



CHAPTER

SAME SEX SEXUAL (SSS) BEHAVIOR IN INSECTS

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ABSTRACT

Same-sex sexual (SSS) behaviour represents an evolutionary paradox and documented in over 1500 species of animals and birds worldwide (Ex: male big horn sheep, giraffes, bottlenose dolphins, killer whales, grey whales, and West Indian manatees, Japanese macaques, mallards, penguins, bats etc.). SSS behaviour has been reported ~100 species of insects and SSS refer here to all evidence of courting, mounting, genital contact, guarding, and pair bonding as SSS behaviour. There are several hypotheses viz., mistaken identity, by-product of heterosexual behaviour, mating interruption, dumping of old sperm, predator avoidance, prison effect, biased sex ratio, indirect sperm translocation, satellite male strategy, practice for heterosexual encounters etc. which provide a base explanation for occurrence of SSS behaviour in insects.

INTRODUCTION

Nature is highly selective and removes the individuals from its arena which seems to be unfit. Still, there are several phenomenon which are not agreeing with the nature's law. Among them, reproductive strategy of organisms is well known and most important one. Organisms follow several types of sexual behavior viz., asexual behavior, parthenogenesis, binary fission, sexual behavior, gynandromorph and most rare sexual behavior i.e., homosexual behavior or same sex sexual behavior where, the individual shows sexual interest in same sex individual rather than opposite sex. Same-sex sexual (SSS) behavior represents an evolutionary paradox which is most common behavior in animals and birds. The classical example is male big horn sheep which live in what are often called "homosexual societies." They bond through genital licking and anal intercourse, which often ends in ejaculation. If a male sheep chooses to not have gay sex, it becomes a social outcast. Ironically, scientists call such straight-laced males "effeminate." Giraffes have all-male orgies. So do bottlenose dolphins, killer whales, gray whales, and West Indian manatees. Japanese macaques, on the other hand, are ardent lesbians; the females enthusiastically mount each other. Bonobos, one of our closest primate relatives, are similar, except that their lesbian sexual encounters occur every two hours. Male bonobos engage in "penis fencing," which leads, surprisingly enough, to ejaculation. They also give each other genital massages. SSS behavior has been reported in most insect orders, and Bagemihl (1999) provides a list of ~100 species of insects demonstrating such behavior. However, it has been suggested that in many cases, especially in invertebrates, SSS behavior does not serve a purpose or contribute to fitness and is merely a byproduct of other behaviors. SSS behaviour is usually performed at some stage during reproduction with a member of the opposite sex, but which is instead aimed towards members of the same sex. SSS is almost parallel to

heterosexual behaviour except the fertilization process, but consists mounting, courtship, copulation, genital organ contact and sometime sperm transfer. But this is a non-reproductive behaviour hence, it is called as Darwinian paradox (It is impossible to maintain genes which do not promote reproduction). Even though, it is non reproductive why still the insects do follow it? We can't say that it is a cost effective process because it do require an equal energy as that of heterosexual behaviour and we can't neglect the fact that, it never produce offspring. Then, the question comes why does they follow this non-reproductive costly behaviour? What is the fate of the homosexual behaviour? To answer this, several hypotheses were proposed.

Mistaken identity/late sex recognition: 80 per cent of the insects shows SSS behaviour because of the mistaken identity. Where, the individual fails to identify the sex of its mating partner and try to mount on the wrong partner. It has been observed in many insects and some of the researchers assume that it is mainly because of

- i. **Lack of experience:** The newly emerged adults and some nymphs try to mount the same sex individual because of the lack of exposure.
- ii. **Overlapping of phenotypic characters of male and female:** It is the case of burying beetle *Nicrophorus vespilloides* Herbst, (Silphidae: Coleoptera) where, the males and females look similar and sometimes they are too close in morphology that it confuses the individuals and leads to mounting of same sex.

The mistaken identity has been observed in Pine cricket, *Xenogryllus marmoratus* (Haan) (Gryllidae: Orthoptera) by Olivero and Robillard (2017) in River bed in Hofu city, Japan, on October 20, 2014 and recorded in the camera.

Same-sex sexual behavior in *Xenogryllus marmoratus* (Haan, 1844) (Grylloidea: Gryllidae: Eneopterinae): Observation in the wild from YouTube (Olivero and Robillard, 2017)

Video link: <https://www.youtube.com/watch?v=C9hF0Fu5A64>
Explanation of the video is given bellow (Table 1 and Plate 1)

Pine cricket, *Xenogryllus marmoratus* (Gryllidae: Orthoptera) found in moist condition and sexual dimorphism can be seen where, males are larger than females (Table 2 and Plate 2). Males of pine cricket provide metanotal gland secretions for the mounted female during mating. The metanotal secretions are rich in nutrients and serve in production of healthy offspring.

Table 1. Sequence of video showing SSS behaviour

Time	Detail of event
0:00–0:54 min.	A male of <i>X. marmoratus</i> sitting on dry herbaceous vegetation (Fig. 1A). It turns around, stops, and then walks left out of the frame, while moving the antennae.
0:54–1:12 min	Same male, seen from above, motionless on plant stems.
1:12 min	Male raises its wings perpendicularly to the body, adopting the usual mating position and exhibiting the metanotal glands (Fig. 1B).
1:20 min	Another cricket's antennae clearly appear at the male's back (Fig. 1B, red circle). Both individuals' antennae briefly touch each other, causing a subtle startle of the first male's body (1:26), but it keeps its forewings raised. The second cricket slowly approaches from behind (Fig. 1C).
1:37 min	The second individual puts one leg on the back of the first one (red circle on Fig. 1D). At this point, the viewer is prepared to observe a mating sequence, but when the second individual moves forward on the first individual's back, it becomes clear that this is not a female, but a second male of <i>X. marmoratus</i> (Fig. 1E) (hereafter male 2).
1:37–1:48 min	Male 2 mounts the back of male 1 up to the exposed metanotal glands (Fig. 1F).
1:48–3:07 min.	Male 2 feeds on the glands of male 1 (Fig. 1F–G), even pushing forward male 1's forewings with its head.
3:08 min	Male 1 reacts by moving the whole body, then becomes still again while male 2 continues feeding on its glands
4:10 min.	Slightly different angle, probably shortly after. Male 2 is still eating from male 1's glands but the latter moves, as if trying to remove male 2 from its back (Fig. 1H).
4:24 min.	The intentions of male 1 become clearer as the apex of its abdomen enters the frame of the video: its genitalia are raised and a spermatophore is ready to be transferred (Fig. 1I). Male 1 attempts mating with male 2.
4:45 min	Slightly different angle, probably shortly after. This angle shows that male 2 is clearly shorter than male 1, as it is entirely sitting on the abdomen of male 1 (Fig. 1J). Both males are motionless, but male 2 continues feeding on male 1's glands.
5:20–6:08 min	Multiple new attempts at mating. Male 1 repeatedly bends its abdomen apex dorsally, but fails to reach male 2, which calmly continues feeding on the other's glands (Fig. 1J).

