context of evolutionary biology (Aiken 1981). Our study underscores the necessity of systematically assessing these costs, akin to how researchers routinely scrutinize the development of mating strategies, as indicated by our empirical data. Furthermore, our results align with the hypothesis that females exhibit less frequent and less costly homosexual behavior in comparison to males, shedding light on the intriguing dynamics of same-sex interactions in this species.

Conclusion

In conclusion, our findings underscore the significance of studying behavioral and life history traits in different environmental contexts, males interacting with females and those interacting with other males exhibited analogous survival curves. Both scenarios displayed shorter median life spans when contrasted with virgin males kept individually isolated. Social circumstances and ecological factors significantly influence sexual differences in lifespan and highlight the complex interplay between reproductive strategies, survival rates, and behavioral costs in CWSBs.

Acknowledgments

The authors express gratitude to the Coffee Board, India, for their funding support for theproject. Special acknowledgment is extended to Dr. Jayarama and Dr. Vinod Kumar PK fromCCRI,

Balehonnur, as well as Dr. Jayanth from BCRL, along with other technical staff, for theirvaluable assistance.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

References

Aiken, R. B. 1980. The role of acoustic signalling in the aggregating and mating behaviour of *Palmacorixa nana* Walley (Heteroptera: Corixidae). Ph.D. thesis, University of Toronto.

Aiken, R. B. 1981. The relationship between body-weight and homosexual mounting in *Palmacorixa nana* Walley (Heteroptera, Corixidae). Florida Entomologist, 64, 267–271.

Andersson, M. 1994. Sexual Selection. Princeton, New Jersey: Princeton University Press.

Bagemihl, B. 1999. Biological Exuberance: Animal Homosexuality and Natural Diversity. New York: St Martin's Press.

Bilde, T., Maklakov, A. A., Meisner, K., la Guardia, L. and Friberg, U. 2009. Sex differences in the genetic architecture of lifespan in a seed beetle: extreme inbreeding extends male lifespan. BMC Evolutionary Biology, 9, 33.

Bonduriansky, R. 2003. Layered sexual selection: a comparative analysis of sexual behaviour within an assemblage of piophilid flies. Canadian Journal of Zoology, 81, 479–491.

Bonduriansky, R. and Rowe, L. 2003. Interactions among mechanisms of sexual selection on male body size and head shape in a sexually dimorphic fly. Evolution, 57, 2046–2053.

Bonduriansky, R., Wheeler, J. and Rowe, L. 2005. Ejaculate feeding and female fitness in the sexually dimorphic fly *Prochylizaxanthostoma* (Diptera: Piophilidae). Animal Behaviour, 69, 489–497.

Cordts, R. and Partridge, L. 1996. Courtship reduces longevity of male *Drosophila melanogaster*. Animal Behaviour, 52, 269–278.

Crudgington, H. S. and Siva-Jothy, M. T. 2000. Genital damage, kicking and early death: the battle of the sexes takes a sinister turn in the bean weevil. Nature, 407, 855–856.

Edvardsson, M. and Tregenza, T. 2005. Why do male *Callosobruchus maculatus* harm their mates? Behavioral Ecology, 16, 788–793.

Fang, J. M. and Clemens, L. G. 1999. Contextual determinants of female-female mounting in laboratory rats. Animal Behaviour, 57, 545–555.

Fox, C. W., Hickman, D. L., Raleigh, E. L. and Mousseau, T. A. 1995. Paternal investment in a seed beetle (Coleoptera: Bruchidae): influence of male size, age, and mating history. Annals of the Entomological Society of America, 88, 100–103.

Fox, C. W., Dublin, L. and Pollitt, S. J. 2003. Gender differences in lifespan and mortality rates in two seed beetle species. Functional Ecology, 17, 619–626.

Fox, C. W., Bush, M. L., Roff, D. A. and Wallin, W. G. 2004. Evolutionary genetics of lifespan and mortality rates in two populations of the seed beetle, *Callosobruchus maculatus*. Heredity, 92, 170–181.