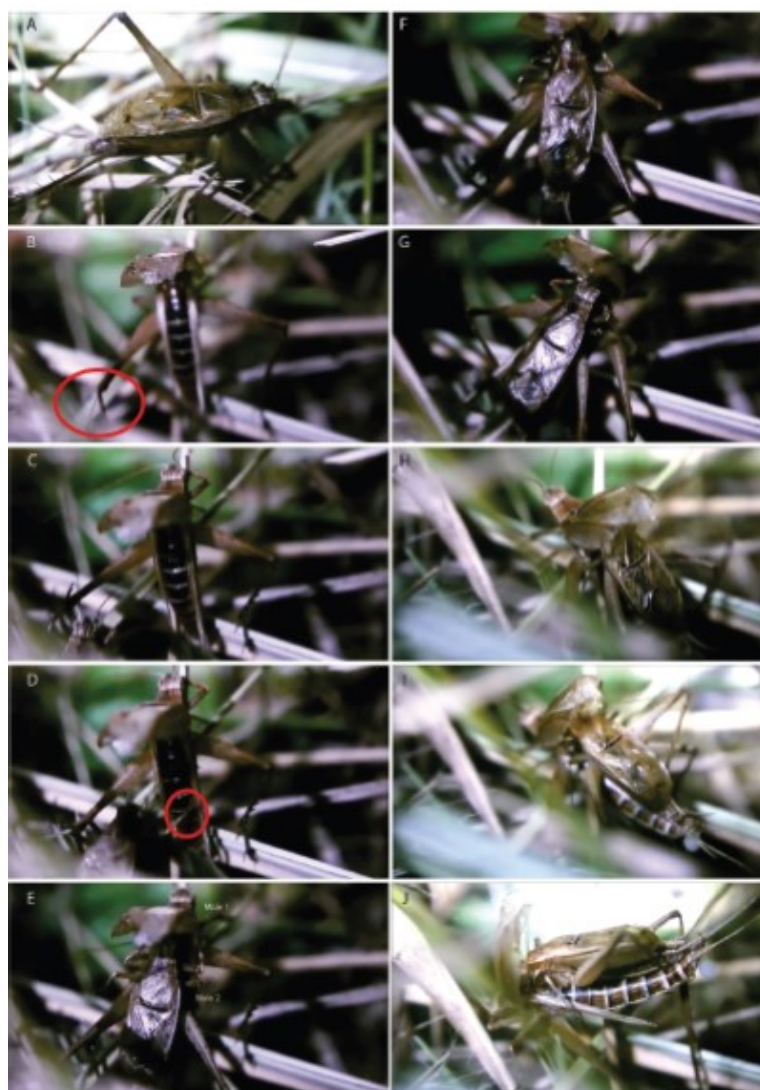
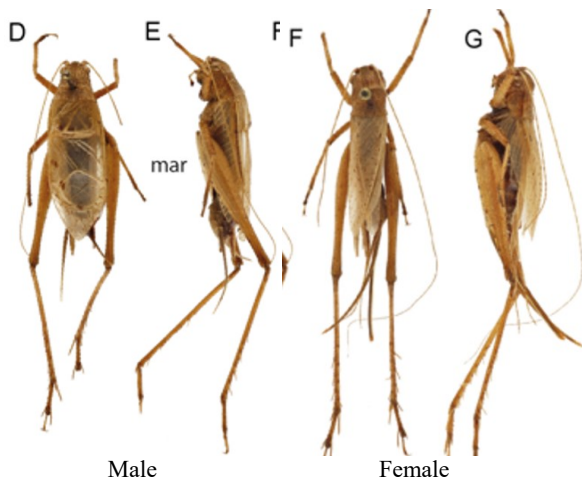


Plate 1. Screenshots of the video showing same-sex sexual behavior between males of *Xenogryllus marmoratus* (Haan)

Table 2. Sexual dimorphism in pine cricket

	Male	Female
Body length	22.1 mm	19.4 mm
Pronotum width	4.3 mm	3.7 mm
Pronotum length	2.9 mm	2.6 mm
FW length	16.5 mm	13.4 mm
FW width	6.5 mm	3.3 mm

**Plate 2. Sexual dimorphism in pine cricket**

The video exhibited the SSS behavior in pine cricket and supports the fact that, SSS occurs in insects and also proved the mistaken identity hypothesis. Here both males failed to identify their mating partner. Male 1 could not reach and insert spermatophore in the male 2 it is because of the smaller size of male 2. Male 1 produced metanotal secretions without proper identification and it costs more for the individual. But, there is an unusual thing happened with this example because how the insects become gender blind? As they are very specific in selection of their mate. Then, researchers found that male 2 followed the strategy called satellite male strategy.

- I. **Satellite male strategy:** Male which is smaller in size use the larger males for its benefit (may be for transport, obtaining resources from the other males etc.). In case of pine cricket, male 2 took advantage of being small and mounted on the male 1 to feed on the nutritious metanotal secretions. The consequence of this may be to make its offspring healthy, but the researchers' opinion is that, smaller males are not preferred by females. Anyhow, male 2 took advantage of its size through SSS behavior.
- II. **Strategy of permissiveness:** Researchers were not supported the gender blindness of the insects as their specificity towards the mating partner is so accurate. Hence, SSS was explained with the help of Acceptance threshold theory given by Reeve in 1989. The theory states that,

If the discriminating male has a high-acceptance threshold and is very restrictive, it will risk rejecting many females, while if it has a low-acceptance threshold and is very permissive, it will accept males as mating partners.

When the male has high threshold i.e., male is so accurate or specific in selecting the female for mating then there is risk of losing the females when male and female has overlapping phenotypes (i.e., male and females look similar). In contrast, when male has low threshold it accept or become more permissive for more individuals irrespective of sex and there is low risk of rejecting the females but high chance of mating with males. The theory well explained in case of burying beetle *Nicrophorus vespilloides* Herbst (Silphidae: Coleoptera).

Acceptance threshold theory can explain occurrence of homosexual behavior

(Engel et al., 2014)

When recognition is not error-proof, the acceptance threshold used by males to recognize potential mating partners should be flexibly adjusted to maximize the fitness pay-off between the costs of erroneously accepting males and the benefits of accepting females. The manipulation of male burying beetles' search time for females and their reproductive potential influenced their perceived costs of making an acceptance or rejection error. As predicted, when the costs of rejecting females increased, males exhibited more permissive discrimination decisions and showed high levels of SSS behaviour; when the costs of accepting males increased, males were more restrictive and showed low levels of SSS behaviour.

MATERIAL AND METHODS

Study organism and maintenance: Burying beetles (*Nicrophorus* spp.) provide elaborate biparental care to their offspring. They compete for and breed on the carcasses of small vertebrates, which are an essential, but rare and ephemeral resource. Females and males mate both on and off a carcass. Descendants of *N. vespilloides* beetles trapped in a deciduous forest in Freiburg, Germany. Experimental beetles were housed in plastic containers filled with moist peat and fed mealworms twice a week. All beetles were maintained at 20°C under a 16 L: 8 D regime.

Treatment groups: In the experiment, immediately after eclosion, males were kept at three different female densities to promote differences in perceived search time: (i) without a female, (ii) with one female and (iii) with three females. To control for general density effects, two additional female-deprived treatment groups were included, where we kept males (iv) with another male and (v) with three males. Males were either kept for 20–22 days under these conditions (20-day treatment) or for 55–60 days (60-day treatment). After these time periods, mating trials were performed in which males were presented with either a male or a female. In situations in which males were kept with one or several females, males perceived a lower searching time for females than the males in female-deprived situations, especially in the 60-day treatment. Hence, the males in the long-lasting female-deprived situations were expected to avoid making any rejection error and be more permissive, whereas the males that perceived a high female encounter rate and therefore low search costs throughout the 60 days of treatment could afford to be choosier and were expected to be more restrictive. In the shorter 20-day treatment, there was no many differences in discrimination decisions between the males of the different social environments, as perceived searching time should generally be low relative to the onset of sexual maturation (approx. on day 12–16). Hence, males of the 20-day treatment were expected to show a low level of SSS behaviour.

RESULTS

After 20 days, only a small proportion (mean+s.e.: 12.22%+7.85) of males were showing SSB and female density had no influence (generalized linear model (GLM) with binomial errors, Wald- χ^2 4,36=3:45, $p=0.49$; figure 1a). However, in the 60-day treatment, the occurrence of SSB was generally higher (mean+s.e.: 34.82%+15.89; GLM, Wald- χ^2 1,131= 4:46, $p=0.035$), and there were large differences between social conditions (GLM, Wald- χ^2 4,86=21:84, $p<0.001$; figure 1b). Males in female-deprived situations engaged significantly more often in SSB than males kept with females (figure 1b). Males maintained with one or three females did not engage in SSB at all. Males kept in complete isolation showed the highest occurrence of SSB. Most males of the 20-day treatment (mean+s.e.: 90.28%+ 2.45) copulated with females and there was no difference between social conditions (GLM, Wald- χ^2 4,36 =0:50, $p<0.97$; figure 1c). Also, a large proportion of males of the 60-day treatment (mean+s.e.: 78.60%+9.65) copulated with females, but in this treatment group, the males' social environment affected the occurrence of matings (GLM, Wald- χ^2 4,87=21:84, $p<0.001$; figure 3d). Males kept with three females copulated less often with a female than males kept in isolation or with a male (Figure 1d).

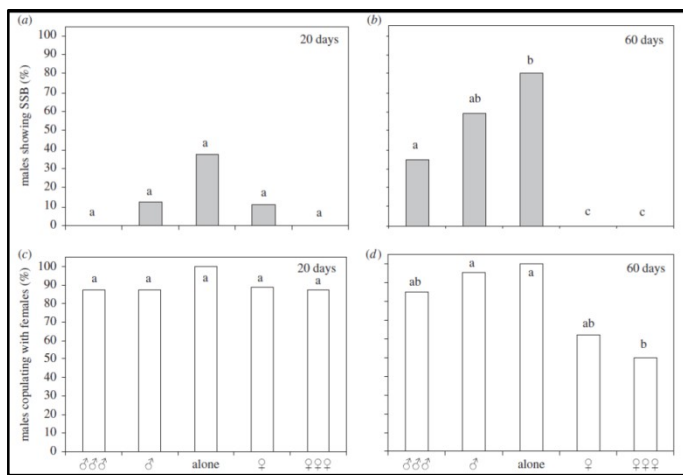


Fig. 1. The influence of a male's social environment on SSB (a,b) and on heterosexual behaviour (c,d) during test trials. Males were kept for either 20 days (a,c) or 60 days (b,d) under different conditions

DISCUSSION

Males in female-deprived situations engaged significantly more often in SSB than males kept with females. In female deprived condition searching time increases and males become more permissive. Males maintained with one or three females did not engage in SSB at all. Males kept in complete isolation showed the highest occurrence of SSB (80.0%). Males kept with three females copulated less often with a female than males kept in isolation or with a male. When more females were offered male becomes choosy and multiple mating cause exhaustion.

Inference: Insects doesn't lose the discriminating ability but follow permissive strategy. When males were exposed for same sex for longer period (60 days) they showed more SSS behaviour than the males reared with females. It is because of the Prison effect.

Prison effect: The phenomenon that the incidence of SSS behaviour increases under the absence of mating opportunity. This is happened in this case because males were kept in absence of females for 60 days and researchers assume that, it is a consequence of a discrimination strategy or mistaken identity. Still, the interrogation is not completed! Because if the insects can't be a gender blind and follows the strategy of permissiveness but why did the male 1 pine cricket produced spermatophore? As the spermatophore is costly, does this happen in all the time when individuals perform SSS behaviour? The answer for these questions was found in red flour beetle *T. castaneum* by Levanet al. (2009).

Testing multiple hypotheses for the maintenance of male homosexual copulatory behaviour in flour beetles (Levanet al., 2009).

Here, they use *Tribolium castaneum* flour beetles, which exhibit frequent male homosexual copulations, to empirically test several hypotheses for the maintenance of such behaviours: (1) establishing social dominance; (2) practice for future heterosexual encounters; and (3) indirect sperm translocation. The main hypothesis- Indirect sperm translocation: When males from two genetic strains mated with females immediately following a homosexual copulation, females produced progeny sired not only by the directly mating male, but also by that male's homosexual partner.

METHODS

Beetle maintenance and general methods all beetles were sexed as pupae and housed in a dark incubator at 29°C and 70% humidity. Adults were provided with excess flour, and kept either individually or

in groups depending on the experimental design. Mating observations were conducted at 29–32°C in plastic arenas with scratched bottoms or filter paper to provide traction. Heterosexual copulations is male dorsally mounting a female in the correct orientation, extending its aedeagus and inserting it into the female's genital opening; during copulation males rapidly rub their legs against the sides of the female's body interspersed with periods of inactivity. Behaviours exhibited during male homosexual copulations were remarkably similar as a male dorsally mounting another male in the correct orientation, extending its aedeagus downward and forward to contact the posterior abdomen of the other male, and rapidly rubbing its legs along the sides of the mounted male's body. The duration of such male homosexual copulations ranged from 0.2 to 11 min.