Fricke, C. and Maklakov, A. A. 2007. Male age does not affect female fitness in a polyandrous beetle, *Callosobruchus maculatus*. Animal Behaviour, 74, 541–548.

Gastal, M. O., Gastal, E. L., Beg, M. A. and Ginther, O. J. 2007. Elevated plasma testosterone concentrations during stallion-like sexual behavior in mares (*Equus caballus*). Hormones and Behavior, 52, 205–210.

Harari, A. R., Brockmann, H. J. and Landolt, P. J. 2000. Intrasexual mounting in the beetle *Diaprepesabbreviatus* (L.). Proceedings of the Royal Society of London, Series B, 267, 2071–2079.

Kotiaho, J. S. 2001. Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. Biological Reviews, 76, 365–376.

Liker, A. & Szekely, T. 2005. Mortality costs of sexual selection and parental care in natural populations of birds. Evolution, 59, 890–897.

Maklakov, A. A., Fricke, C. andArnqvist, G. 2007. Sexual selection affects lifespan and aging in the seed beetle. Aging Cell, 6, 739–744.

Martin, O. Y. and Hosken, D. J. 2004. Copulation reduces male but not female longevity in *Saltellasphondylli* (Diptera: Sepsidae). Journal of Evolutionary Biology, 17, 357–362.

Parker, G. A. 1968. Sexual behaviour of blowfly *Protophormia terrae*-novae R-D. Behaviour, 32, 291–308.

Perneger, T. V. 1998. What's wrong with Bonferroni adjustments? British Medical Journal, 316, 1236–1238.

Roff, D. A. and Fairbairn, D. J. 2007. The evolution of trade-offs: where are we? Journal of Evolutionary Biology, 20, 433–447.

Rup, P. J. 1986. Mating and its attendant behaviour in *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Journal of Stored Products Research, 22, 77–79.

Savalli, U. M. and Fox, C. W. 1999. The effect of male mating history on paternal investment, fecundity and female remating in the seed beetle *Callosobruchus maculatus*. Functional Ecology, 13, 169–177.

Serrano, J. M., Castro, L., Toro, M. A. & Lopez-Fanjul, C. 1991. The genetic properties of homosexual copulation behavior in *Triboliumcastaneum*: diallel analysis. Behavior Genetics, 21, 547–558.

Serrano, J. M., Castro, L., Toro, M. A. and Lopez-Fanjul, C. 2000. Inter- and intraspecific sexual discrimination in the flour beetles *Triboliumcastaneum* and *Triboliumconfusum*. Heredity, 85, 142–146.

Sommer, V. and Vasey, P. L. 2006. Homosexual Behaviour in Animals: an Evolutionary Perspective. Cambridge: Cambridge University Press.

Srivastava, A., Borries, C. and Sommer, V. 1991. Homosexual mounting in free ranging female *Hanuman langurs* (Presbytis entellus). Archives of Sexual Behavior, 20, 487–512.

Stearns, S. C. 1992. The Evolution of Life Histories. New York: Oxford University Press.

Switzer, P. V., Forsythe, P. S., Escajeda, K. and Kruse, K. C. 2004. Effects of environmental and social conditions on homosexual pairing in the Japanese beetle (*Popillio japonica*Newman). Journal of Insect Behavior, 17, 1–16.

Thornhill, R. and Alcock, J. 1983. The Evolution of Insect Mating Systems. Cambridge, Massachusetts: Harvard University Press.

Trivers, R. 1972. Parental investment and sexual selection. In: Sexual Selection and the Descent of Man 1871–1971 (Ed. by B. Campbell), pp. 136–179. Chicago: Aldine.

Van Gossum, H., De Bruyn, L. and Stoks, R. 2005. Reversible switches between male male and male–female mating behaviour by male damselflies. Biology Letters, 1, 268–270.

Vasey, P. L. 1995. Homosexual behavior in primates: a review of evidence and theory. International Journal of Primatology, 16, 173–204.

Vasey, P. L., VanderLaan, D. P., Rains, D., Duckworth, N. andKovacovsky, S. D. 2008. Inter-mount social interactions during heterosexual and homosexual consortships in Japanese macaques. Ethology, 114, 564–574.