Male heterosexual copulation rate was measured by providing males with a succession of individual virgin females. Not all copulations result in successful the male insemination success was measured (proportion of copulations leading to insemination) by isolating each mated female for 2 weeks and scoring insemination based on the presence or absence of larva that would develop only if insemination had occurred. Experimental beetles came from two *T. castaneum* genetic strains differing in adult body colour: a wild-type strain (+ / +) homozygous for a chestnut body allele, and Chicago black (b / b) which is homozygous for an autosomal, semidominant black body colour allele. The body colour phenotype of adult progeny was used to distinguish between progeny sired by either black or wild-type males. Data were analysed using SPSS 16.0, (SPSS Inc Chicago IL, USA) after verifying assumptions of statistical tests.

The experiment was conducted using males from different genetic strains to examine whether *T. castaneum* males engaging in homosexual copulations might indirectly inseminate females through a male intermediary (Plate. 3 & 4). Randomly selected, virgin wild-type males were paired with randomly selected, virgin black males and these male pairs were allowed to copulate once (n = 86 pairs; both strains were represented approximately equally as mounting males). Immediately after each male homosexual copulation, males were separated and then both the mounting and mounted males were isolated to copulate once with a virgin black female (total of 172 matings). These mated females were isolated to oviposit with weekly transfers to new containers for 2 weeks. To control for any progeny that might arise due to back / forward mutations between the black and wild type alleles, mated single black or wild-type males with virgin black females (n = 65 matings), and collected eggs from each female as above. The body colour phenotypes (as b / b or b / +) was scored of all adult progeny from matings of the mounting, mounted and control males (27 180 total progeny were scored). To detect progeny fertilized by any sperm translocated during the initial homosexual copulation, the progeny showing unexpected body colour phenotypes based on the known genotype of the directly mating males was used as indication.



Plate 3. Mounting male is releasing a spermatophore from its extended genitalia

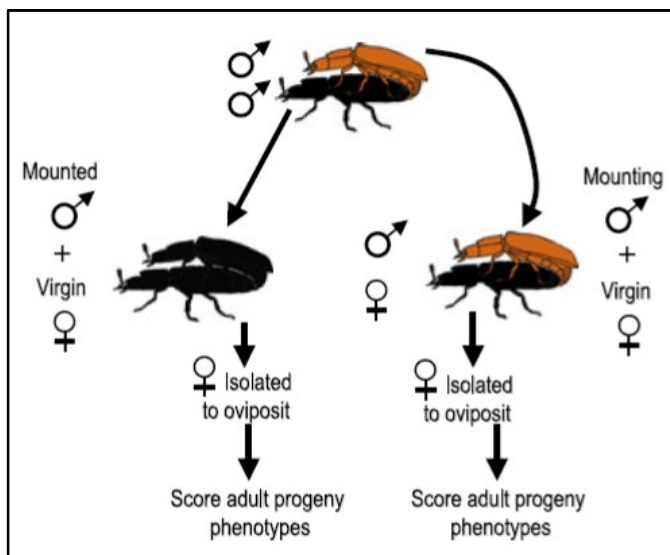


Plate 4. Experimental design used to test the indirect sperm translocation hypothesis

RESULTS

- In six of 86 pairs (7%), females produced progeny with body colour phenotypes indicating that they were sired indirectly by their mate's previous homosexual partner
- In case of Chicago black male and female combination, wild type of progenies produced
- None of the 65 control matings produced any unexpected progeny
- In each case only a single progeny was indirectly sired, representing <0.5% of each females' total progeny. None of the 65 control matings produced any unexpected progeny (of 11 765 total progeny scored), indicating a low probability that these represent back mutations from black to wild-type or vice versa.

DISCUSSION

The results provide limited support for the hypothesis of indirect sperm translocation, which proposes that males engaging in homosexual copulations might indirectly transfer sperm to females through a male intermediary. In six instances, females that mated with males immediately following a homosexual copulation produced progeny whose body colour phenotype suggested they were sired not by the directly mating male, but rather by that male's homosexual partner: however, only a single unexpected progeny was observed in each case, representing ~0.5% of each females' total progeny. In addition, the indirect sires were equally likely to be mounted or mounting males, suggesting the possibility of bidirectional sperm transfer. These unexpected progeny are unlikely to represent genotyping mistakes, as +/b heterozygotes are easily distinguished from b/b homozygotes. In addition, unexpected progeny are unlikely to have arisen from mutations, as the observed rates are substantially above the typical mutation rates for phenotypic mutations of 10^{-4} – 10^{-6} per locus per generation (Futuyma, 2005).

Inference: Insect use SSS as tool for indirect sperm translocation:

The other side of this hypothesis turns to be a controversial because the question "Why insect has to use the other male to transfer the spermatophore as it is capable of doing it?" the answer by the researchers for this question was surprising. The reason behind the indirect sperm translocation is Sperm dumping.

Sperm dumping: It is process of discarding the aged sperm of poor performance (low motility, fertilizing ability and sperm competitive

ability). This is because females prefer the younger sperms for fertilization since, they are capable of removing spermatophore from their genital tract. The older sperms would be removed and made space to be available for younger sperms. Sperm dumping may also be selected when males cannot down-regulate their sperm production rates. *Tribolium* beetles have life-history features likely to result in selection for continuous sperm production, as they are income breeders whose gamete production is fuelled by adult nutrient input and adults mate repeatedly during their 1- to 2-year adult lifespan. When many females are available (e.g. under high population densities), high sperm production rates provide a selective advantage; indeed, *T. castaneum* males can mate with up to seven different females within 15 min. On the flip side, however, these males may be physiologically incapable of down-regulating their sperm production when lower population densities are encountered. Thus, homosexual copulations may provide *T. castaneum* males with a mechanism for discarding sperm. Hence, SSS is a strategic process not an unintentional behaviour.

Physiological evidences for SSS behaviour: There is no evidences for physiological causes for SSS behaviour but certain genetic evidences showed that, mutation of sex linked genes can induce the SSS behaviour (Table 3).

Table 3. Genes responsible for SSS behaviour

Genes	Function	Reference
<i>fruitless</i>	Associated with courtship	Hall, 1994; Yamamoto <i>et al.</i> , 1996
<i>dissatisfaction</i>	Associated with courtship	Finley <i>et al.</i> , 1997
<i>prospero</i>	Associated with courtship	Balakireva <i>et al.</i> , 1998
<i>quick-to-court</i>	Associated with courtship	Gaines <i>et al.</i> , 2000
<i>tra^F</i>	Associated with courtship	Ferveuret <i>et al.</i> , 1995; O'Dell <i>et al.</i> , 1995
mini-white transgene (mw)	Associated with courtship	Zhang and Odenwald, 1995; Hing and Carlson, 1996

Substantial male–male courtship has been found in flies with mutation of *fruitless* (*fru*) *dissatisfaction*, *prospero* or *quick-to-court* genes. The ectopic expression of a female-dominant form of the transformer gene (*tra^F*) and the presence of the mini-white transgene (*mw*) are also associated with male–male courtship. Several brain regions involved in male–male courtship behavior have been identified by targeted expression of *tra^F* and *fru* in male brains under the control of specific galactosidase-4 (*GAL4*) lines. For example, when *tra^F* was expressed in either antennal lobes or in mushroom bodies, feminized male flies showed high male–male courtship behavior.

Consequence of SSS behavior in insects: The SSS behaviour is not in favour of some insects, mainly, bed bugs *Cimex lectularis* (L.).

Homosexual interactions in bed bugs: alarm pheromones as male recognition signals (Ryne, 2009):

Common bed bugs are nocturnal, blood-feeding ectoparasites of humans that mate and lay eggs in close proximity to their hosts. The only mode of mating is through traumatic insemination in which the male pierces the female through the abdomen at a specific site and ejaculates into the haemocoel. The female has evolved a secondary genital opening (spermatheca) that decreases the immunological cost of being wounded (pierced) during insemination. Mating is closely associated with feeding, since feeding causes an increase in body size and males are attracted to any large individual regardless of sex: it is the bloated body that increases the attractiveness. Mating behaviour does not include long-distance attraction; instead males rapidly mount any large, newly fed nearby individual. The male then folds his abdomen underneath the mounted individual and probes with the paramere (penis). After this sequence of mounting, the male decides either to continue to mate or to dismount; thus sex identification is likely to occur after mounting (Reinhardt & Siva-Jothy 2007). Male–male interactions and mounting often result in the clearly detectable emission of alarm pheromones. The characteristic smell is, however, rarely detected in male–female

interactions. Thus, the release of alarm pheromone and the behavioural responses to this signal appear to differ between males and females.

METHODS

Experimental Insects: The bed bugs were reared on weekly meals of fresh, defibrinated chicken blood. The blood was heated to 35°C and the jars were placed on the blood with the parafilm barrier between the blood and the bugs, enabling the bed bugs to pierce the parafilm to reach the blood. The culture was kept in a climate chamber at 70% relative humidity, 25°C, and 12:12 h light:dark. To obtain virgins of similar age, moulting of final instar nymphs with a blood meal was induced (Carayon 1966), and the nymphs were placed into individual jars. The sex of newly moulted adults was determined under a stereomicroscope. All adults were fed 7–10 days prior to experiments, as this is the normal feeding interval of bed. all individuals in all tests for presence of sperm from mating under a stereomicroscope after the experiments was examined.

Mating and Mounting Behaviour: Both males and females require a blood meal to produce gametes and to initiate mating behaviour. *Cimex lectularius* live for approximately 200–300 days and mate throughout adult life, with mating activity associated with feeding events. The optimal feeding frequency for *C. lectularius* is every 7–10 days; thus all males that performed the mounting in these experiments were fed at least 7 days prior to experiments. Recently fed males are unable to perform mating behaviour because of the fully bloated body, but females are mounted frequently directly after a blood meal. Experimental (focal) males and females were fed directly before the experiments to ensure that they were in the most attractive state. Mounting behaviour in this study consisted of the combined behaviours of mounting and probing with the male paramere. The two behaviours occur regardless of the focal individuals' sex and in short time intervals. Whether probing led to actual piercing was not possible to distinguish in all interactions, since the male abdomen is curved underneath the female (or male), but the duration of a mounting leading to piercing and sperm transfer is approximately 110 s in the first mating after a female blood meal (Siva-Jothy 2006). Mountings not leading to sperm transfer are usually shorter, but piercing may still occur.

Male–Male Interactions: The 2–3-week-old virgin males (focal) were placed dorsally in a petri dish with a silicone elastomer bottom covered with plastic foil for immobilization. The males were randomly divided into two groups: (1) operated (treatment) and (2) sham operated (control). I applied nail polish to the two large metathoracic glands between the first and second pair of legs through a small hole in the plastic foil to cause a mechanical blockage of the glands, which hinders the emission of alarm pheromones (operated group, blocked glands). The control group (sham operated) had nail polish applied between the second and third pair of legs. When the nail polish was dry, I allowed the males to feed from my arm immediately prior to the experiment. The experiment was conducted at room temperature in dimmed light. One fully satiated male (focal) was placed in a petri dish with a filter paper covering the bottom to facilitate movement, and an unfed male was introduced. Each mounting on the focal male was timed and the number of mountings was recorded in all treatments since all focal males were mounted several times as the introduced male's attention remains unchanged between mountings. The experiment lasted 3 min. Petri dishes were thoroughly cleaned and the filter paper was replaced between replicates.

Male–Female Interactions

Extracts and delivery system: Extracts were obtained by submerging 10 virgin males, 1 month old, in 1 ml of hexane (99% purity) for 30 min. The solvent was recovered and transferred into a new glass vial and was kept in a freezer between trials. Pure hexane (10 ml; treatment 2) or extract (treatment 3) was placed on a 0.5 cm² filter paper inside

glass pipettes with a 5 ml rubber bulb as a delivery system for the volatile chemicals during mounting behaviour.

Behavioural experiments: I used 2–3-week-old males and females, fed at least 7 days prior to experiments. Mating trials were conducted in dimmed light and at normal room temperature. The focal female bed bugs were randomly divided into three treatment groups: (1) male & female (control group), (2) male & female with hexane stimulus (solvent control), and (3) male & female with male extract stimulus (treatment). All the females were blood fed directly before the experiments. The fed female was introduced to a virgin male, which performed the mounting behaviour. The chemicals were delivered by a continuous manual puffing when the male mounted the female, creating an intermittent air flow containing volatile chemicals. Only the first mounting duration was timed in all three treatments, as male attention decreased after first mounting/mating.

Male Mating Scars: One-week-old males, previously held individually, were marked with correction fluid (Tip-ex) and randomly divided into two groups, each with 10 males. The males from one group (control) were individually kept, until death, in 3 ml jars containing a piece of filter paper. The males from the other group (treatment group) were placed in individual 30 ml jars, each containing a filter paper and four additional males. Additional males were replaced if found dead during the experiment, that is, treatment males had four additional males present at all times. I fed the males from the two groups (singly held control males and marked, focal males from the treatment group) on my arm every 7–10 days. At death, longevity was recorded and males were placed in 70% ethanol prior to examination for piercing scars. Males were photographed with a digital camera fitted on a stereomicroscope.

RESULTS

Male–Male Interactions: Data from male–male interactions were not significantly different from normal distributions (Kolmogorov–Smirnov: $Z < 0.949$, $P > 0.329$). Mounting behaviour was affected by treatment (MANOVA: Wilk's $\lambda = 0.694$, $F_{2,19} = 4.198$, $P = 0.031$). Univariate between-subjects effects analyses showed that 'duration per mounting' ($F_{1,20} = 7.560$, $P = 0.012$) and 'total duration of all mountings' ($F_{1,20} = 4.205$, $P = 0.054$), but not 'number of mountings' ($F_{1,20} = 1.121$, $P = 0.302$; Fig. 2), were significantly affected by treatment. Sperm transfer was not observed in any groups. Scars on male's abdomen was recorded (Plate 5).

Male–Female Interactions: Male mounting was significantly reduced in the presence of male extract in the air stream applied to the bed bug pair (KruskalWallis test: $\chi^2 = 17.155$, $P < 0.001$, followed by Mann–Whitney U tests; Fig. 3). The percentages of mountings resulting in mating with observed sperm transfer were normal mating 100%, hexane treatment 76% and alarm pheromone 48%. Mounting duration and the treatments had significant effects on the sperm transfer (log likelihood: mounting duration: $G_1 = 43.110$, $P < 0.001$; treatment: $G_2 = 7.253$, $P = 0.027$).

DISCUSSION

Males use the alarm pheromone as a tool to stop the mating by other males as like females. The experiment depicts that, SSS behaviour occurs in male bed bugs and also the scars on the male abdomen reveals that, the SSS behaviour is not a safe process. The scars in the males' abdomen are the result of lacking a special adaption of spermatheca which is present in females. Presence of scars indicate that SSS behaviour occurs in male bed bugs. It may be because of miss identification with bloated body of males. The other side of the SSS is in the favour of insects where, the adult longevity was increased in case of seed beetle *Acanthoscelides obtectus* (Say) (Chrysomelidae: Bruchinae: Coleoptera).

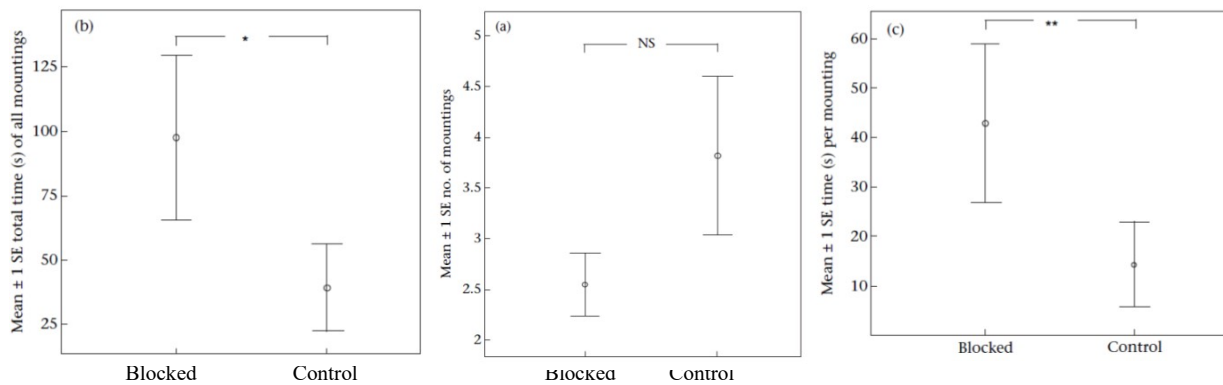


Fig. 2. Duration per mounting event in male-male interactions

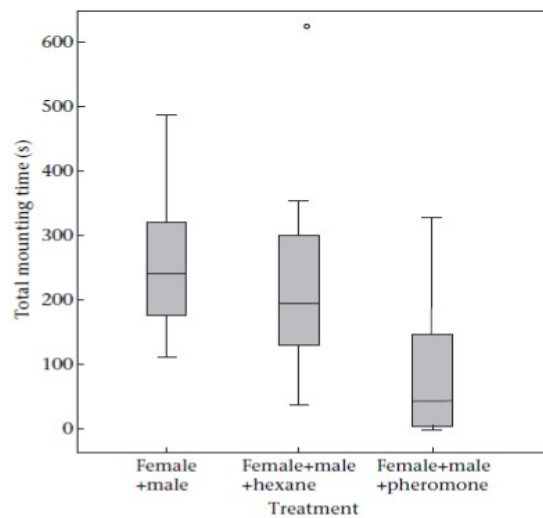


Fig. 3. Male-female mounting interactions

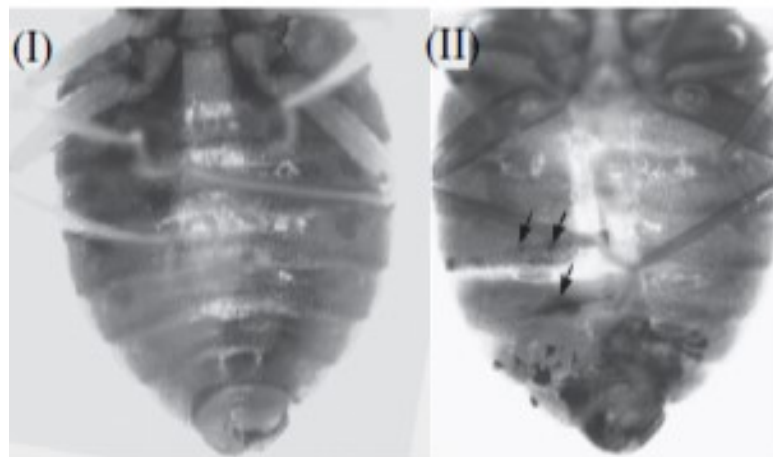


Plate 5. Male Mating Scars (The arrows point to the most obvious melanized tissue from damage of the cuticle)

Homosexual behaviour and its longevity cost in females and males of the seed beetle *Acanthoscelides obtectus* (Stojkovic *et al.*, 2010)

MATERIALS AND METHODS

Insects and experimental conditions: Populations of the seed beetle *A. obtectus* (Coleoptera: Chrysomelidae: Bruchinae) obtained from a laboratory population established in 1986. This population ('base'; hereafter referred to as B) had been produced by the mass-mating of equal numbers of adults from three local populations of *A. obtectus* captured in the vicinity of Belgrade, Serbia. The B population was maintained at large size (approximately 5000 individuals) in large bottles with approximately 5000 common bean

seeds for an approximately 40-day interval. All experiments were conducted in a dark incubator at 30°C and 70% relative humidity. All bean seeds were brought from one source and frozen before their use in the experiments. No food or water was offered to the experimental adults.

Experimental lines

All selection lines were derived from the B population. Experimental females and males were obtained from the lines of *A. obtectus* that were selected for reproduction in either early or late life. Four replicates per selection treatment were maintained under adult aphy. At the onset of the early reproduction regime, each replicate line was

initiated from approximately 500 newly-emerged females and males, randomly chosen from the B population. Females and males were kept together in ten separate Petri dishes at low adult density (approximately 50 individuals per 9-cm dish) with 50 bean seeds and females were allowed to lay eggs for 48 h after emergence. During the course of the experiment (225 generations), procedures were identical to those described for the first generations. The number of adults per replicate line per generation used as parents for the next generation varied in the range 300–500. Given that females of this beetle species do not remate during this 48-h period, the E selection treatment inadvertently created conditions for the evolution of monoandrous mating system. Indeed, the rate of female remating within the E treatment was very low, at approximately 13%. This treatment gave rise to beetles with enhanced fitness during early life and a short life span. Late reproduction lines were obtained from beetles selected for 158 generations. For each generation, over 1000 females and males in the L treatment were kept together in ten separate vials (diameter 3 cm, height 4 cm) at high adult density (i.e. approximately 100 individuals per vial) without beans and were allowed to mate from emergence until death. The beetles were introduced into bottles with approximately 100 bean seeds at day 10 and thus eggs laid prior to this day did not contribute to the next generation. Adults of both sexes from the L treatment were larger and lived longer than those from the E treatment. Because females and males were kept together during their whole lives, there was an opportunity for the remating of the females and for the development of a polyandrous mating system.

All experiments described below were performed using four-way crosses within each selection regime. These crosses were obtained by crossing the F1 generation of different pairs of replicate lines within each selection regime (E1 × E2) × (E3 × E4), where the subscript number refers to the specific replicate line]. The out crossing of replicate lines should have removed the effect of inbreeding depression and diminished any epistatic interaction among genes originating during the long-term selection as a result of Wahlund's effect or mutation pressure. In addition, the first and second generations of crosses were made from 1-day-old beetles allowed to mate and reproduce during their entire life span. This procedure ensured that the selected lines (i.e. their crosses) would pass two generations under relaxed selection and common housing regimes.

Longevity assays: The longevities of homosexual pairs were compared with individually housed virgin controls. In addition, to separate the costs of homosexual behaviour from the cost of producing gametes and associated materials and copulation costs, it was necessary to estimate the longevity of the heterosexual mating pairs. The longevities of eight different groups of females and males were therefore established within the B population and the E and L lines: (i) virgin females housed alone (V); (ii) virgin males housed alone; (iii) pairs of virgin females from the same selection treatment (homosexual–homotypic females; Ho1); (iv) pairs of virgin males from the same selection treatment (homosexual–homotypic males; Ho1); (v) pairs of virgin females from the E and L selection treatments (homosexual–heterotypic females; Ho2); (vi) pairs of virgin males from the E and L selection treatments (homosexual–heterotypic males; Ho2); (vii) females from heterosexual pairings (He); and (viii) males from heterosexual pairings (He). Each of the groups consisted of 100 individuals. The longevity assays of females and males from the heterosexual pairings were performed using mated pairs in separate Petri dishes with one bean seed. The presence of host seed, as well as mating, induces oocyte maturation and oviposition and, thus, longevity reduction as a consequence of heterosexual interactions represented a measure of the full cost of reproduction. The numbers of dead beetles were counted daily.

RESULTS

Longevity costs of female homosexual interactions: Females that copulated and laid eggs (He) lived on average shorter than unmated females kept alone (V) in all three experimental groups (Fig. 4), indicating a significant reproductive cost incurred by such females. It

is worth noting that the cost of cohabitation with other females (Ho1) appeared to be negligible in the B population, whereas, in both the E and L lines, homosexual females generally suffered a reduced longevity compared with females kept alone (6.9% and 23.5% in the E and L lines, respectively). The magnitudes of these reductions were, nevertheless, much smaller than those observed in the full cost of reproduction (i.e. the sum effect of mating itself and egg production on longevity), which amounted to 42.1% and 50.9% for the E and L females, respectively.

The comparison of the longevities of homosexual–homotypic pairs (Ho1; both females stemmed from the same line) and homosexual–heterotypic pairs (Ho2; one female from the E and another from the L line) revealed different patterns in the E and L lines. The E females from the Ho1 homosexual cohorts lived 12% longer than those E females kept together with L females (i.e. Ho2). By contrast, there was no significant difference in the longevity between Ho1 and Ho2 homosexual cohorts in the L line. The multiple comparisons between longevity means (Tukey's test; Fig. 8) revealed the patterns: $V > Ho1 > Ho2 > He$ and $V > (Ho1 = Ho2) > He$, for the E and L females, respectively. The overall shapes of the mortality functions between experimental female groups were fully consistent with the observed trend on the mean longevities.

Longevity costs of male homosexual interactions: In terms of the longevity costs, the patterns seen for the males were very similar to those observed for the females (Fig. 5). Selection for either early or late reproductive effort produced a more pronounced effects on the longevity of homosexual males compared with the B population. Compared with the males kept alone, the longevities of homosexual males were reduced by 14%, 24% and 31% in the B, E and L lines, respectively. It is worth noting that these decreases in longevity for males kept together with other males were higher than in the case of homosexual females. As in females, however, the homosexual interactions had a much smaller effect on male survival than heterosexual interactions, where the longevity loss were 43%, 54% and 55% in the B, E and L lines, respectively.

DISCUSSION

- Longevity was high in individuals which are Virgin and shows Homosexuality
- Homosexuality and being virgin has no significant difference
- Heterosexuality resulted in reduction in longevity

In case of heterosexual interaction, individuals have to spend more energy in finding their mate, pheromone production, in courtship behavior, egg production, etc. probably, this might be the reason for decrease in longevity of the insects in heterosexual behavior. The saved energy in the homosexual insects was utilized for increasing longevity.

Inference: Homosexuality results in more longevity

Some other hypotheses for occurrence of SSS behavior in insects

- I. By-product of heterosexual behavior:** It is a kind of mistaken identity where, the male insects confused by the odour or sex pheromone attached to the cuticle of other male during mating with the female and blindly try to mount on the mated male. Here mated males usually targeted by other males because of the attached pheromone. It is mainly seen in cockroaches, flies, wasps, beetles (Iguchi, 1996).
- II. Mating interruption:** This is the intentional homosexual behaviour where, male damage the genital organ of other male by forceful mating with an intention to be a dominant male and court more females. This is evidenced in flies, wasps, butterflies (He, 2008).
- III. Practice for heterosexual encounters:** This is the special case of homosexual behaviour mainly seen in red flour beetles where the young male individuals would use another male for practicing the mating process. Some of the supporting studies

stated that, the individuals with pre-mating practice with other males showed high mating efficiency in hetero sexual encounter (Levanet *et al.*, 2009)

IV. Biased sex ratio: This is the exceptional case or occur in controlled condition where, particular individual is exposed for a same sex for a longer period or if the population of one sex is high compared to other sex the individual shows SSS towards same sex. This is well explained in fruit fly *Drosophila melanogaster* Meigen where, the males of *D. melanogaster* were separated from the females for longer period and the observations were taken on the males' interaction among them. The isolated males showed more SSS behaviour than the males reared with females (Machiano *et al.*, 2017).

In case of dragonflies and damsel flies the common heterosexual behavior is male holds the female's head by its hook like projections (epiprocts) that makes the scars/ injury on the head region of females sometime the same will be observed in male's head too (which perform SSS behavior). Hence, heterosexualis a tradeoff process where one individual have to invest more for the success but not in case of SSS behavior. Nature has accepted this unusual behavior of living organisms and we can't frame the survival of the fittest theory to this phenomenon. Because it is the situation based expression of behavior but neither evolutionary nor the hereditary. Moreover, the one message from this behavior is that, we need to accept the nature as how it is.

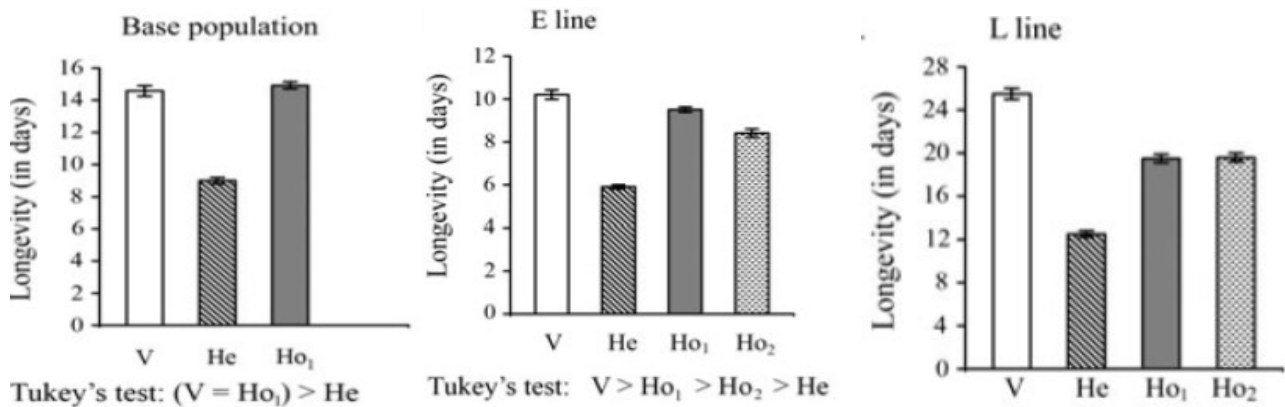


Fig. 4. Mean \pm SE longevity of female *Acanthoscelidesobtectus* from (A) the base population, (B) the early (E) line and (C) the late (L) line. V, virgin females housed alone; He, females from heterosexual pairings; Ho₁, pairs of virgin females from the same experimental treatment; Ho₂, pairs of virgin females from the E and L selection treatments

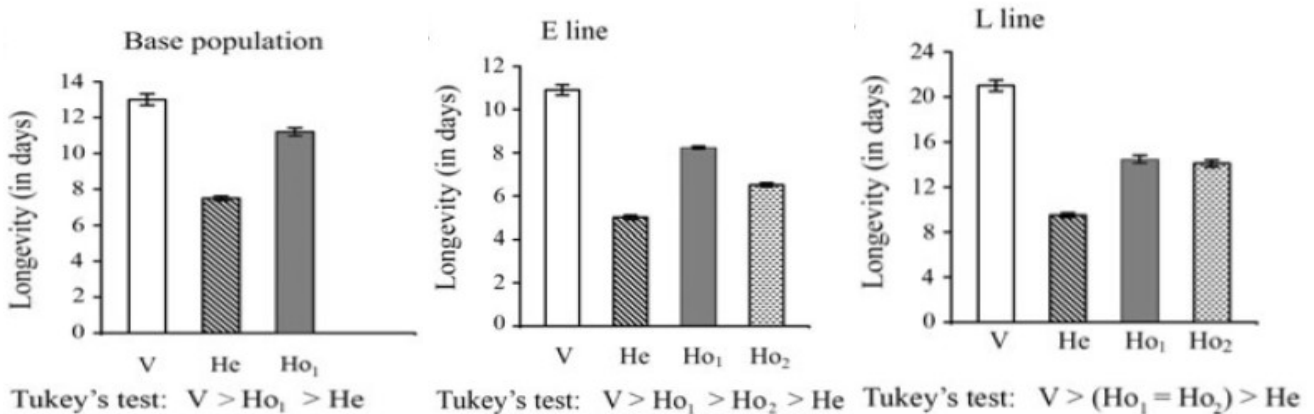


Fig. 5. Mean \pm SE longevity of male *Acanthoscelidesobtectus* from (A) the base population, (B) the early (E) line and (C) the late (L) line. V, virgin males housed alone; He, males from heterosexual pairings; Ho₁, pairs of virgin males from the same experimental treatment; Ho₂, pairs of virgin males from the E and L selection treatments

CONCLUSION

There is no evolutionary evidences for the occurrence of SSS behavior but there must be a proximate and ultimate causes for the occurrence of any behavior. The researchers' hypothesis is that, homosexual behavior is derived from heterosexual behavior because except the fertilization all the sequence of mating is present in homosexual behavior. When we see the different cases of the SSS behavior in insects, the SSS have been used for different purposes (ex: sperm dumping, satellite strategy, to avoid losing of females etc.) it means SSS is a strategic process but not a un intentional process. As SSS behavior increases the longevity and safer to the individuals it has become obligate and mostly preferred by the insects ex: 50% of female bruchids (*Callosobruchus* spp.) performs the SSS behavior to avoid being hurt by toothed aedeagus hence, they mount on other females and kick the approaching males.

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